Open Forum Infectious Diseases
MAJOR ARTICLE
IDSA Infectious Diseases Society of America | hivma hiv medicine association | OXFORD

# Preclinical Evidence for the Protective Capacity of Antibodies Induced by Lyme Vaccine Candidate VLA15 in People

**Urban Lundberg,[1] Romana Hochreiter,[1] Yekaterina Timofoyeva,[2] Isis Kanevsky,[2] Andreas Meinke,[1] Annaliesa S. Anderson,[2] and Raphael Simon[2]**

[1]Valneva Austria GmbH, Vienna, Austria, and [2]Vaccine Research and Development, Pfizer Inc., Pearl River, New York, USA

***Background.*** Vaccine candidate VLA15 is designed to protect against the dominant *Borrelia* genospecies-causing Lyme disease in North America and Europe. Active immunization with VLA15 has protected in the mouse model of tick challenge. VLA15 is currently under evaluation in clinical studies for the prevention of Lyme borreliosis.

***Methods.*** Mice were passively administered sera from clinical trial participants vaccinated with VLA15, or normal human serum from unvaccinated individuals as control. Posttransfer serum anti-outer surface protein A (OspA) immunoglobulin G titers were assessed by enzyme-linked immunosorbent assay. Following passive transfer, mice were challenged with *Ixodes* ticks colonized with *Borrelia burgdorferi* (OspA serotype 1) or *Borrelia afzelii* (OspA serotype 2) and infection was determined by serology for VlsE C6 or by polymerase chain reaction and culture to assess the presence of *Borrelia* bacteria.

***Results.*** Passive transfer of immune sera prevented transmission of *Borrelia* from the tick vector and protected mice against challenge. Posttransfer protective threshold immunoglobulin G antibody titers were observed in this animal model of 131 U/mL for *B burgdorferi* (OspA serotype 1) and 352 U/mL for *B afzelii* (serotype 2).

***Conclusions.*** Passive transfer of sera from trial participants immunized with VLA15 protected mice from borreliosis in a tick challenge model. This indicates that VLA15 induces functional immune responses in people that can be linked to efficacy in a stringent preclinical model.

**Keywords.** antibody; lyme disease; passive transfer; vaccine; mouse model.

## BACKGROUND

Lyme disease is caused by several genospecies of the human pathogenic spirochete *Borrelia burgdorferi* sensu lato group that are carried within *Ixodes* ticks. It is the most common vector-borne disease in Northern latitudes, with recent estimates of at least 476 000 and 130 000 cases annually in the United States and Europe, respectively [1].

Outer surface protein A (OspA) is a membrane-anchored lipoprotein expressed by *Borrelia* while in the tick midgut where it is required for stable persistence but turned off with transmission to the host [2]. OspA is an established vaccine target for prevention of Lyme disease, whereby anti-OspA antibodies ingested by the tick during feeding bind to *Borrelia* in the midgut and prevent transmission to the host [3].

Individual *Borrelia* genospecies express different OspA serotypes (ST). In North America, *B burgdorferi* sensu stricto (OspA ST1) is the dominant genospecies. In Europe, a broader range of genospecies circulate, that include principally *B afzelii* (ST2), *B garinii* (STs 3, 5, 6), *B bavariensis* (ST4), and *B burgdorferi* sensu stricto. Hexavalent Lyme disease vaccine candidate VLA15 includes OspA serotypes 1–6, with coverage for the vast majority of *Borrelia* isolates causing Lyme disease in North America and Europe [4–6]. VLA15 is currently being evaluated in human clinical studies [7].

We previously reported that active immunization with VLA15 or passive transfer of murine VLA15 immune sera protected mice against challenge with *Ixodes* ticks bearing OspA ST1 or ST2 *Borrelia* [4–6]. A key unanswered question was whether protective antibodies are also induced by VLA15 in humans. We report herein that immune sera from vaccinated VLA15 clinical trial participants protected after passive transfer in a mouse model of experimental Lyme borreliosis.

Received 21 May 2024; editorial decision 08 August 2024; accepted 15 August 2024; published online 17 August 2024

Correspondence: Raphael Simon, PhD, Vaccine Research and Development, Pfizer Inc., 401 N. Middletown Road, Pearl River, NY 10965 (Raphael.Simon@pfizer.com); Annaliesa S. Anderson, PhD, Vaccine Research and Development, Pfizer Inc., 401 N. Middletown Road, Pearl River, NY 10965 (Annaliesa.Anderson@pfizer.com).

