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

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		Title Page
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4 5 6	1	TITLE
7	2	Burden of Lyme Disease (BOLD) Prospective Incidence Epidemiology Study Methods: Conducting
8 9	3	Active Surveillance in Endemic Areas of Six European countries
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4	1	ABSTRACT
5	2	Introduction:
6	3	This study is being conducted to inform the Phase 3 efficacy study for Pfizer and Valneva's
/ 8	4	investigational Lyme Disease Vaccine, VLA15. Previous Lyme vaccine efficacy studies exclusively
9	5	involved US sites. VLA15 Phase 3 will be conducted in US and Europe due to the vaccine's expanded
10	6	serotype coverage and the public health burden of LD in Europe. In Europe the existence and
11	7	location of study sites that have access to populations with high LD annual incidence is uncertain.
12 13	8	This active, prospective surveillance study assesses annual LD incidence of GP/primary care clinical
14	9	trial sites in high-incidence regions. This will allow for Phase 3 site vetting and better
15	10	characterization of LD burden in selected regions for study size calculations
16	10	characterization of 20 barach in selected regions for stady size calculations.
17 18	11	Methods and analysis:
19	12	The Burden of Lyme disease (BOLD) study will assess LD incidence at 15 GP/primary care practices in
20	13	endemic areas of 6 European countries from Spring 2021 until December 2022. Suspected LD cases
21	14	identified from each site's practice panel are documented on Screening Logs. Clinical diagnoses are
22 23	15	recorded, alongside LD clinical manifestations and standard of care Lyme diagnostic results. In the
24	16	initial 12-month enrolment phase, suspected LD cases are offered enrolment. Participants undergo
25	17	interview and clinical assessments to establish medical history, final clinical diagnosis, clinical
26 27	18	manifestations, and impact of LD on quality of life. Study specific procedures include LD serology,
27 28	19	skin punch biopsies and photographs of Lyme manifestations. For every enrolled participant
29	20	diagnosed with LD, 6-10 age-matched controls are randomly selected and offered enrolment for an
30	21	embedded LD risk factor analysis. Persistent symptoms or post-treatment Lyme Disease will be
31 22	22	assessed at follow-up visits up to two years after initial diagnosis while patients remain
32 33	23	symptomatic.
34		
35	24	Ethics and dissemination:
36 27	25	This study has been approved by all sites' local ethics committees. Results will be presented at
38	26	conferences and published in peer-reviewed journals.
39	27	
40		
41 42	28	STRENGTHS AND LIMITATIONS
43	29	• The quality and quantity of LD incidence data from European countries varies due to consensus
44	30	case definitions not being consistently used and differing reporting procedures. This study uses
45 46	31	consistent LD case definitions to establish comparative LD incidence from high-incidence areas
40 47	32	across 6 European countries.
48	33	• This study conducts LD surveillance in clearly defined populations (i.e., the practice panel of a
49	34	primary care provider), which will be used as the denominator for LD incidence calculations.
50 51	35	allowing for accurate calculation of incidence rates.
51 52	36	The study will follow enrolled LD cases post antibiotic treatment to assess the proportion of
53	37	natients that have persistent symptoms or Post Treatment Lyme Disease (PTLD)
54	20	 The study can only canture ID diagnoses that the study site staff are aware of and as such may
55 56	20	miss some or all events only treated outside of the practice due to travel or other reasons
57	70	depending on the completeness of practices' routing systems to identify such events
58	40	depending on the completeness of plactices routine systems to identify such events.
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1 2		
3	1	Pre-season baseline serology specimens will not be available from the study population to assess
4	1 2	for scrosony oreion across the lymp space and on this basis, asymptomatic lymp disease
5	2	iof seroconversion across the contine season and, on this basis, asymptomatic Lyme disease
6 7	3	infections will also not be captured.
7 8		INTRODUCTION
9	4	INTRODUCTION
10	5	Lyme Disease (LD) is the most frequent tick-borne disease in the moderate climates of the northern
11	6	hemisphere.[1] LD is caused by infection with <i>Borrelia burgdorferi sensu lato (B.b.s.l.)</i> . There are 18
12	7	documented <i>Borrelia</i> genospecies, but only a subset has been associated with human disease.[2]
14	8	Serotypes (ST) are determined by outer surface protein A (OspA) types. In North America, almost all
15	9	LD (>98%) is due to <i>B. burgdorferi senso stricto</i> (ST1), with minor contribution from <i>B. mayonii</i> (1-
16	10	2%). In Europe, <i>B. afzelii</i> (ST2) and <i>B. garinii</i> (ST3,5,6) are predominant, but <i>B. burgdorferi</i> s.s. (ST1)
1/ 10	11	and <i>B. bavariensis</i> (ST4) are also documented.[3] To address the high burden of LD, Pfizer and
19	12	Valneva are jointly developing a 6-valent vaccine (VLA15) for the prevention of LD caused by Borrelia
20	13	strains expressing outer surface protein A (OspA) ST 1-6 by active immunization.
21		
22	14	This prospective epidemiology study will collect key information to support VLA15's Phase 3 efficacy
25 24	15	study. While two successful Phase 3 efficacy studies have been previously conducted for other
25	16	investigational Lyme vaccines, they exclusively involved US sites because those vaccines included
26	17	Serotype 1 only.[4,5] Due to high medical need in Europe and US, VLA15 includes expanded serotype
27	18	coverage, so its Phase 3 efficacy study will be conducted in both the US and Europe. In the US, it is
28 29	19	established that at least a 1% annual incidence of acute LD is present in high-risk areas.[3] However,
30	20	due to less developed LD surveillance in Europe, uncertainty exists regarding the existence and
31	21	location of potential Phase 3 efficacy trial sites that would have access to a population with high
32	22	annual incidence of acute LD from which to enrol Phase 3 study participants. On this basis, this
33 24	23	active, prospective surveillance study will identify discrete GP/primary care practice-based sites in
35	24	potential high-incidence geographical regions and assess their annual LD incidence. This will allow
36	25	for vetting of potential Phase 3 sites and better characterization of the burden of LD in the region for
37	26	use in study size calculations.
38		
39 40	27	BOLD study sites are embedded in GP/primary care practices to provide accurate denominators for
41	28	LD incidence measurements (i.e., the practice panel is a clearly delineated source population) and
42	29	comprehensive event capture for the incidence numerator (i.e., primary care physicians should be
43	30	aware of Lyme events among their patients diagnosed at their practice sites as well as in other
44 45	31	clinical settings such as urgent care centers and hospitals). To estimate LD incidence by LD risk
46	32	factors, 6-10 randomly selected potential control participants from the site's practice panel will be
47	33	approached for participation for each enrolled case with newly diagnosed LD to create a comparison
48	34	group for a nested case control analysis.
49 50		
51	35	BOLD will also deliver epidemiologic data on high-risk geographic areas for uses beyond the Phase 3
52	36	trial. The quality and quantity of LD incidence data from European countries varies due to consensus
53	37	case definition not being consistently used and differing reporting procedures.(Burn L, Tran TMP, Pilz
54	38	A, et al. Incidence of Lyme Borreliosis in Europe from National Surveillance Systems (2005-2020).
55 56	39	Vector Borne Zoonotic Dis 2022 submitted) Annual LD incidence is reported as up to 632 per 100,000
57	40	population in Sweden,[6] and the population-weighted LD incidence in western Europe has been
58	41	estimated at 22 cases per 100,000 person-years among all ages.[7] However, these composite
59	42	estimates and national incidence estimates are limited by under-reporting and marked intra-country
00		

