Safety and immunogenicity of a novel multivalent OspA vaccine against Lyme borreliosis in healthy adults: a double-blind, randomised, dose-escalation phase 1/2 trial

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Summary

Background Lyme borreliosis is caused by *Borrelia burgdorferi* sensu stricto in the USA and by several *Borrelia* species in Europe and Asia, but no human vaccine is available. We investigated the safety and immunogenicity of adjuvanted and non-adjuvanted vaccines containing protective epitopes from *Borrelia* species outer surface protein A (OspA) serotypes in healthy adults.

Methods Between March 1, 2011, and May 8, 2012, we did a double-blind, randomised, dose-escalation phase 1/2 study at four sites in Austria and Germany. Healthy adults aged 18–70 years who were seronegative for *B burgdorferi* sensu lato were eligible for inclusion. Participants were recruited sequentially and randomly assigned to one of six study groups in equal ratios via an electronic data capture system. Participants and investigators were masked to group allocation. Participants received three vaccinations containing 30 μg, 60 μg, or 90 μg OspA antigen with or without an adjuvant, with intervals of 28 days, and a booster 9–12 months after the first immunisation. The coprimary endpoints were the frequency and severity of injection-site and systemic reactions within 7 days of each vaccination, and the antibody responses to OspA serotypes 1–6, as established by ELISA. This study is registered with ClinicalTrials.gov, number NCT01504347.

Findings 300 participants were randomly assigned: 151 to adjuvanted vaccines (50 to 30 μg, 51 to 60 μg, and 50 to 90 μg doses), and 149 to non-adjuvanted vaccines (50 to 30 μg, 49 to 60 μg, and 50 to 90 μg doses). Adverse reactions were predominantly mild, and no vaccine-related serious adverse events were reported. The risk of systemic reactions (risk ratio 0·54 [95% CI 0·41–0·70]; p<0·0001) and of moderate or severe systemic reactions (0·35 [0·13–0·92]; p=0·034) was significantly lower for adjuvanted than non-adjuvanted formulations. The 30 μg adjuvanted formulation had the best tolerability profile; only headache (five [10%, 95% CI 4–20] of 50), injection-site pain (16 [32%, 21–45]), and tenderness (17 [34%, 23–47]) affected more than 6% of patients. All doses and formulations induced substantial mean IgG antibody titres against OspA serotypes 1–6 after the first three vaccinations (range 6944–17 321) and booster (19 056–32 824) immunisations. The 30 μg adjuvanted formulation induced the highest antibody titres after the booster: range 26 143 (95% CI 18 906–36 151) to 42 381 (31 288–57 407).

Interpretation The novel multivalent OspA vaccine could be an effective intervention for prevention of Lyme borreliosis in Europe and the USA, and possibly worldwide. Larger confirmatory formulation studies will need to be done that include individuals seropositive for *Borrelia burgdorferi* sensu lato before placebo-controlled phase 3 efficacy studies can begin.

Funding Baxter.

Introduction

Lyme borreliosis is a multisystem inflammatory disease caused by infection with tick-borne bacterial spirochetes of the *Borrelia burgdorferi* sensu lato species complex.[1,2] The most common clinical manifestation of the disease is an expanding local skin lesion that can be accompanied by fatigue, fever, headache, arthralgia, and myalgia. Infected individuals can also develop more serious manifestations affecting the skin (lesions, atrophy, and fibrous nodule formation), nervous system (facial palsy, meningitis, myelitis, and encephalitis), joints (recurrent or persistent large joint synovitis), or heart (eg, conduction abnormalities and carditis).[3]

Lyme borreliosis can be successfully treated with antibiotics,[1,2] but patients and their primary physicians can be unaware of infection until the onset of severe disease symptoms, even where the disease is endemic.[4] About 10% of patients in the USA do not respond clinically to antibiotic treatment.[1] Moreover, patients who do recover after antibiotic treatment are vulnerable to reinfection.[5] Lyme borreliosis is the most common arthropod-borne disease in the temperate northern hemisphere,[6] and, in 2011, was the sixth most commonly reported notifiable infectious disease in the USA.[7] 85 000 cases are reported every year in Europe,[8] and about 30 000 cases registered annually in the USA,[7] where it

accounts for more than 95% of reported cases of vector-borne illness.[2]

Vaccination would be the most effective intervention for prevention of Lyme borreliosis, but no vaccine is available for human use.[2] In US clinical trials,[9,10] vaccines based on bacterial outer surface protein A (OspA) serotype 1 derived from *B burgdorferi* sensu stricto (the only *Borrelia* species causing Lyme borreliosis in the USA) were safe and effective. However, disease in Asia and Europe is caused by several *Borrelia* species with antigenically distinct OspA proteins.[1,2] Therefore, an OspA vaccine for use worldwide would have to induce antibodies against several serotypes.

A previously marketed monovalent vaccine based on OspA serotype 1 (OspA-1) was associated with safety concerns,[2] specifically speculation that arthritis in some recipients of the vaccine was triggered by molecular mimicry between an epitope of OspA-1 for T-helper cells and an epitope of human lymphocyte function associated antigen 1 (LFA-1).[11,12] Although no evidence supported this hypothesis and a subsequent study[13] suggested that molecular mimicry does not have a role in Lyme arthritis, this association was one of several factors that contributed to poor acceptance of the vaccine by the public and by physicians, and the decision to withdraw the vaccine from the market in 2002.[4,12]

We have used knowledge of OspA structure and function[14,15] to develop a new vaccine, which comprises three recombinant OspA antigens. Each recombinant OspA antigen contains protective epitopes from two different OspA serotypes: OspA-1 and OspA-2 (*B burgdorferi* sensu stricto and *Borrelia afzelii*); OspA-5 and OspA-3 (both *Borrelia garinii*); and OspA-6 and OspA-4 (*B garinii* and *Borrelia bavariensis*). In a proof-of-concept study,[12] one chimeric molecule (OspA-1 and OspA-2) elicited antibody responses that protected mice against infection with either *B burgdorferi* sensu stricto (OspA-1) or *B afzelii* (OspA-2). The new multivalent vaccine is designed to protect against all major disease-causing *Borrelia* species in the USA (OspA-1) and Europe (OspA serotypes 1–6), and potentially throughout the world. The hypothetical risk of T-cell cross-reactivity has been eliminated with the replacement of the putative cross-reactive OspA-1 epitope with the corresponding OspA-2 sequence.[16] We did a dose-finding study to investigate the safety and immunogenicity of adjuvanted and non-adjuvanted vaccine formulations in healthy adults.