**Open Forum Infectious Diseases®**

© The Author(s) 2024. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.

https://doi.org/10.1093/ofid/ofae467

## METHODS

### Clinical Study

Samples were obtained from volunteers enrolled in VLA15-202 (NCT03970733). Participants were randomized to receive either placebo or 180 µg VLA15 at 0, 2, 6, and 18 months. Sera taken 1 month after the 4th dose were assessed for anti-OspA titers by enzyme-linked immunosorbent assay (ELISA) and selected high-titer sera were pooled or given individually based on available volume for passive transfer experiments. The trial was conducted in compliance with Good Clinical Practice guidelines and the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

### Mouse Passive Transfer and Challenge Experiments

***Ethics.*** Animal procedures were conducted in accordance with good scientific practice guidelines and national legislation and have been approved by the corresponding authority department (MA58/GZ:73618/2017/17).

Experimental procedures were approved by Valneva's animal welfare committee and performed by trained personnel that all completed a Federation of Laboratory Animal Science Association B course. The animals were housed at the Valneva Austria GmbH animal facility in Vienna. No mortalities occurred before the conclusion of the experiments and were also not expected, because mice are the natural reservoir of *Borrelia* in nature.

*Borrelia* strains and infection of *Ixodes* ticks with *B burgdorferi*. The *Borrelia* strains used in this study were *B burgdorferi* Pra1 (OspA ST1; Valneva Austria GmbH) and *B afzelii* IS1 (OspA ST2; Insect Services, Germany) [4]. To generate infected nymphs, *Ixodes ricinus* larvae were fed on the respectively infected gerbils (Insect Services) and allowed to molt. The extent of nymphal tick infection was determined by OspA polymerase chain reaction (PCR) on DNA extracted from a representative subset of ticks selected at random from each batch. The nymphs from the gerbils infected with the same strain were randomized and used for challenge of mice as described [4].

Passive transfer protection studies in mice. All animal experiments were conducted as described [4] and used 6- to 8-week-old female C3H/HeN mice (Janvier, France) (10/group). Animals were anesthetized by a 0.3-mL intraperitoneal injection of a 10% ketamine (Ketamidor, Richter Pharma AG) and 3% xylazine (Rompun, Bayer Healthcare, Germany) mixture before the ventilated containers were mounted as described [6]. For tick application and terminal blood collection, isoflurane (Baxter Healthcare) was administered using an HNG-6 Anesthesia machine (H. Holzel Laboratory Equipment GmbH, German). On study day 1, mice were administered 500 µL of pooled or single human sera intraperitoneally. On day 2, blood samples were collected from mice via the facial vein to obtain serum for measurement of posttransfer anti-OspA titers by ELISA, and the hair on the back of mice was removed with Veet Cream (Reckitt Benckiser, United Kingdom) after which a small, ventilated container was glued to the skin (Pattex, Germany). One day later, 2 (IS1) or 3 (Pra1) infected *I ricinus* nymphs were placed in the ventilated container and were allowed to feed until repletion. The number of ticks applied to each mouse was dependent on the infection rate of the respective batch of ticks such that there was a > 95% likelihood that at least 1 of the ticks applied was colonized with *Borrelia*. The feeding status of the ticks was monitored daily and only mice where at least 1 (IS1) or 2 (Pra1) fully fed tick(s) was collected were included in the final readout. After 4 weeks, mice were bled to obtain serum for measurement of anti-VlsE antibodies and then euthanized to collect tissue for direct detection of *Borrelia* infection. The infection status of mice was determined by VlsE ELISA and quantitative PCR targeting *recA* (nucleotide 334 to 524) as described previously [6]. Cultivation of spirochetes from one ear was accomplished by adding the dissected ear to 10 mL BSK-II medium and incubating for 4 weeks at 35 °C, with the presence of spirochetes determined microscopically. Assessment of residual *Ixodes* colonization with *Borrelia* was accomplished by quantitative PCR for the *recA* gene as described previously. Ticks that had fed to repletion were frozen until further processed. DNA was extracted from fed ticks using the Qiagen DNeasy Blood and Tissue Kit (Cat. No. 69506) as described by the manufacturer (Qiagen, German). Results are expressed as the proportionate number of colonized ticks compared to the total number that fed to repletion.