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 <i>Borrelia</i> genospecies/OspA serotype distribution of LD in persons of all age 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 diseas 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 age 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 ag 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 Phas 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

1		
2 3 4		efficacy trial), overall, by age group, and by site.
6 7 8 9 10 11 12	• 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.
13 14 15 16	• Describe LD diagnostic testing practices under current standard of care.	• Proportion of participants with standard of care LD diagnostic testing, overall and by type.
17 18 19 20 21	• 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.
22 23 24 25 26 27 28 20	• 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.
29 30 31 32 33 34 35 36 37 38	• 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
39 1 40 2 41 2 42 43		
44 45 46 47		
48 49 50 51		
52 53 54 55 56		
57 58 59 60		
	6 For peer review only - http://bmjopen.bm	nj.com/site/about/guidelines.xhtml

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 clin manifestation (Erythema migrans ver disseminated LD that subsequently develop persistent symptoms, includ 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 (incl PTLD) by clinical manifestation (Eryth migrans and disseminated LD) among suspected LD cases: Pain severity (Sh Form McGill Pain Questionnaire [SF-I and the pain subscale of the Medical Outcomes Survey Short Form-36 [SF- Fatigue Severity (Fatigue Severity Sca [FSS]); Cognitive impairment (Cogniti 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 manifesta (Erythema migrans and disseminated)
 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 (PTLE cases compared to treated LD cases symptoms not meeting PTLD case definition, and participants with othe LD diagnosis. SF-36, SF-MPQ, FSS and 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 type of medical visits and therapeuti procedures, and frequency of hospitalization, and mean length of several s

4 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.