Methods

Study design and participants

Between March 1, 2011, and May 8, 2012, we did a double-blind, randomised, dose-escalation, phase 1/2 study at four sites in Austria and Germany. Healthy adults aged 18–70 years who were seronegative for *B burgdorferi* sensu lato (tested by C6 ELISA) were eligible for inclusion. Individuals were excluded according to our exclusion criteria (appendix), such as when they had active Lyme borreliosis or chronic illness related to Lyme borreliosis, had received antibiotic treatment for Lyme borreliosis within the previous 3 months, or had received any live vaccine within 4 weeks or inactivated vaccine within 2 weeks of enrolment.

The study was done in accordance with the International Conference on Harmonisation Guideline for Good Clinical Practice. An independent data monitoring committee of three external medical experts reviewed data. All participants provided written informed consent.

Randomisation and masking

Participants were recruited sequentially and randomly assigned to one of six study groups in equal ratios. Randomisation was done centrally via an electronic data capture system. Participants and investigators were masked to group allocation.

Procedures

Participants were assigned to receive vaccines containing 30 μg, 60 μg, or 90 μg OspA antigen with or without aluminium hydroxide adjuvant (1 mg aluminium hydroxide per dose). The vaccines contained three lipidated OspA chimeric molecules produced in *Escherichia coli* strain HMS174 (DE3) with a T7 expression system. The proximal portion of an OspA-1 sequence (strain B31) is fused to the distal portion of an OspA-2 sequence (strain PKo), replacing the putative cross-reactive OspA-1 epitope with the corresponding OspA-2 sequence to produce the recombinant OspA antigen containing OspA-1 and OspA-2. The molecular mimicry hypothesis was based on partial homology between LFA-$1_{\alpha 332\text{-}340}$ and the immunodominant T-cell epitope $OspA_{165\text{–}173}$ of *B burgdorferi* sensu stricto. This epitope differs in six of the nine core residues in OspA-1 and OspA-2, and the aminoacids necessary for T-cell reactivity are absent on the OspA-2 antigen.[17] The strategy used to fuse the proximal and distal parts of two OspA antigens maintains the overall three-dimensional structure of the native OspA molecules, ensuring that key protective epitopes are maintained. Similarly, for the recombinant OspA antigen containing OspA-6 and OspA-4, an OspA-6 sequence (strain K48) is fused to a OspA-4 sequence (strain pTroB), and for the recombinant containing OspA-5 and OspA-3, an OspA-5 sequence (strain W) is fused to an OspA-3 sequence (strain PBr). Each of the novel antigens were generated by cloning OspA fragments prepared from complementary overlapping oligonucleotides.

We selected the doses on the basis of previous experience with monovalent OspA vaccines that contained 30 μg adjuvanted or non-adjuvanted OspA.[9,10] Both formulations elicited substantial antibody responses and were safe and efficacious.[9,10] Therefore, we chose a 30 μg dose of the multivalent OspA vaccine for Lyme borreliosis (10 μg of each bivalent antigen) as the lowest dose for this study. The highest dose was 90 μg of the multivalent OspA vaccine (30 μg of each bivalent antigen).

See Online for appendix

Participants were to receive three intramuscular immunisations with intervals of 28 days, and a booster 9–12 months after the first dose. Blood was drawn before the first immunisation, 7 days and 28 days after each immunisation, 6 months and 9 months after the first immunisation, and before the booster immunisation.

We did antibody screening with a commercially available C6 ELISA assay (Immunetics, Boston, MA, USA; used according to the manufacturer's instructions) to assess whether participants had been infected with *B burgdorferi* sensu lato before randomisation and before the booster immunisation. The C6 ELISA measures the response to a highly conserved antigen of the VlsE surface protein, and, as such, was not used for investigation of the immune response to vaccination. The antigen used in the C6 assay is a 26 aminoacid synthetic peptide derived from the invariable region of VlsE surface protein, which is highly immunogenic and specific for Lyme borreliosis,[18] and is conserved in the four major *Borrelia* species associated with human disease in Europe and the USA (ie, *B burgdorferi* sensu stricto, *B afzelii*, *B bavariensis*, and *B garinii*). Serum samples that tested positive by C6 ELISA before the booster were also verified with a highly specific immunoblot assay (Euroimmune, Lübeck, Germany), according to the manufacturer's instructions.

The primary safety endpoint was the frequency and severity of injection-site and systemic reactions within 7 days of each vaccination. Participant diaries were used to obtain daily oral body temperature, solicited injection-site and systemic reactions, and other adverse events, which were assessed using the Food and Drug Administration's toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials[19] as guidance.

The primary immunogenicity endpoint was the antibody response to OspA serotypes 1–6 28 days after the third vaccination, as established by ELISA with affinity-purified recombinant OspA antigens representing each OspA serotype. We used individual OspA antigens rather than the chimeric vaccine immunogens, because these antigens are equivalent to the form of the antigen expressed by the borrelia strains to be targeted by the vaccine. Antibody titres to the chimeric vaccine immunogens would not allow measurement of serotype specific responses. We defined IgG antibody titres as the highest serum dilution at which the optical density was at least three times higher than background. The detection limit of the assay is a titre of 100; titres lower than 100 were recorded as a titre of 50.