### ELISA

OspA ELISA. For the evaluation of human anti-OspA specific antibodies in mouse serum, microtiter plates were coated with individual full-length OspA proteins of various serotypes. Diluted sera were added to the plates (3-fold serial dilutions, 1:40–1:87480), with binding detected using an anti-human immunoglobulin G (IgG) HRP-enzyme conjugate (diluted 1:5000 for ST1/ST4 or 1:10 000 for ST2/ST3/ST5/ST6; Dako, Denmark) followed by addition of the TMB (3,3', 5, 5'—tetramethylbenzidine; SeraCare, US) substrate. The amount of protein-specific IgG was calculated using a reference substance curve by 4-parameter logistic fit and parallel line analysis.

VlsE ELISA. Determination of infection status of mice by VlsE (C6 peptide) ELISA was as described previously [6].

### Statistical Analyses

Statistical significance between groups after challenge was determined by 2-sided Fisher exact test ($\alpha = 0.05$).

**Table 1. Passive Transfer of Immune Sera Followed by *B burgdorferi* Tick Challenge**

| Human Serum Used For Transfer | Geomean Titer Post Transfer U/mL OspA ST1 IgG (Range) | Infected/Total[a] | *P* Value | Tick Colonization |
|---|---|---|---|---|
| Undiluted VLA15 immune serum pool | 1196 (1043–1419) | 0/9 | .0090 | 6% |
| 1:2 VLA15 immune serum pool | 559 (507–655) | 0/10 | .0031 | 11% |
| 1:4 VLA15 immune serum pool | 256 (224–325) | 1/8 | .0498 | 19% |
| 1:8 VLA15 immune serum pool | 131 (106–152) | 1/9 | .0498 | 33% |
| Human nonimmune serum pool | 20[b] | 6/9 | n/a | 61% |

Abbreviations: IgG, immunoglobulin G; n/a, not applicable; OspA, outer surface protein A.

[a]Infection was confirmed with at least 1 positive test (polymerase chain reaction, culture, serology).

[b]Enzyme-linked immunosorbent assay IgG titers measured below the lower limit of quantification of 40 U/mL were reported with 20 U/mL.

**Table 2. Passive Transfer of Immune Sera Followed by *B Afzelii* Tick Challenge**

| Human Serum Used For Transfer | Geomean Titer Post Transfer U/mL OspA ST2 IgG (Range) | Infected/Total[a] | *P* Value | Tick Colonization |
|---|---|---|---|---|
| 1:2 VLA15 immune serum pool | 693 (599–745) | 4/10 | .0108 | 53% |
| 1:4 VLA15 immune serum pool | 352 (302–388) | 4/9 | .0294 | 75% |
| 1:8 VLA15 immune serum pool | 178 (168–191) | 10/10 | ns | 94% |
| 1:16 VLA15 immune serum pool | 82 (71–95) | 8/8 | ns | 100% |
| Human nonimmune serum pool | 20[b] | 9/9 | n/a | 67% |

Abbreviations: IgG, immunoglobulin G; n/a, not applicable; ns, not significant; OspA, outer surface protein A.

[a]Infection was confirmed with at least one positive test (polymerase chain reaction, culture, serology).

[b]Enzyme-linked immunosorbent assay IgG titers measured below the lower limit of quantification of 40 U/mL were reported with 20 U/mL.

## RESULTS

Mice passively administered human non-immune sera were susceptible to infection with *B burgdorferi* (ST1) from the tick vector (Table 1). By comparison, transfer of VLA15 immune sera containing anti-OspA antibodies (Supplementary Table 1) protected against *B burgdorferi* (ST1) infection. Near-complete protection was achieved at the lowest OspA titer serum administered that resulted in a posttransfer circulating serum GMT of 131 U/mL anti-OspA ST1 IgG. Protection at the ~130 U/mL level posttransfer was confirmed in a follow-up experiment, and although not statistically significant, results also indicated a trend for loss of protection when a higher dilution was administered that resulted in posttransfer titers of ~60 U/mL anti-OspA ST1 IgG (not shown). Significant protection was also found for mice passively administered individual high-titer VLA15 human immune sera (Supplementary Table 2), or lower titer sera reproducing posttransfer anti-OspA ST1 IgG levels within the range of the highest serum pool dilution tested (Supplementary Table 3).

Though not obligate for blocking transmission, *Borrelia* spirochetes can be eradicated from the tick vector in the presence of anti-OspA antibodies [8]. Accordingly, clearance of *Borrelia* from the tick vector after feeding can serve as an additional measure for the potency of the anti-OspA immune response. The proportion of *Borrelia* colonized ticks postfeeding was reduced for those fed on mice administered VLA15 immune sera compared to those fed on control animals receiving nonimmune sera (Table 1). Notably, reduced tick colonization postfeeding corresponded in a dose-dependent manner with the circulating anti-OspA IgG concentration posttransfer.

