

Design and evaluation of a novel OspA-based vaccine for the prevention of Lyme borreliosis

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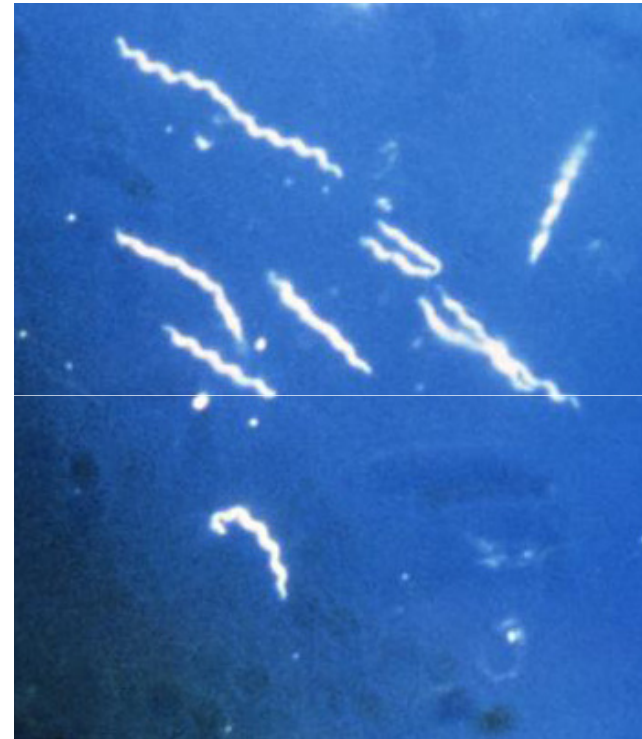
Lyme Borreliosis (LB)



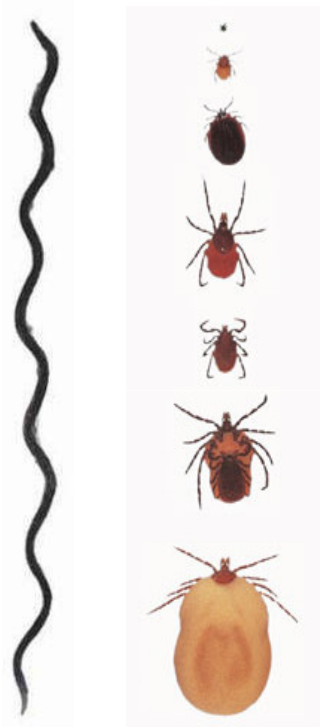
Introduction to vaccine

Biology of Borrelia

- + At least 14 species, where 3 cause the majority of clinical cases
- + Helical shape, 5-20 μm long
- + Very motile (periplasmic flagella)
- + Generation time; 12 – 24 h
- + “Gram negative-like”; No LPS, but multiple lipoproteins on surface
- + Segmented genome (B31, Bb: 1.5 Mbp)
 - › 1 linear chromosome
 - › 21 plasmids; 12 linear and 9 circular plasmids
- + Many genes are uncharacterized and have no homolog in other species
- + Several OspA serotypes
- + Rather low homology between species and within *B. garinii*
- + Only transmitted by *Ixodes* ticks



B. burgdorferi grown in culture medium (CDC) and EM of *B. garinii* isolated from a neuroborreliosis patient (Sweden)



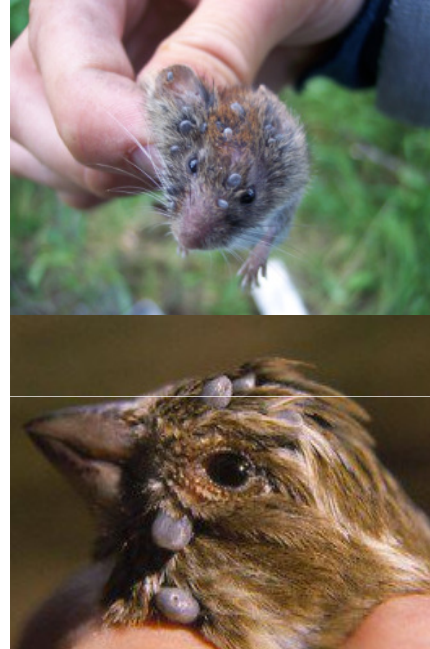
developmental stages of *Ixodes ricinus* ticks