	Cond	ition	ICD9 Code	ICD10 Code
	Lvme	disease	088.81	A69.2
	Lyme	borreliosis	NA	NA
	Ervth	ema migrans	NA	NA
	Acrod chron	lermatitis atrophicans lica	701.8	L90.4
	Lyme	arthritis	NA	A69.23, M01.2
	Lyme	neuroborreliosis	NA	A69.22
	Borre	lial lymphocytoma	NA	NA
	Lyme	carditis	NA	NA
	Lyme- inflan	-related intraocular	NA	NA
	Bell's	Palsy	351.0	G51.0
	Vario and n	us neuritis, neuropathy, erve disorders	351.8-357.89	G51.8-G61.89
	NA =	not available		
3				
4	Eligibi	lity		
5	LD cas	e participant Inclusion (Criteria	
6	During	the 12-month enrolment	period, patients must me	et all of the following criteria to be
7	enrolle	rolled in the consented portion of the study:		
8	1.	1. Member of participating patient practice.		
	2.	Suspected or confirmed	newly diagnosed LD durin	g enrolment period regardless of ti
9				
9 10		infection.		
9 10 11	2	infection. Evidence of a personally	signed and dated informe	ed consent and assent (when age-
9 10 11 12	3.	infection. Evidence of a personally appropriate and per loca	signed and dated informe al requirements) documen	ed consent and assent (when age- it indicating that the patient (or a le
9 10 11 12 13	3.	infection. Evidence of a personally appropriate and per loca acceptable representation	r signed and dated informe al requirements) documen ve) has been informed of a	ed consent and assent (when age- it indicating that the patient (or a le all pertinent aspects of the study an
9 10 11 12 13 14	3.	infection. Evidence of a personally appropriate and per loca acceptable representation they agree to participate	signed and dated informe al requirements) documen ve) has been informed of a e.	ed consent and assent (when age- it indicating that the patient (or a le all pertinent aspects of the study an
9 10 11 12 13 14 15	3. There a	infection. Evidence of a personally appropriate and per loca acceptable representation they agree to participate are no exclusion criteria for	signed and dated informe al requirements) documen ve) has been informed of a e. or the LD case participants	ed consent and assent (when age- at indicating that the patient (or a le all pertinent aspects of the study ar
9 10 11 12 13 14 15 16	3. There a	infection. Evidence of a personally appropriate and per loca acceptable representativ they agree to participate are no exclusion criteria for ol Participant Inclusion (r signed and dated informe al requirements) documen ve) has been informed of a e. or the LD case participants Criteria	ed consent and assent (when age- at indicating that the patient (or a le all pertinent aspects of the study an
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9 10 11 12 13 14 15 16 17 18	3. There a Contro Study:	infection. Evidence of a personally appropriate and per loca acceptable representativ they agree to participate are no exclusion criteria for ol Participant Inclusion (I participants must meet a	r signed and dated informe al requirements) documen ve) has been informed of a e. or the LD case participants Criteria all of the following inclusio	ed consent and assent (when age- at indicating that the patient (or a le all pertinent aspects of the study ar s.

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2 requirements) indicating that the patient (or a legally acceptable representative) has been 3 informed of all pertinent aspects of the study and that they agree to participate.

4 **Control Participant Exclusion Criteria**

- 5 Control participants meeting any of the following criteria will not be included in the study:
 - 1. Active Lyme Disease in last 90 days.

8 **Study Visits**

9 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 1ª	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-
Screening, Demographics, and Informed Consent/assent	Х		Х	Х	Х	Х	Х
Confirm eligibility (inclusion criteria)	Х					Х	
Patient (or parent/legal guardian) interview, including symptoms and LD risk factors	х	х	х	х	х	х	
Study blood sample for Lyme serology, and scavenge residual SOC cerebral spinal fluid and synovial fluid specimens if available	х	х	х				
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	х	х	х	х	Х		
Collect pre-specified medical history of clinical significance							
including past LD diagnoses	Х	Х	Х			х	Х
Collect SOC LD diagnostic laboratory testing results	Х	Х	Х	Х	Х		
Collect LD treatment and healthcare utilization, and LD event outcome	х	x	x	х	х		
Record clinical diagnosis and LD manifestation categories							
experienced based on clinical assessment	Х	Х	Х				
Record clinical assessment of persistent symptoms/PTLD			Х	Х	Х		
Collect Charlson Comorbidity Index (CCI) information			Х				Х
Collect health survey outcome information							Х
Assess Adverse Events (AEs) 2 hours after blood draw and 24							
hours after skin punch biopsy	Х	Х	Х	x	Х	Х	
Assess interest in participation in follow-up studies, and the potential for the participant to meet Phase 3 exclusion criteria	х	x	х			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