We established the ability of vaccine-induced antibodies to bind to and to promote the killing of borrelia in vitro by surface binding and borreliacidal killing assays, using borrelia strains expressing OspA-1 (strain B31), OspA-2 (strain Arcon), OspA-3 (strain PBr), OspA-4 (strain DK6), OspA-5 (strain W), and OspA-6 (strain KL11). We used flow cytometry to quantify antibody binding to borrelia via phycoerythrin-labelled anti-IgG antibodies and a DNA-specific dye. We defined the surface-binding titre as the highest dilution at which fluorescence was at least three times higher than that of the negative control. We used a luciferase-based cell-viability assay to quantify borrelia killing. We incubated heat-inactivated serum samples with an exogenous complement source (guinea pig or rabbit serum) for 3–8 days at 33°C. Borreliacidal activity was expressed as the reciprocal of the highest dilution factor resulting in at least a 50% reduction in luminescence relative to the negative control.

Statistical analyses

A sample size of 300 participants (50 in each group) was judged sufficient to record dose-dependent and formulation-dependent differences in immunogenicity and adverse reactions, which commonly have a close temporal association with vaccination. However, the study was not powered to detect uncommon or rare adverse events, and, as such, we included no placebo group. The sample size would enable detection of an adverse event with a true underlying prevalence of 1% with a probability of 95%. We used Hanley's[20] simple approximation: $\Pi \approx 3/N$, in which Π is the underlying incidence that can be detected and N is the total sample size. With the assumption that 10% of participants will drop out, about 45 individuals in each study group would be available for immunogenicity assessment, such that the 90% CI limits of the seroconversion frequency would extend no more than 12% from the reported frequencies if they are about 90%.

The safety dataset contains all participants who were vaccinated at least once. The immunogenicity dataset contains all participants who were vaccinated at least once and had baseline and at least one titre measurement after vaccination. We analysed the risks of injection-site and systemic reactions in a period of 7 days after each vaccination, applying the general estimating equation method for parameter estimation, accounting for fixed effects of vaccine dose, use of adjuvant, and time; assuming a binomial distribution of the response variable; and accounting for the repeated subject effect. We omitted the interaction terms between the fixed effects from the final analysis model because they were non-significant at the 10% level. We back-transformed estimated model variables and 95% CI limits into risk ratios (RR; calculated by log link).

For injection-site and systemic reactions, we estimated the risks of a moderate or severe adverse event within a period of 7 days after the first vaccination and compared them with the risk of a mild adverse event in the same category. The model accounted for the fixed effects of vaccine dose and adjuvant, and the dependent variable was maximum severity for each participant, pooled into two categories: 1 (mild) and 2 (moderate or severe). For seroconversion, we computed point estimates and Clopper-Pearson 90% CIs, assuming a theoretical

seroconversion (increase of ELISA titre by four times) frequency of 90%. We did a longitudinal analysis for log-transformed antibody titres within a repeated mixed-model ANCOVA framework, accounting for the effect of vaccine dose, adjuvant, time, and baseline titre as covariates, and for the random subject effect. We estimated least-square mean differences and 90% CIs within the mixed framework and then back-transformed them into ratios of geometric mean titres (GMTs) by exponentiation. We made no adjustment for multiplicity. All analyses were done in SAS (version 9.1.3).

This study is registered with ClinicalTrials.gov, number NCT01504347.

Role of the funding source

The study was designed and funded by Baxter. Baxter employees were responsible for study design, data collection, data analysis, data interpretation, writing of