Introduction to vaccine

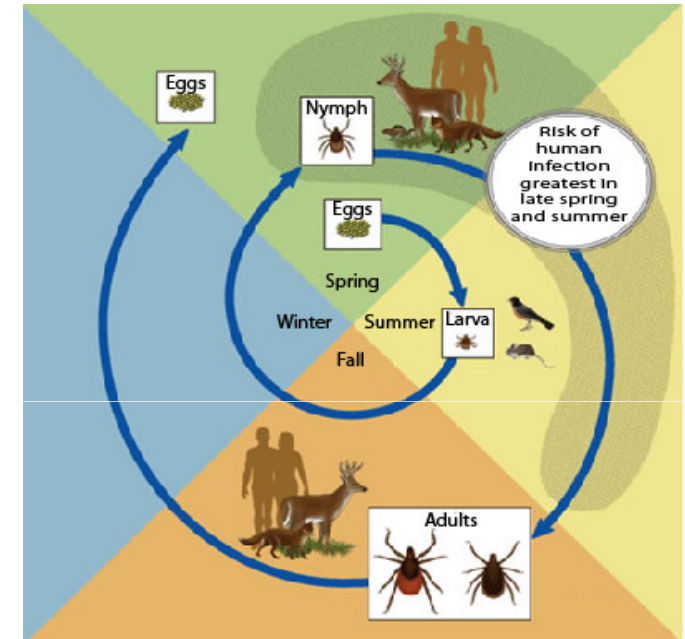
Infection cycle of *Borrelia*



One female tick can lay >1000 eggs



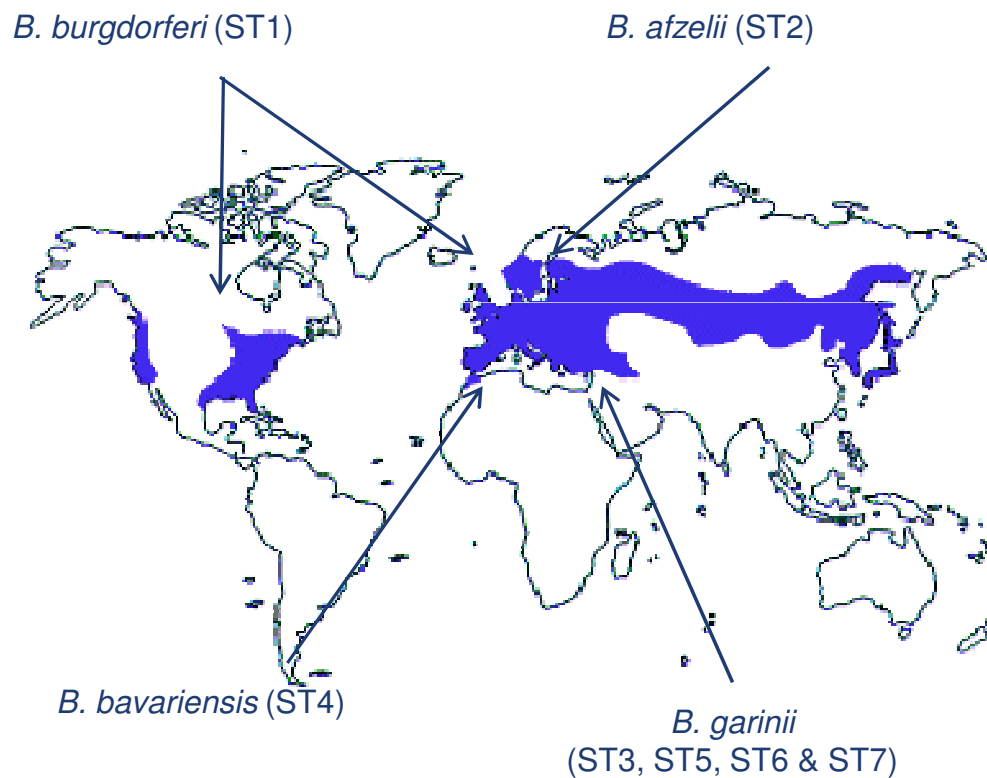
Rodents and birds are natural *Borrelia* reservoirs