15 10 OspA serotype by sequencing. SOC Lyme diagnostic laboratory results are collected including but not

16
 11 limited to serology with ELISA and/or immunoblots, culture and PCR for *Borrelia* from specimen,
 12 histology and neurological, dermatological and/or rheumatological assessments. If CSF and/or

18 12 Instology and hedrological, definatological and/or medinatological assessments. If CSF and/or
 19 13 synovial fluid samples are obtained from a participant as a part of SOC testing, site staff should

- ²⁰ 14 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
- 16 for the presence of borrelial molecules by different biochemical techniques, immunoassays and/or
- 17 nucleic acid sequences by PCR. Specimen processing and testing will be conducted at designated
- 25
 18 central laboratories and/or Pfizer (401 N Middletown Rd, Pearl River, NY 10965, United States).
 26
 26
- 19 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).
- 49
 50 35 Participants may decline study-specific procedures, however, remain enrolled to allow for complete
 51 36 tracking of all clinical diagnoses of LD and capture of standard of care diagnosis data.
 52
- 53 37 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.

1 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 BbsI 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 BbsI result from biopsy OR Positive Culture of BbsI from biopsy
Lyme Neuroborreliosis ^ь	 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 Bbsl from cerebrospinal fluid

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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 Bbsl 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 Bbsl from ocular fluid
 a. Laboratory confirmation w and skin biopsy specimens, b. These clinical manifestatio disseminated if there are n 	ill primarily come from dedicated specimens) ns categories are disseminated Lyme manife nultiple EM lesions.	collected specifically for the study (ie, serum

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.

8 Persistent symptoms and PTLD Case Definition

9 Participants are considered to have PTLD at Visit 3 (9-10 months after Visit 2) if they continue to

10 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]
- 12 The case definition for PTLD is described in Table 6.

16

1 Table 6 PTLD Case Definition

Pres	entation	Sign/Symptom (Detailed Definition)	Evaluations(s)
Post Lym	-treatment e 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 Subjective symptoms are so severe that result in substantial reduction in activities No other co-morbidities that can explain illness 	 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 result in reduction of activities as assessed by either the "limitations in physical activities" subscale (score 55 or lower) of the SF-36.[12]
2 3 Persi	stent Sympt	oms of Lyme Disease	
4 The ir 5 qualit 6 (inclu 7 quest 8 perfo 9 criter 0 • 1	ncidence and and any of life and l ding confirma- ionnaires and rms a clinical ia. Patient had antibiotic i	severity of persistent symptoms (includ nealth resource use associated with per ed) enrolled LD cases are assessed. This d by patient (or parent(s)/legal guardiar assessment at Visit 3 to determine if th d a final diagnosis of Lyme disease and regimen with resolution or stabilization	ling PTLD) by clinical manifestation, and sistent symptoms among suspected is evaluated at Visit 3 by standardized h(s)) interview. In addition, the investigator he patient meets the following PTLD completed treatment with an appropriate of objective manifestations of Lyme
2 3 • 4 5	disease. Patient suf fatigue, ge months af	fers from debilitating (results in substa neralized musculoskeletal pain, or cogr ter completing therapy and lasting for a	ntial reduction in activities) symptoms of nitive difficulties having onset within 6 nt least 6 months after onset.

- No concurrent comorbidities can otherwise explain the patient's subjective symptoms.
- 2 At Visits 4 and 5, participants with a final diagnosis of LD who had any persistent symptoms
- 3 documented at the previous visit (Visit 3 or Visit 4, respectively) are asked to return for participant
- 4 interview, medical record review and to re-assess LD impact on participant-reported physical and
- 5 mental functions and quality of life.

6 Patient and Public Involvement

7 No patients were involved.

8 ETHICS AND DISSEMINATION

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

13 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.

24 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.

29 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.