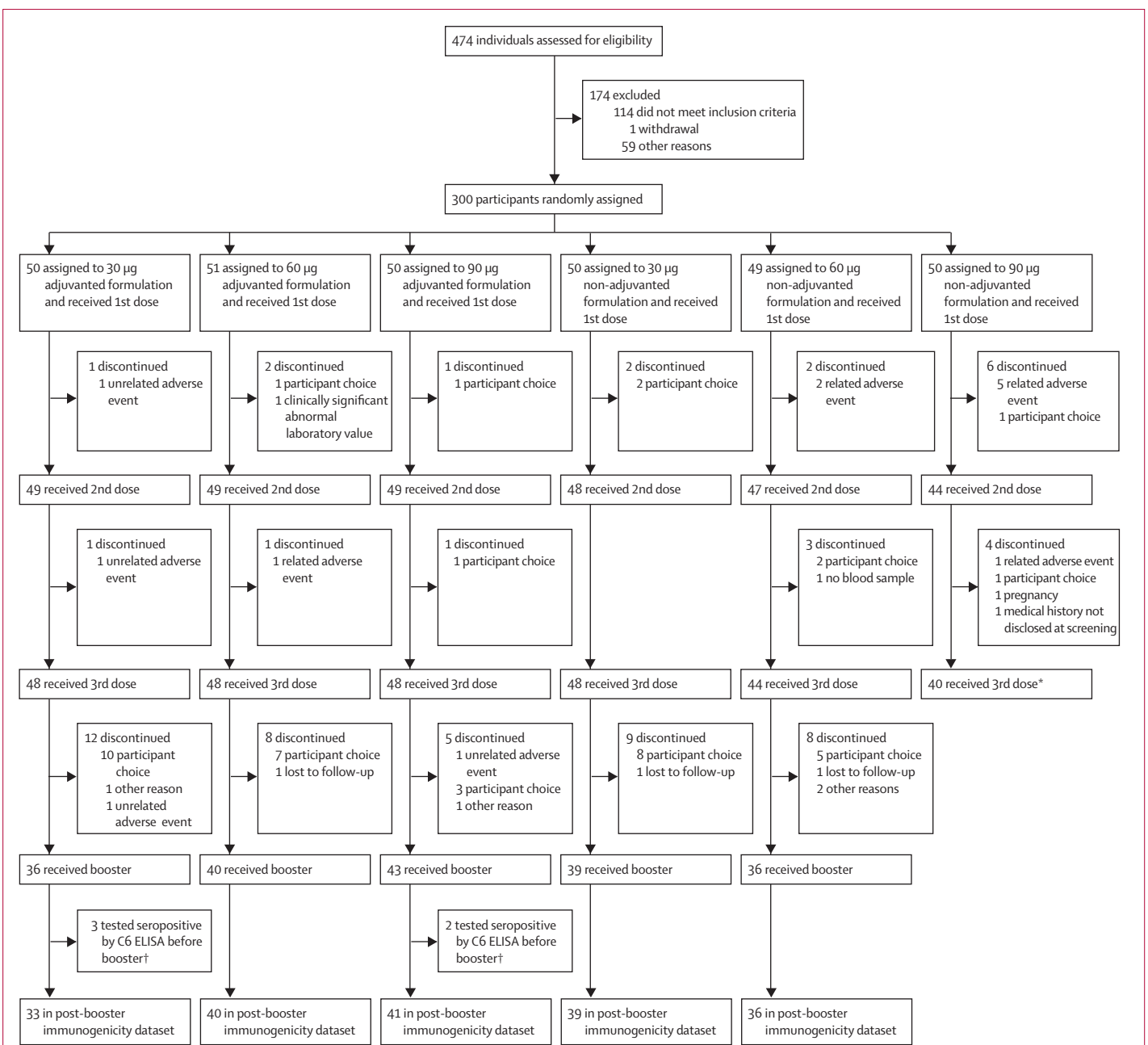

Figure 1: **Study profile**

*No booster given in this group because of high frequency of systemic reactions. †No positive C6 ELISA results were subsequently confirmed by immunoblot.

the report, and the decision to submit for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. All study data were available to all authors on request.

Results

300 participants were randomly assigned (figure 1). Baseline characteristics were similar across the groups (table 1). Most adverse events occurred within 24 h of immunisation, were mild in severity, and resolved spontaneously within 72 h (appendix). The risk of systemic reactions (RR 0·54, 95% CI 0·41–0·70; p<0·0001) and of moderate or severe systemic reactions (0·35, 0·13–0·92; p=0·034) was significantly lower for adjuvanted than for non-adjuvanted formulations (appendix). Successive vaccinations were generally associated with a decrease in risk of systemic reaction; risk was significantly higher after the first vaccination (RR 2·33, 1·63–3·33; p<0·0001) and second vaccination (1·61, 1·10–2·36, p=0·014) than after the booster (appendix). Participants receiving 90 µg non-adjuvanted formulation had the most systemic reactions after the first immunisations (table 2, appendix) and were excluded from the booster immunisation.

The most common injection-site reactions within 7 days of the first immunisation were pain and tenderness (table 2). Fatigue, headache, myalgia, and malaise were more common in recipients of non-adjuvanted formulations than in those of adjuvanted vaccines (table 2). Most solicited reactions occurred infrequently in recipients of the 30 µg adjuvanted formulation (table 2)—only headache, injection-site pain, and tenderness affected more than 6%—and most

	Adjuvanted formulations			Non-adjuvanted formulations		
	30 µg (n=50)	60 µg (n=51)	90 µg (n=50)	30 µg (n=50)	60 µg (n=49)	90 µg (n=50)
Age (years)	37·4 (15·3)	39·8 (14·7)	42·7 (14·6)	39·6 (12·8)	41·6 (14·4)	38·3 (13·7)
Weight (kg)	72·2 (15·3)	71·9 (15·0)	77·4 (16·8)	75·3 (13·0)	73·8 (13·0)	77·5 (18·3)
Height (cm)	170·0 (9·5)	171·0 (9·5)	173·1 (8·4)	173·6 (8·3)	171·6 (10·1)	174·1 (8·9)
Body-mass index (kg/m²)	24·8 (4·2)	24·5 (4·1)	25·6 (4·3)	27·9 (3·7)	25·0 (3·6)	25·4 (4·5)
Sex						
Male	21 (42%)	23 (45%)	25 (50%)	24 (48%)	20 (41%)	27 (54%)
Female	29 (58%)	28 (55%)	25 (50%)	26 (52%)	29 (59%)	23 (46%)

Data are mean (SD) or n (%).

Table 1: **Baseline characteristics**

	Adjuvanted formulations			Non–adjuvanted formulations		
	30 µg (n=50)	60 µg (n=51)	90 µg (n=50)	30 µg (n=50)	60 µg (n=49)	90 µg (n=50)
Local reactions						
Any	24 (48%, 36–61)	30 (59%, 46–71)	23 (46%, 34–59)	30 (60%, 47–72)	23 (47%, 35–60)	25 (50%, 38–62)
Swelling	0 (0%, 0–6)	1 (2%, 0–9)	2 (4%, 1–12)	2 (4%, 1–12)	2 (4%, 1–12)	3 (6%, 2–15)
Induration	0 (0%, 0–6)	2 (4%, 1–12)	4 (8%, 3–17)	2 (4%, 1–12)	1 (2%, 0–9)	7 (14%, 7–25)
Redness	1 (2%, 0–9)	1 (2%, 0–9)	3 (6%, 2–15)	2 (4%, 1–12)	0 (0%, 0–6)	4 (8%, 3–17)
Injection-site pain	16 (32%, 21–45)	15 (29%, 19–42)	15 (30%, 20–42)	22 (44%, 32–57)	16 (33%, 22–45)	18 (36%, 25–49)
Tenderness	17 (34%, 23–47)	25 (49%, 37–61)	15 (30%, 20–42)	18 (36%, 25–49)	18 (37%, 25–50)	15 (30%, 20–42)
Systemic reactions						
Any	9 (18%, 10–29)	13 (25%, 16–37)	10 (20%, 11–32)	27 (54%, 42–66)	22 (45%, 33–58)	29 (58%, 45–70)
Malaise	0 (0%, 0–6)	2 (4%, 1–12)	1 (2%, 0–9)	10 (20%, 11–32)	12 (25%, 15–37)	16 (32%, 21–44)
Fatigue	2 (4%, 1–12)	4 (8%, 3–17)	3 (6%, 2–15)	12 (24%, 15–36)	12 (25%, 15–37)	14 (28%, 18–40)
Headache	5 (10%, 4–20)	7 (14%, 7–24)	2 (4%, 1–12)	11 (22%, 13–34)	10 (20%, 12–32)	17 (34%, 23–47)
Nausea	0 (0%, 0–6)	1 (2%, 0–9)	0 (0%, 0–6)	1 (2%, 0–9)	7 (14%, 7–25)	8 (16%, 8–27)
Vomiting	0 (0%, 0–6)	0 (0%, 0–6)	0 (0%, 0–6)	1 (2%, 0–9)	1 (2%, 0–9)	2 (4%, 1–12)
Myalgia	3 (6%, 2–15)	4 (8%, 3–17)	7 (14%, 7–25)	10 (20%, 11–32)	11 (22%, 13–34)	14 (28%, 18–40)
Arthralgia	0 (0%, 0–6)	1 (2%, 0–9)	0 (0%, 0–6)	2 (4%, 1–12)	11 (22%, 13–34)	8 (16%, 8–27)
Fever (>38·0°C)	0 (0%, 0–6)	0 (0%, 0–6)	1 (2%, 0–9)	0 (0%, 0–6)	4 (8%, 3–18)	4 (8%, 3–17)