Infected nymphs attack humans mainly during spring and summer

Introduction to vaccine

Epidemiology of Borrelia



Country	Average annual incidence per 100,000	Average number of cases per year
Germany	25	20,700
Austria	130	14,000
France	16.5	10,022
Lithuania	25	8,500
Sweden (S.)	80	7,223
Netherlands	43	4,890
Latvia	16	3,680
Bulgaria	55	3,500
Slovenia	155	3,096
Czech Rep.	29	2,962
Switzerland	30.4	2,264
Poland	4.8	1,832
Slovakia	18.4	1,000
Finland	12.7	700
Estonia	35	500
U.K.	0.7	423
Norway	2.8	128
Ireland	0.6	30
Total	-	85,450

Estimated average annual LB incidence (over 10 years) in selected European countries (Lindgren and Jaenson, 2006). *Note: Some countries do not use standardized diagnostic tests or accepted case definitions, therefore data may be subject to considerable error.*

Introduction to vaccine

Distribution of OspA serotypes in clinical isolates

- + Spirochetes cultivated from clinical samples collected from LB patients
 - › V. Fingerle (Munich, Germany) collection 236 strains and Baxter data from 359 isolates
- + Strains were typed by sequencing of the *ospA* gene
- + Invasive cases defined when strains were isolated from CSF and synovia

Borrelia sp.	OspA serotype	Human isolates	
		Total	Invasive
<i>B. afzelii</i>	2	53% (126)	24% (19)
<i>B. bavariensis</i>	4	15% (36)	22% (17)
<i>B. burgdorferi</i>	1	13% (31)	27% (21)
<i>B. garinii</i>	6	8% (19)	15% (12)
<i>B. garinii</i>	5	4% (10)	5% (4)
<i>B. garinii</i>	3	3% (8)	5% (4)
<i>B. spielmanii</i>	snd	2% (4)	0% (0)
<i>B. garinii</i>	7	<1% (2)	1% (1)

Analysis of strain collection from V. Fingerle. 236 human isolates from Germany of which 78 were isolated from people with invasive disease. Number of isolates in brackets.

Borrelia sp.	OspA serotype	Human isolates	
		Total	Invasive
<i>B. afzelii</i>	2	57% (204)	12% (7)
<i>B. garinii</i>	6	16% (57)	38% (23)
<i>B. burgdorferi</i>	1	12% (42)	12% (7)
<i>B. garinii</i>	5	7% (26)	15% (9)
<i>B. bavariensis</i>	4	4% (16)	12% (7)
<i>B. garinii</i>	3	2% (7)	9% (5)
<i>B. spielmanii</i>	snd	1% (4)	0% (0)
<i>B. garinii</i>	7	<1% (3)	3% (2)

BAXTER analysis of 359 human isolates from 16 European countries of which 60 were isolated from people with invasive disease. Number of isolates in brackets.

- + *B. afzelii* most common isolate in both strain collections
- + Variation between strain collections in OspA serotypes involved in invasive disease