The capacity of VLA15 human immune sera to prevent transmission of *B afzelii* (ST2) was next assessed. Whereas all mice receiving human nonimmune sera were productively infected after being fed on by *B afzelii*–colonized ticks, transfer of pooled VLA15 human immune sera prevented transmission in ~55% of mice; for which a circulating geometric mean antibody titer of at least 352 U/mL was required to significantly prevent infection in this preclinical mouse model (Table 2). Additionally, reduction in tick colonization with *B afzelii* was seen in a dose-dependent fashion with transfer of lower serum dilutions producing greater levels of eradication of *Borrelia* from the tick. However, the level of tick colonization recorded in the nonimmune serum transfer group exceeded that seen for groups receiving different dilutions of immune-serum groups other than the lowest dilution tested.

## DISCUSSION

OspA is a validated vaccine target for the prevention of Lyme borreliosis caused by *B burgdorferi,* having proven protective in two prior clinical efficacy studies that assessed full-length native monovalent OspA ST1 Lyme disease vaccines among residents living in Lyme-endemic areas in the Northeastern United States [9, 10]. The mechanism of action for anti-OspA antibodies within the tick is not completely understood, and may include serum bactericidal activity, aggregation, and potentially antagonism of OspA binding to its

cognate ligand Tick Receptor for OspA [11, 12]. Additionally, determinants such as antibody avidity as well as posttranslational antibody modifications such as glycosylation may further affect observed protective function. The results reported herein provide important evidence for the capacity of VLA15 to induce qualitatively functional antibodies in people against 2 different *Borrelia* genospecies covered by the vaccine, that are the most common causes of Lyme disease in North America (*B burgdorferi*, OspA ST1) and Europe (*B afzelii*, OspA ST2) [1, 13, 14]. The mouse tick challenge model may be used to characterize the transmission-blocking potential of vaccine-induced antibodies in people because of several important considerations. First, the mechanism of action for OspA antibodies occurs within the *Ixodes* vector and not the animal host, thus obviating potential differences in host–pathogen interactions. Additionally, mice are a natural host for *Borrelia*, and mouse tick challenge models faithfully reproduce the key events associated with *Borrelia* transmission to humans [2]. Finally, the use herein of passively administered serum anti-OspA antibodies from vaccinated trial participants may reflect the potential for the vaccine in humans.

We found that posttransfer efficacy in mice was maintained for OspA ST1 *Borrelia* down to circulating titers of approximately 131 U/mL. A protective threshold based on anti-OspA IgG ELISA titer was reported for the prior LYMErix monovalent OspA ST1 vaccine [15]. The anti-OspA ST1 titers shown to be protective preclinically for VLA15 herein cannot be compared to this prior work however, as the vaccine constructs are very different (VLA15 includes only the C-terminal protein domain [amino acids 126–273], whereas LYMErix composes the whole protein) and the immunoassays are not directly comparable [6]. Additionally, the preclinical challenge model used herein measures protection directly proximal to the time of exposure. By comparison, the LYMErix protective threshold was for a full Lyme disease season and accounted for the effect of natural waning of antibody titers. An additional caveat remains that the protective titers are derived from an animal challenge model that may be more stringent than natural exposure, and it is thus possible that the required minimal level of VLA15-induced anti-OspA IgG may be overestimated. Importantly, the putative protective threshold identified in this model can be achieved in people after vaccination with VLA15 [7].

Higher apparent antibody titers were required to block transmission of *B afzelii*. However, as the ELISA is not cross-standardized, the relative titers across OspA serotypes cannot be directly related. *B burgdorferi* is effectively vectored by *I ricinus* ticks and is epidemiologically relevant in Europe [1]. Findings for *B burgdorferi* are anticipated to translate to the context of transmission from *I scapularis,* the relevant vector in the United States; however, this should be confirmed in future studies.

VLA15 is currently being assessed in a phase 3 efficacy study C4601003 (NCT05477524, VALOR) conducted among individuals living in Lyme-endemic regions in North America and Europe. VLA15 is immunogenic in people, inducing measurable IgG antibody titers against the 6 OspA serotypes covered by the vaccine [7], at levels congruent with those reported here to be protective in the context of an animal challenge model. The results described here demonstrating protection in a relevant animal model following passive transfer of human immune sera corroborates preclinical findings from prior studies that evaluated active immunization in mice. These results provide, for the first time, evidence that functional transmission-blocking antibodies can be formed in humans after vaccination with VLA15 [4–6]. Protection against Lyme disease in people following VLA15 vaccination will need to be confirmed in the ongoing VALOR study, for which the preclinical protective thresholds determined herein may be additionally evaluated.

## Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

## Notes

***Acknowledgments.*** The authors wish to express their gratitude to Sandra Jost and Ursula Bartuschka for their technical support, and to Christina D'Arco for editorial support.

***Patient consent statement***. Written informed consent was obtained from all participants enrolled in clinical study VLA15-202 (NCT 03970733). The trial was conducted in compliance with Good Clinical Practice guidelines and the ethical principles of the Declaration of Helsinki. This study was approved by an Institutional Review Board and conformed to all ethical standards applicable in the United States.

***Financial support***. Funding was from Pfizer and Valneva.

***Potential conflicts of interest***. Authors are current or former employees of Pfizer or Valneva and may, as a consequence, be shareholders of Pfizer Inc. and Valneva SE, respectively. Pfizer and Valneva were involved in the study concept and design, the collection, analysis and interpretation of data, the drafting of the manuscript, and the decision to submit the manuscript for publication.

## References

1. Steere AC, Strle F, Wormser GP, et al. Lyme borreliosis. Nat Rev Dis Primers **2016**; 2:16090.
2. Kurokawa C, Lynn GE, Pedra JHF, Pal U, Narasimhan S, Fikrig E. Interactions between Borrelia burgdorferi and ticks. Nat Rev Microbiol **2020**; 18:587–600.
3. Federizon J, Lin YP, Lovell JF. Antigen engineering approaches for Lyme disease vaccines. Bioconjug Chem **2019**; 30:1259–72.
4. Comstedt P, Schuler W, Meinke A, Lundberg U. The novel Lyme borreliosis vaccine VLA15 shows broad protection against Borrelia species expressing six different OspA serotypes. PLoS One **2017**; 12:e0184357.
5. Comstedt P, Hanner M, Schuler W, Meinke A, Schlegl R, Lundberg U. Characterization and optimization of a novel vaccine for protection against Lyme borreliosis. Vaccine **2015**; 33:5982–8.
6. Comstedt P, Hanner M, Schuler W, Meinke A, Lundberg U. Design and development of a novel vaccine for protection against Lyme borreliosis. PLoS One **2014**; 9:e113294.

7. Bezay N, Hochreiter R, Kadlecek V, et al. Safety and immunogenicity of a novel multivalent OspA-based vaccine candidate against Lyme borreliosis: a randomised, phase 1 study in healthy adults. Lancet Infect Dis **2023**; 23:1186–96.
8. Gipson CL, de Silva AM. Interactions of OspA monoclonal antibody C3.78 with Borrelia burgdorferi within ticks. Infect Immun **2005**; 73:1644–7.
9. Steere AC, Sikand VK, Meurice F, et al. Vaccination against Lyme disease with recombinant Borrelia burgdorferi outer-surface lipoprotein A with adjuvant. Lyme Disease Vaccine Study Group. N Engl J Med **1998**; 339:209–15.
10. Sigal LH, Zahradnik JM, Lavin P, et al. A vaccine consisting of recombinant Borrelia burgdorferi outer-surface protein A to prevent Lyme disease. Recombinant Outer-Surface Protein A Lyme Disease Vaccine Study Consortium. N Engl J Med **1998**; 339:216–22.
11. Frye AM, Ejemel M, Cavacini L, et al. Agglutination of Borreliella burgdorferi by transmission-blocking OspA monoclonal antibodies and monovalent fab fragments. Infect Immun **2022**; 90:e0030622.
12. Schwendinger MG, O'Rourke M, Traweger A, et al. Evaluation of OspA vaccination-induced serological correlates of protection against Lyme borreliosis in a mouse model. PLoS One **2013**; 8:e79022.
13. Ruzic-Sabljic E, Maraspin V, Bogovic P, et al. Microbiologic findings in a cohort of patients with erythema migrans. Microorganisms **2024**; 12:185.
14. Strnad M, Honig V, Ruzek D, Grubhoffer L, Rego ROM. Europe-wide meta-analysis of Borrelia burgdorferi sensu lato prevalence in questing Ixodes ricinus ticks. Appl Environ Microbiol **2017**; 83:e00609-17.
15. Van Hoecke C, Lebacq E, Beran J, Parenti D. Alternative vaccination schedules (0, 1, and 6 months versus 0, 1, and 12 months) for a recombinant OspA Lyme disease vaccine. Clin Infect Dis **1999**; 28:1260–4.