Data are n (%, 90% CI).

Table 2: **Participants with solicited injection-site and systemic reactions within 7 days of first immunisation**

were rated as mild (appendix). Ten serious adverse events were reported in eight participants: moderate infective bursitis (participant received 60 µg adjuvant dose); severe pulmonary embolism (90 µg non-adjuvant); severe cerebral metastasis and bleeding, and lung cancer (30 µg adjuvant); cubital tunnel syndrome (60 µg non-adjuvant); ligament rupture (30 µg non-adjuvant); intravertebral disc protrusion (30 µg non-adjuvant); pulmonary embolism and deep vein thrombosis (90 µg adjuvant); and elective abortion (90 µg non-adjuvant; defined as serious adverse event because required admission to hospital). All were judged to be unrelated to vaccination. No symptoms suggesting Lyme borreliosis or immune arthritis were reported.

Substantial GMTs were induced after the three vaccinations in all groups (range 6944–17 321; appendix). There was a significant positive effect of dose on antibody titres for all OspA serotypes after three vaccinations and a significant negative effect of adjuvantation; the highest mean GMTs after three vaccinations were elicited by the 90 µg non-adjuvanted formulation (appendix). We recorded little apparent dose response after adjuvanted vaccines; significant differences in GMTs were recorded only for the 90 µg dose compared with the 30 µg dose for OspA-1 and OspA-5 (appendix).

Antibody titres were effectively increased after booster vaccination (mean GMT range 19 056–32 824; appendix). After the booster immunisation, use of adjuvant had a significant positive effect on antibody induction for all OspA serotypes (appendix). For five of six serotypes, we recorded a significant inverse relation between dose and antibody titre, independent of the effect of adjuvant. The 30 µg adjuvanted formulation induced the highest antibody titres after the booster: range 26 143 (95% CI 18 906–36 151) to 42 381 (31 288–51 407; appendix). The difference between the antibody titres elicited by the 30 µg and 60 µg adjuvanted doses was significant for OspA-2, OspA-4, and OspA-5, and that between the titres elicited by the 30 µg and 90 µg adjuvanted doses was significant for OspA-1, OspA-3, OspA-4, and OspA-5 (appendix). On the basis of the favourable tolerability profile and high post-booster OspA antibody titres induced, we identified the 30 µg adjuvanted vaccine as the best dose and formulation.

GMTs against the six individual OspA serotypes after three immunisations with the 30 µg adjuvanted formulation and geometric mean fold increases (GMFIs) of OspA IgG titres compared with baseline were substantial (table 3). After booster vaccination, GMTs and GMFIs further increased (table 3). After the first three vaccinations, the proportion of participants receiving the 30 µg adjuvanted dose who seroconverted (increase of ELISA titre by four times) ranged from 95·7% (95% CI 86·9–99·2) to 100% (93·7–100·0) against the different OspA serotypes; this proportion increased to 100% (91·3–100) for all OspA serotypes after the booster immunisation (appendix).

After the first three vaccinations, most participants who had received the 30 µg adjuvanted formulation had antibody titres of at least 1000 against the different OspA serotypes, with at least 52% achieving antibody titres of at least 5000 (figure 2). After the booster immunisation, the proportion with antibody titres of at

	OspA serotype 1	OspA serotype 2	OspA serotype 3	OspA serotype 4	OspA serotype 5	OspA serotype 6
Geometric mean IgG titre						
Baseline (n=49)*	123 (98–154)	87 (73–103)	106 (88–128)	129 (106–157)	86 (75–99)	114 (98–134)
28 days after first dose (n=49)	239 (187–305)	141 (114–175)	289 (226–369)	181 (146–225)	229 (183–286)	355 (285–441)
28 days after second dose (n=49)†	2858 (2188–3733)	1705 (1302–2233)	5439 (4185–7069)	2169 (1688–2787)	4368 (3348–5699)	3929 (3057–5048)
28 days after third dose (n=46)	6400 (5087–8052)	4699 (3770–5857)	9328 (7609–11 436)	5588 (4500–6941)	9258 (7500–11 427)	7727 (6366–9379)
6 months after first dose (n=44)	1224 (994–1508)	1062 (868–1300)	1613 (1343–1937)	1177 (927–1493)	1718 (1366–2160)	1389 (1131–1705)
9 months after first dose (n=45)‡	844 (700–1018)	485 (395–595)	691 (575–830)	948 (782–1149)	1114 (917–1354)	616 (505–750)
Immediately before booster (n=33)	713 (555–915)	509 (410–633)	713 (593–856)	596 (464–766)	767 (605–973)	676 (549–834)
28 days after booster (n=33)	31 254 (23 407–41 730)	26 143 (18 906–36 151)	34 352 (28 225–41 808)	32 939 (24 635–44 041)	42 381 (31 288–57 407)	31 917 (23 519–43 313)
Geometric mean fold increase compared with baseline						
28 days after first dose (n=49)	1·9 (1·5–2·4)	1·6 (1·4–2·0)	2·7 (2·1–3·5)	1·4 (1·2–1·7)	2·7 (2·1–3·3)	3·1 (2·5–3·8)
28 days after second dose (n=49)†	23·3 (16·8–32·2)	19·6 (14·6–26·3)	51·4 (38·1–69·4)	16·8 (13·1–21·6)	50·7 (38·1–67·4)	34·3 (26·5–44·6)
28 days after third dose (n=46)	52·2 (37·7–72·3)	54·6 (41·8–71·4)	87·8 (65·7–117·4)	44·2 (34·6–56·5)	106·0 (82·5–136·2)	67·5 (54·4–83·6)
6 months after first dose (n=44)	10·4 (7·7–14·0)	12·6 (10·0–16·0)	15·9 (12·1–20·8)	9·1 (7·1–11·8)	20·7 (16·2–26·6)	12·2 (9·8–15·2)
9 months after first dose (n=45)‡	7·1 (5·5–9·0)	5·8 (4·6–7·4)	6·6 (5·1–8·6)	7·5 (5·9–9·4)	13·2 (10·5–16·5)	5·4 (4·4–6·7)
28 days after booster (n=33)	261·4 (179·8–380·2)	339·9 (239·4–482·7)	336·4 (239·0–473·3)	267·0 (198·0–359·9)	517·4 (374·7–714·4)	278·4 (206·9–374·7)
28 days after booster§ (n=33)	43·9 (33·2–58·0)	51·3 (39·1–67·4)	48·2 (38·3–60·7)	55·2 (43·5–70·1)	55·2 (43·3–70·5)	47·2 (36·3–61·4)