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3	1	Dissemination plan
4	2	This study has been approved by all sites' local ethics committees in participating countries. Results
5	2	from this study will be published in peer-reviewed international journals and presented at relevant
7	2	notional and international conferences. Driver owners to publication has Driveria international prosting to a Driver (DI) of
8	4	national and international conferences. Prizer supports publication by a Principal investigator (PI) of
9	5	the results of the study based on information collected or generated by the PI, however, the first
10	6	manuscript will be a joint publication covering all sites.
11		
12	7	AUTHORS' CONTRIBUTIONS:
13	8	The study concept and design was developed by FB, AP, JS, BG, NB, ALB, KA and BP. The protocol
14	0	manuscript was drafted by MB and critically reviewed by EB, KH, ALB, AD, BC, KA and IS
15	9	manuscript was drafted by MB and Childany Tevlewed by LB, KH, ALB, AF, BG, KA and JS.
17		
18	10	FUNDING STATEMENT:
19	11	This study is co-funded by Pfizer and Valneva. Pfizer is the sponsor of the study. No specific grant or
20	12	award funded this research.
21		
22	13	COMPETING INTERESTS' STATEMENT.
23	1/	BB ALB AP EB IS KH LH BG and KA are employees of Pfizer Inc. and may own Pfizer stock NB is
24 25	15	on employee of Velacus Austria Crabil, DD and MD were University students on placement at Dfirer
25	15	an employee of valneva Austria GmbH. BP and MB were University students on placement at Pfizer
27	16	UK during the BOLD Study.
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59	41	after treatment for Lyme porreliosis: study protocol for an observational, prospective cohort 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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1		Title Page
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5	1	TITLE
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7	2	A Prospective Incidence Epidemiology Study Protocol: Conducting Active Surveillance to assess the
8	3	Burden of Lyme disease (BOLD) in Primary Care Practices in Endemic Areas of Six European countries
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42	22	Address: Prizer vaccines, 9 Riverwark, Citywest Business Campus, Dublin 24
43	23	KEYWORDS
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51	27	figures and tables)
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3 1	1	ABSTRACT
5	2	Introduction:
6	3	Lyme Disease (LD) is the most frequent tick-borne disease in the moderate climates of Europe. This
7 o	4	study will inform the Phase 3 efficacy study for Pfizer and Valneva's investigational Lyme Disease
o 9	5	Vaccine, VLA15, VLA15 Phase 3 will be conducted in US and Europe due to the vaccine's serotype
10	6	coverage and public health burden of LD. In Europe the existence and location of sites that have
11	7	access to populations with high LD annual incidence is uncertain. This active, prospective
12 13	8	surveillance study assesses annual LD incidence at GP/primary care sites, allowing for Phase 3 site
14	9	vetting and better characterization of LD burden in selected regions for study size calculations
15	5	
16 17	10	Methods and analysis:
17	11	This Burden of Lyme disease (BOLD) study will assess LD incidence overall and by site at 15
19	12	GP/primary care practices in endemic areas of 6 European countries from Spring 2021 until
20	13	December 2022 and will be summarized with counts (n), percentages (%), and associated 95%
21	14	confidence intervals. Suspected LD cases identified from site's practice panels are documented on
22	15	Screening Logs, where clinical LD manifestations, diagnoses, and standard of care diagnostic results
24	16	are recorded. In the initial 12-month enrolment phase, suspected LD cases are offered enrolment.
25	17	Participants undergo interview and clinical assessments to establish medical history, final clinical
26 27	18	diagnosis, clinical manifestations, and quality of life impact. Study specific procedures include LD
28	19	serology, skin punch biopsies and Lyme manifestation photographs. For every enrolled participant
29	20	diagnosed with LD, 6-10 age-matched controls are randomly selected and offered enrolment for an
30	21	embedded LD risk factor analysis. Persistent symptoms or post-treatment LD will be assessed at
31 32	22	follow-up visits up to two years after initial diagnosis while patients remain symptomatic.
33	22	Tables and discontinuation.
34	23	Ethics and dissemination:
35 36	24	This study has been approved by all sites' local ethics committees. Results will be presented at
37	25	conferences and published in peer-reviewed journals.
38	26	
39 40		
40 41	27	STRENGTHS AND LIMITATIONS
42	28	This study uses consistent LD case definitions to establish comparative LD incidence from high-
43	29	incidence areas across 6 European countries.
44 45	30	• LD surveillance is conducted in clearly defined populations (i.e., the practice panel of a primary
46	31	care provider), allowing for accurate calculation of LD incidence rates.
47	32	• The study will follow enrolled LD cases post antibiotic treatment to assess persistent symptoms
48	33	or Post Treatment Lyme Disease (PTLD).
49 50	34	• The study can only capture LD diagnoses that study site staff are aware of, and thus may miss
51	35	some or all events only treated outside of the practice due to travel or other reasons.
52	36	• Pre-season baseline serology specimens will not be available to assess for seroconversion,
53 54	37	therefore asymptomatic Lyme disease infections will not be captured.
54 55		
56	38	INTRODUCTION
57	39	Lyme Disease (LD) is the most frequent tick-borne disease in the moderate climates of the northern
58 59	40	hemisphere.[1] LD is caused by infection with Borrelia burgdorferi sensu lato (B.b.s.l.). There are 18
60	41	documented Borrelia genospecies, but only a subset has been associated with human disease.[2]