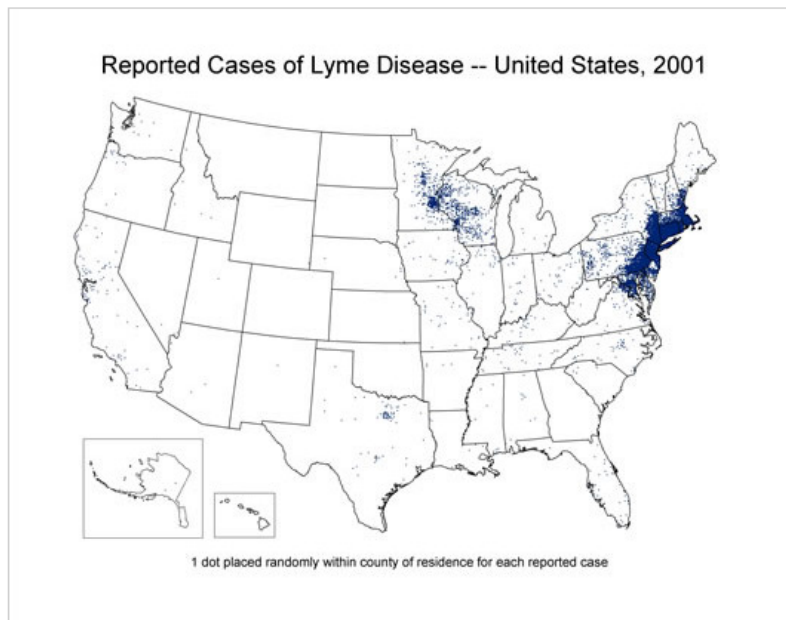
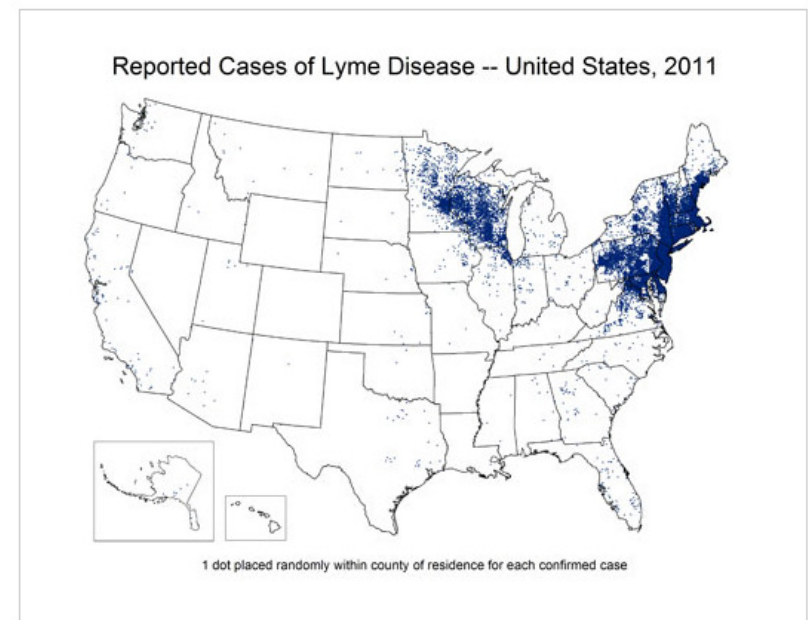
Snd – OspA serotype not defined

Lyme borreliosis in the United States

Distribution of reported LB cases over an eleven years period

+ Only OspA serotype 1 present in US: *Borrelia burgdorferi*

- › Between 2001 and 2011 the number of reported cases went from 17,029 to 24,364, making LB the sixth most common reportable disease in the US
- › In endemic areas 2nd (New England) and 3rd (Mid Atlantic states) most common
- › These numbers are an underestimate, as preliminary results from CDC indicate that over 300,000 Americans are diagnosed with LB each year (makes LB third most common reportable disease in the US after Chlamydia and Gonorrhea)



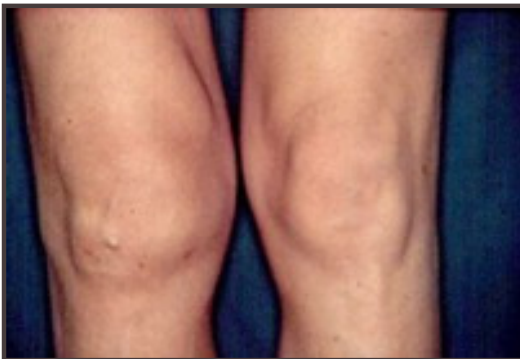
Introduction to vaccine

Clinical manifestations of LB

- + Localized infection
 - › Skin: Erythema migrans (~80% of infections)
- + Disseminated infection
 - › Joints: Lyme arthritis (*B. burgdorferi*)
 - › CNS: Neuroborreliosis (*B. garinii* and *B. bavariensis*)
 - › Skin: Acrodermatitis chronica atrophicans (*B. afzelii*)