Data in parentheses are 90% CI. *No titre measurement after vaccination for one participant who received the first dose, because they withdrew; therefore, they were not included in immunogenicity dataset. †Blood obtained for immunogenicity assessment from one patient but no third dose given because of an unrelated adverse event. ‡One more participant at 9 months than at 6 months because one blood sample was missed at 6 months. §Compared with antibody titres before booster.

Table 3: **Geometric mean IgG titre to six OspA serotypes and geometric mean fold increase of OspA IgG titres compared with baseline for participants given 30 µg adjuvanted vaccine**

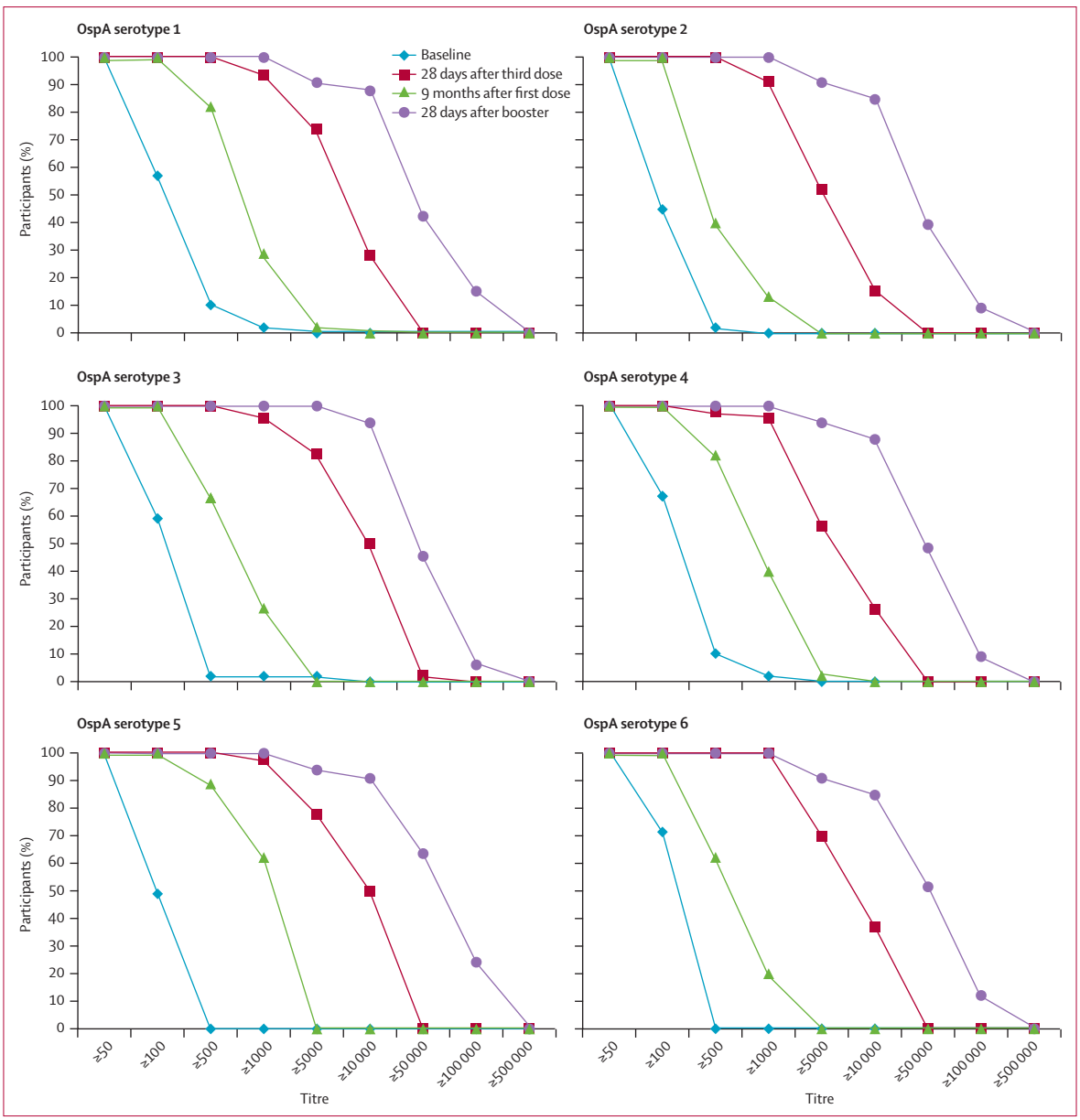

Figure 2: **Reverse cumulative distribution of OspA titres in participants receiving the 30 µg adjuvanted formulation**

least 5000 increased further (figure 2). Additionally, more than 80% of participants had OspA titres of at least 10 000 (figure 2).