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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

- 43 cases. The study also aims to assess the impact of LD on quality of life by comparing suspected LD

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

OBJECTIVES AND ENDPOINTS

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

for occurrence on the second

1 Table 1 – BOLD Objectives and Endpoints

Pri	imary Objective:	Prir	nary 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.		
Sec	condary 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 <i>Borrelia</i> 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.		
Exp	ploratory Objectives:	Exp	oloratory 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.		

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•	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
Obje inclu	ectives for assessment of persistent symptoms of LD uding PTLD:	Enc of l	lpoints for assessment of persistent symptoms .D 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	•	Duration of persistent symptoms (including PTLD) by clinical manifestation (Erythema migrans and disseminated LD)
	that do not meet PILD case definition		inigrans and disseminated ED
•	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.

1 METHODS AND ANALYSIS

2 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.

13 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.

31 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,

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3	1	starting from each sites' activation, is an enrolment phase when all suspected LD cases identified are
4	2	offered study enrolment (Fig. 1). During this period, the Screening Log also includes information
5	3	relating to patient consent and enrolment.
7	0	
8	4	
9 10	5	Fligibility
10	5	
12	6	LD case participant inclusion Criteria
13	7	During the 12-month enrolment period, patients must meet all of the following criteria to be
14	8	enrolled in the consented portion of the study:
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17	9	1. Member of participating patient practice.
18	40	
19	10	2. Suspected or confirmed newly diagnosed LD during enrolment period regardless of timing of
20	11	infection.
21		
22	12	3. Evidence of a personally signed and dated informed consent and assent (when age-
24	13	appropriate and per local requirements) document indicating that the patient (or a legally
25	14	acceptable representative) has been informed of all pertinent aspects of the study in an age-
26	15	appropriate manner and that they agree to participate.
27		
28	16	There are no exclusion criteria for the LD case participants.
29 30		
31	17	Control Participant Inclusion Criteria
32	18	Control participants must meet all of the following inclusion criteria to be eligible for inclusion in the
33	19	study:
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35 26	20	1. Member of participating patient practice at time of associated case diagnosis.
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38	21	2. Evidence of informed consent and assent (when age-appropriate and per local
39	22	requirements) indicating that the patient (or a legally acceptable representative) has been
40	23	informed of all pertinent aspects of the study and that they agree to participate.
41	_	
42 43	24	Control Participant Exclusion Criteria
44	25	Control participants meeting any of the following criteria will not be included in the study:
45	20	
46	26	1. Active Lyme Disease in last 90 days
47		
48 70	27	Controls were selected as soon as feasible after enrolment of the related LD case and those who
50	28	later became a LD case were retained as a control if there were no LD associated symptoms at the
51	29	time of control enrolment or other evidence of infection (e.g., serological seroconversion)
52	25	
53	30	Study Visits
54	21	Study charge in a second star and star to five visits for LD cases (Table 2) Visits 4 E are for
56	27	norticipants with a final diagnosis of LD who had any participants with a final diagnosis of LD who ha
57	32	participants with a final diagnosis of LD who had any persistent symptoms documented at the
58	33	previous visit. Controls are seen at Contact 1, and a selection of controls will have Contact 2.
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Table 2 – BOLD Study Specific Procedures

		LD Ca	se Participants			Con	trols
Procedure/ Assessment	Visit 1 Day 1ª	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 Month 16
Screening, Demographics, and Informed Consent/assent	Х		Х	Х	Х	х	Х
Confirm eligibility (inclusion criteria)	Х					х	
Patient (or parent/legal guardian) interview, including symptoms and LD risk factors	х	х	х	х	х	х	
Study blood sample for Lyme serology, and scavenge residual SOC cerebral spinal fluid and synovial fluid specimens if available	Х	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	х	х	х	х	х		
Collect pre-specified medical history of clinical significance						1	
including past LD diagnoses	х	х	Х			х	х
Collect SOC LD diagnostic laboratory testing results	Х	Х	Х	Х	Х		
Collect LD treatment and healthcare resource utilization, and LD event outcome	х	x	x	х	х		
Record clinical diagnosis and LD manifestation categories							
experienced based on clinical assessment	Х	Х	Х				
Record clinical assessment of persistent symptoms/PTLD			Х	Х	Х		
Collect Charlson Comorbidity Index (CCI) information			Х				Х
Collect health survey outcome information							Х
Assess Adverse Events (AEs) 2 hours after blood draw and 24							
hours after skin punch biopsy	Х	Х	Х	x	Х		
Assess interest in participation in follow-up studies, and the potential for the participant to meet Phase 3 exclusion criteria	X	x	Х			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

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- 1 with final LD diagnosis, six practice panel patients without current LD are approached regarding
- 2 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
- ⁷ 4 leisure exposures), past history for tick-borne disease including LD, TBE, tick bite prophylaxis and
 ⁸ 5 known tick bites, interest in investigational LD vaccine study participation, and assessment of
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 6 meeting potential Phase 3 exclusion criteria (Table 2). If the proportion of potential control
- 11 7 participants declining participation is higher than anticipated, the number of potential controls
- ¹² 8 approached will be increased to 10 so approximately 4 control participants are enrolled per LD
- 13149 event. The Screening Log tracks control selection, consent and enrolment.
- 15 10 To assess the impact of LD on quality of life, for each enrolled LD case participant with a final 16 11 diagnosis of LD, one of the age-matched control participants who had Contact 1 performed is re-17 18 12 consented 16-18 months later to collect health survey outcome information. This includes a 36-Item 19 13 Short Form Survey (SF-36), degree of fatigue measured by Fatigue Severity Scale (FSS), degree of 20 14 cognitive difficulties measured by the Cognitive Failures Questionnaire (CFQ), and degree of pain 21 22 15 measured by the Short Form McGill Pain Questionnaire as well as to assess pre-specified medical 23 16 history and comorbidities. 24
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28 29 19 Sample Size Estimates

- 30 20 Study size is based on feasibility, not on hypothesis testing as this is a descriptive study. It is
- 21 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
- 26 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
- 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.
- 42
 43 29 6-10 potential control participants are approached for each enrolled participant with a final clinical
 44 30 diagnosis of LD, with approximately 75% (4.5 controls per case) expected to enrol. If 500 participants
- 45 31 are enrolled, approximately 90% of these will have final clinical diagnosis of LD (no laboratory
- 46
 47 32 confirmation required), yielding an estimate of 2,025 controls enrolled.

48 49 33 **Data Analysis**

50 51 34 Analysis of Endpoints

- 52 35 For proportion endpoints, data will be summarized with counts (n), percentages (%), and associated 53 36 95% confidence interval (CI)s, which will be calculated using the Clopper-Pearson method.
- 54 55 Frequency of Research Related Injuries (RRIs) and adverse events (AEs) following study procedures
- 38 will be tabulated. Results for cases and control participants will be presented separately. For the
- 57 39 primary endpoint, data will be summarized overall, by site, by country and by province. The
- ⁵⁸ 40 population denominator will be based on the size of the primary care practices' patient panels. All
- 41 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 Bbsl 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 BbsI result from biopsy OR Positive Culture of BbsI from biopsy
Lyme Neuroborreliosis ^ь	 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 Bbsl from cerebrospinal fluid

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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 BbsI 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 BbsI result from ocular fluid OR Positive Culture of BbsI from ocular fluid
a. Laboratory confirmatio and skin biopsy specim b. These clinical manifest disseminated if there a	n will primarily come from dedicated specimens ens) ations categories are disseminated Lyme manife re multiple EM lesions.	s collected specifically for the study (ie, serum
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 consid	ered to have PILD at Visit 3 (9-10 mon oms 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 Subjective symptoms are so severe that result in substantial reduction in activities No other co-morbidities that can explain illness 	 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 result in reduction of activities as assessed by either the "limitations in physical activities" subscale (score 55 or lower) of the SF-36.[15]
2			
3 F 4 T 5 c 6 (7 c 8 F 9 c .0	Persistent Sympt The incidence and quality of life and h (including confirme questionnaires and performs a clinical criteria. • Patient had antibiotic	oms of Lyme Disease severity of persistent symptoms (incluc nealth resource use associated with per ed) enrolled LD cases are assessed. This d by patient (or parent(s)/legal guardiar assessment at Visit 3 to determine if th d a final diagnosis of Lyme disease and a regimen with resolution or stabilization	ding PTLD) by clinical manifestation, and esistent symptoms among suspected is evaluated at Visit 3 by standardized n(s)) interview. In addition, the investigator be patient meets the following PTLD completed treatment with an appropriate of objective manifestations of Lyme
12 13 14	 Patient suf fatigue, ge months aff 	fers from debilitating (results in substa neralized musculoskeletal pain, or cogr ter completing therapy and lasting for a	ntial reduction in activities) symptoms of nitive difficulties having onset within 6 nt least 6 months after onset.