In addition to the high titres of total IgG OspA antibodies achieved by the multivalent OspA vaccine, potent antibody responses were generated, which bound to or promoted the killing of *B burgdorferi* sensu stricto (OspA-1), *B afzelii* (OspA-2), *B bavariensis* (OspA-4), and *B garinii* (OspA-3, OspA-5, and OspA-6; table 4). Surface binding and borreliacidal antibody responses induced by vaccination returned to near-baseline levels before the booster immunisation; after the booster immunisation, they increased substantially (table 4).

Discussion

We have shown that a novel multivalent chimeric OspA vaccine is safe, well tolerated, and immunogenic in healthy adults. Local and systemic reactions in our study were predominantly mild in severity. Both non-adjuvanted and adjuvanted formulations induced high titres of OspA antibodies after the first three vaccinations, but systemic reactions were more common and of higher severity in recipients of non-adjuvanted formulations than in those of adjuvanted formulations. Although adjuvant did not have a positive effect on antibody induction after the first three vaccinations, it did for all OspA serotypes after booster immunisation.

	OspA serotype 1*	OspA serotype 2†	OspA serotype 3‡	OspA serotype 4§	OspA serotype 5‡	OspA serotype 6‡
Surface-binding antibodies						
Baseline (n=49)	12 (9–17)	3 (3–3)	13 (13–13)	3 (3–4)	7 (6–8)	8 (6–8)
28 days after third dose (n=46)	77 (50–117)	38 (26–57)	767 (582–1012)	11 (9–15)	38 (29–50)	25 (17–36)
Immediately before booster (n=33)	26 (18–39)	7 (5–9)	34 (27–43)	4 (3–5)	17 (13–21)	11 (9–13)
28 days after booster (n=33)	1239 (837–1834)	466 (314–692)	3928 (3072–5021)	115 (77–172)	429 (307–598)	377 (268–531)
Borreliacidal antibodies						
Baseline (n=49)	35 (28–43)	28 (25–32)	ND	33 (25–42)	31 (24–41)	33 (26–43)
28 days after third dose; n=46)	131 (85–201)	101 (70–145)	ND	65 (46–92)	52 (38–73)	152 (89–260)
Immediately before booster (n=33)	58 (39–86)	34 (27–42)	ND	37 (28–49)	32 (25–41)	49 (32–75)
28 days after booster (n=33)	889 (634–1248)	654 (470–909)	ND	706 (459–1085)	228 (158–329)	774 (454–1319)

Data in parentheses are 90% CI. The killing assay could not be done for *Borrelia garinii* expressing OspA serotype 3 because of their inherent complement sensitivity. ND=not done. **Borrelia burgdorferi* sensu stricto. †*Borrelia afzelii*. ‡*B garinii*. §*Borrelia bavariensis*.

Table 4: **Antibody titres for specific OspA serotypes induced by the 30 µg adjuvanted formulation capable of binding to the surface of and killing *Borrelia* spp**

The reasons for the apparent absence of a dose response in recipients of adjuvanted formulations after the primary immunisations and the inverse dose response after the booster are unclear. Several other adjuvanted vaccines have shown an absence of or inverse dose response.[21,22] The size of the antibody response might be determined by the ratio of adjuvant to antigen rather than by the antigen dose per se.[23] With respect to the inverse dose response after the booster immunisation, a reduced antigen dose might be sufficient to induce substantial antibody titres. However, in previous studies of a monovalent OspA-1 vaccine, substantially higher antibody titres were reported for a 30 µg adjuvanted dose[24] than for a 10 µg adjuvanted dose.[25]

The safety and immunogenicity of monovalent OspA-1 vaccines formulated with or without adjuvant have been investigated in several previous studies.[9,10,24,26–29] Retrospective comparison of data is difficult, but the novel multivalent OspA vaccine in our study seems to be at least as well tolerated as previous monovalent OspA vaccines.[24,27,29] The improved tolerability of the adjuvanted formulations might be explained by masking of the lipid portion of OspA by aluminium hydroxide, hindering its interaction with Toll-like receptors, which are known to be key mediators of the inflammatory response to lipoproteins.[30] A small phase 1 study of a monovalent OspA-1 vaccine[31] showed that systemic reactions were less common with an adjuvanted formulation than with a non-adjuvanted one. Improved tolerability of formulations adjuvanted with aluminium hydroxide has also been described for other lipid-containing vaccines.[32] Masking of the lipid portion of the OspA protein might explain the lower antibody titres induced by the adjuvanted formulations than by the non-adjuvanted ones after the first three immunisations, because interaction with Toll-like receptors has a central role in the immune response to OspA.[33] However, adjuvantation with aluminium hydroxide could lead to more effective induction of memory B cells.[34] Low-dose antigen exposure might induce B cells with high affinity receptors, whereas, at increased antigen doses, B cells with lower affinity receptors are stimulated.[35] This notion would be consistent with our finding of a positive effect of dose on antibody titres after the first three doses but an inverse dose effect after the booster.

A previously marketed monovalent OspA-1 vaccine was licensed as a two-dose primary immunisation schedule, with a 12 month booster immunisation (panel).[27] How the antibody titres reported in our study relate to those for this previous vaccine is unclear, because different ELISA assays were used. However, the increases in OspA-1 antibody titres and longevity of serum antibodies between the first three immunisations and booster in our study are highly consistent with those reported for the previously licensed monovalent formulation given in a dose schedule of three primary immunisations.[24] In this previous study,[24] a third primary immunisation—as used in our study—substantially increased GMTs before and after the booster compared with a two-dose primary immunisation schedule used in the phase 3 efficacy trial.[10] Moreover, in our study, the mean increases of antibody titres to OspA serotypes 2–6 elicited by the multivalent vaccine after the primary and booster immunisations were, in most cases, similar or even higher than the mean increase to OspA-1. For the previously licensed monovalent OspA-1 vaccine, an antibody titre of 1100 enzyme immunoassay units per mL was an absolute correlate of protection.[38] However, because of the differences in the ELISA assay used in our study and in previous studies of the monovalent vaccine, we cannot predict a protective antibody titre for the novel multivalent vaccine. Additionally, how the previously established correlate of protection for the monovalent OspA-1 vaccine would translate to the other OspA serotypes in the multivalent vaccine is unclear.