1 2		
2	1	 No concurrent comorbidities can otherwise evolain the nationt's subjective symptoms
4	Т	• No concurrent comorbidities can otherwise explain the patient's subjective symptoms.
5 6	2	At Visits 4 and 5, participants with a final diagnosis of LD who had any persistent symptoms
7	3	documented at the previous visit (Visit 3 or Visit 4, respectively) are asked to return for participant
8	4	interview, medical record review and to re-assess LD impact on participant-reported physical and
9 10	5	mental functions and quality of life.
11		
12	6	Patient and Public Involvement
13 14	7	No patients were involved.
15	0	
16	8	ETRICS AND DISSERVINATION
17	9 10	The study is conducted in accordance with the protocol, legal and regulatory requirements, and the
19	10	general principles set for international Errical Guidennes for Biomedical Research Involving
20	12	GCP and the Declaration of Helsinki
21 22	12	der, and the Declaration of Heisinki.
23	13	The ethics committees that approved this study are as follows:
24		
25 26	14	Ethical commission of IKEM and FTN, Faculty of Thomayer Hospital (Prague, Czechia)
27	15	Ethics Committee at the State Medical Association of Hesse (Frankfurt, Germany) Schleswig-
28	16	Holstein (Bad Segeberg, Germany), and Saxony (Dresden, Germany)
29 30		
31	17	Bioethics Committee at the Lublin Medical Chamber (Lublin, Poland), Medical University of Bialystok
32	18	and Regional Bialystok Medical Chamber (Bialystok, Poland)
33 34	19	Ethical Commission of the Trencin Self-governing region (Trencin, Slovakia)
35		
36 27	20	The Commission of the Republic of Slovenia for Medical Ethics (Ljubljana, Slovenia)
38	21	The Ethics Review Authority Box 2110 (Uppsala, Sweden)
39		
40 41	22	Consent and assent
42	23	The Informed Consent Documents (/assent documents) and any participant recruitment materials
43	24	follow ICH GCP, local regulatory requirements, and legal requirements, including applicable privacy
44 45	25	laws. The investigator, or a person designated by the investigator, obtains written informed consent
46	26	from each participant (or the participant's legally acceptable representative, parent[s], or legal
47	27	guardian and the participant's assent, when applicable) before any study-specific activity is
48 ⊿o	28	performed. Participants may withdraw from the study at any time at their own request, or they may
5 0	29	be withdrawn at any time at the discretion of the investigator or sponsor for safety, behavioural, or
51	30	administrative reasons. If the participant withdraws from the study and withdraws consent for
52	31	disclosure of future information, no further evaluations should be performed, and no additional data
55 54	32	is collected.
55	20	Confidentiality
56 57	27	Measures are taken to ensure protection of participant personal data. Participant pares or other
58	24	directly identifiable data on any sponsor forms reports publications or in any other disclosures are
59	55	ancetty identifiable data on any sponsor rorms, reports, publications, or in any other disclosures, are
60		

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

3 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
- spontalleously 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
- 12 7 period if related to a study procedure are recorded in the CRF. Any SAE that an investigator suspects 13 8 may be related to any Driver product used by the participant under reutine and during and outside
- 15 8 may be related to any Pfizer product used by the participant under routine care during and outside
- 9 the active collection period is reported immediately upon awareness, and under no circumstance
 10 exceeding 24 hours. All processes comply with country specific regulatory requirements relating to
- 10 exceeding 24 hours. All processes comply with country specific regulatory requirements relating to
 11 safety reporting to the regulatory authority, IRBs/ECs, and investigators. Reporting of exposure to
- 10 12 any Pfizer product during pregnancy or breast feeding applies throughout the active collection
- 12 any Prizer product during pregnancy or breast reeding applies throughout the a
 20 13 period; when required, is reported within 24 hours of investigator awareness.

15 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.

21 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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43 28 **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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13 14	8	FIGURE LEGEND:
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19	12	SUPPLEMENTAL TABLES AND FIGURES:
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21	14	Supplemental Table - Lyme Disease and related diagnoses with relevant International Classification of
22	15	Diseases (ICD: procedure codes used in modical billing) codes if available
24	15	Diseases (ICD, procedure codes used in medical bining) codes in available.
25	16	Supplemental Figure – Active Surveillance Process within GP practice panels.
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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	ΝΑ	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.