Our primary ELISA-based immunogenicity data are supported by the finding that vaccine-induced antibodies

Panel: Research in context

Systematic review

On Jan 23, 2013, we searched PubMed for reports published at any time previously, with the term "Lyme vaccine clinical trial". Previous clinical trials of OspA vaccines were restricted to various doses of monovalent outer surface protein A (OspA) serotype 1 (OspA-1) antigens with or without adjuvant.[9,10,24,26,28,29,31,36,37] These previous vaccines were designed to protect against only Lyme borreliosis caused by infection with *Borrelia burgdorferi* sensu stricto, which is the only *Borrelia* species that causes disease in the USA. Clinical studies[10,24,26,28,29,37] showed that a previously marketed monovalent OspA-1 vaccine was well tolerated and immunogenic in adults and children. A phase 3 trial[10] showed that two 30 μg doses of this vaccine followed by a booster after 12 months had a clinical efficacy of 49% in the first tick season and 76% in the second tick season. An alternative immunisation schedule, with a third primary immunisation induced substantially higher OspA-1 antibodies than did the two-dose schedule investigated in the efficacy trial.[24]

Interpretation

We showed that a novel multivalent vaccine designed to protect against several *Borrelia* species and strains is at least as well tolerated as the previously licensed monovalent vaccine. Moreover, the increases in antibodies against the other five OspA serotypes induced by the multivalent vaccine are generally similar to or higher than those induced against OspA-1. Vaccine-induced antisera were biologically active and could bind to and kill strains from all major human pathogenic *Borrelia* species. Our data suggest that the multivalent vaccine could be an effective intervention to prevent Lyme borreliosis caused by infection with all clinically relevant *Borrelia* sensu lato species, for which no human vaccine is presently available.

can also bind to and promote the killing of *B burgdorferi*, *B afzelii*, *B bavariensis*, and *B garinii*. OspA antibodies could prevent tick-to-host transmission by various mechanisms, such as aggregation or interference with one of the many specific functions attributed to OspA (eg, promotion of bacterial dissemination,[39] binding of TROSPA in the tick gut,[40] and protection from acquired host immunity in the tick,[41] all of which require the recognition and binding of OspA). Importantly, the results of the surface-binding assay show that antibodies induced by the multivalent OspA vaccine can bind to strains expressing OspA serotypes 1–6, which are representative of all major human pathogenic *Borrelia* species. These data, together with the preclinical evidence for protection against challenge in mouse infection models,[16] suggest that the vaccine has the potential to induce protective antibody responses.

Our initial phase 1/2 study was restricted to investigation of the safety and immunogenicity of the novel vaccine in a healthy adult population and, as such, our conclusions have several limitations. Assessment of long-term safety and rare adverse events in vaccine recipients will be necessary. Placebo-controlled phase 3 studies are planned to assess the clinical efficacy of the multivalent vaccine against clinical manifestations of Lyme borreliosis caused by several *Borrelia* species in both Europe and the USA, where the large sample size will also enable assessment of possible uncommon and rare adverse events. It will also be important to establish that sufficiently high antibody titres are maintained throughout an entire tick season and can be boosted by subsequent vaccinations. Additional studies are planned to investigate and define the optimum booster intervals necessary to maintain protective antibody concentrations throughout a tick season. A study of the previously marketed monovalent OspA vaccine[26] showed that antibody titres are further augmented by additional booster immunisations after 24 or 36 months. Finally, our conclusions cannot be extended to children or individuals seropositive for *Borrelia burgdorferi* sensu lato; however, previous OspA vaccines were also safe and immunogenic in these populations.[36,37,42]

Incidence and geographical distribution of Lyme borreliosis has increased substantially since the disease was first described in 1977.[1,8] Furthermore, prevalence is predicted to increase as changes in climate and other factors increase the contact between people, reservoir hosts, and vector ticks.[8] A novel multivalent chimeric OspA vaccine has the potential to be an important intervention to mitigate the growing effect of this potentially serious disease.

Contributors

NW, E-MP, GA, AL-B, SF, IL, BAC, BL, HJE, and PNB contributed to study concept and design, and analysed and interpreted data. NW, E-MP, GA, AL-B, SF, IL, BAC, HJE, and PNB supervised the study. DP, MS, PB, AP, CF,BS, MZ, MM, HK, MP-K, ME, and PGK acquired and analysed data. DP and CF interpreted data. SF, TD and JS did statistical analyses. All authors contributed to the drafting and revision of the report, and approved the final version.

Conflicts of interest

NW, E-MP, GA, DP, AL-B, SF, IL, BAC, MS, PB, AP, TD, JS, CF, HJE, and PNB are Baxter employees. NW, E-MP, GA, AL-B, SF, IL, BAC, MS, PB, TD, JS, HJE, and PNB own stock and share options in Baxter. NW, GA, IL, BAC, MS, and PNB have planned, pending, or issued patents for Lyme vaccines. BL has received patent payments from Baxter on behalf of Stony Brook University; has received consulting fees, travel support, fees for participation in review activities and consultancy from Baxter; has received royalties from the Research Foundation for the State University of New York; and has received grant support from the National Institutes of Health and Centers for Disease Control and Protection, on behalf of Stony Brook University. BS received support from Baxter on behalf of Health Center Mainz for this study. MZ, MM, and HK received grant support from Baxter on behalf of the Medical University of Vienna for this study. HK has also previously received payment for participation in the H5N1 focus group and the development of educational presentations for the International Scientific Working Group on Tick-Borne Encephalitis in 2012. ME and PGK received grant support from Baxter on behalf of the Universtity of Tübingen for this and a previous study. MPK declares that she has no conflicts of interest.

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