Erythema migrans



Lyme arthritis



Facial nerve palsy



Acrodermatitis chronica atrophicans

Lyme Borreliosis vaccine



Introduction to vaccine

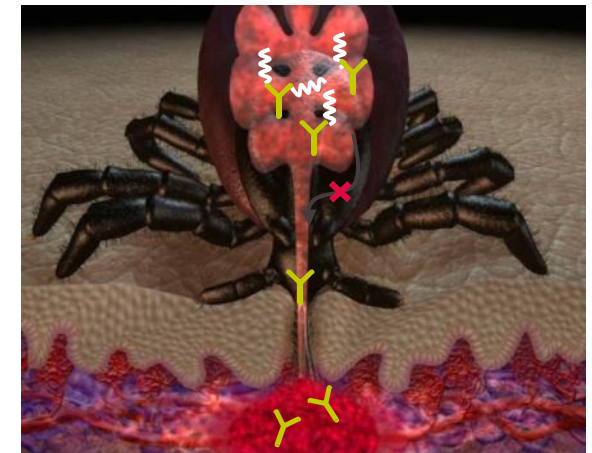
Mechanism of action of an OspA based vaccine

+ Expression of OspA

1. OspA is expressed when spirochetes are located in the mid-gut of an unfed tick
2. During feeding, the incoming blood elicits the down regulation of OspA expression
3. Down regulation of OspA allows the spirochetes to migrate to the salivary glands and into the blood of the vertebrate host

+ Function of anti-OspA antibodies

1. The anti-OspA antibodies in the blood of the host bind to the spirochetes before OspA expression is down regulated
2. Migration to the salivary glands is blocked and borreliae are killed in a complement independent manner* in the tick
3. Thus, only antibodies that bind OspA are needed for elimination of spirochetes in the tick



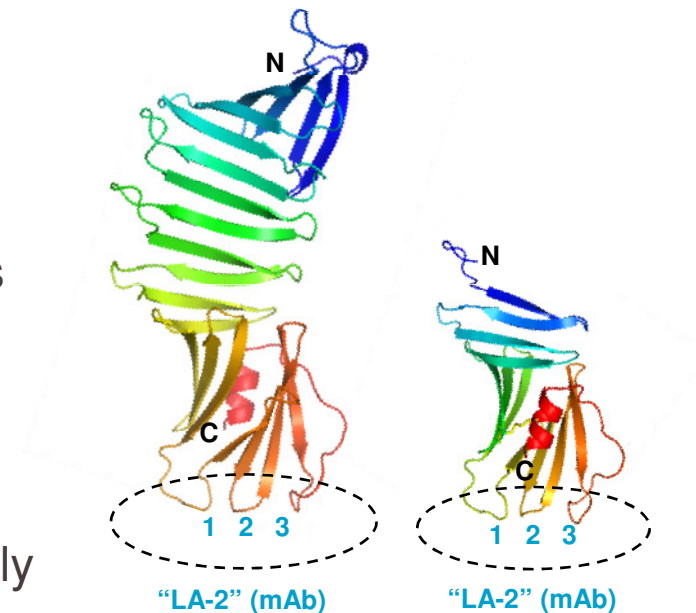
* Valenzuela *et al.*, JMB 2000 ,Rathinavelu *et al.*, Inf. Imm. 2003, Gipson *et al.*, Inf. Imm. 2005

The OspA strategy

Learnings from other studies

- + *B. afzelii* (OspA-ST2) is the most common cause of LB in Europe
- + Pronounced cross-protection is not expected due to sequence variation among the OspA serotypes
- + Crystal structure of OspA *B. burgdorferi* (OspA-ST1) has been determined*
- + Anti-OspA antibodies block the transmission of spirochetes from ticks to the vertebrate host
- + Studies have claimed that epitope LA-2 (OspA-ST1) correlates with protective immunity after vaccination**
- + The C-terminal part of OspA (aa131-273) from *B. burgdorferi* (ST1) is protective in mice, but not structurally stable. Point mutations facilitating formation of hydrophobic interactions restore stability and vaccine efficacy***

+ Focus on C-terminal region of OspA



Left: Crystal structure of full-length OspA from *B. burgdorferi* serotype 1 when bound to mAb LA-2 (not shown). Right: C-terminal part of OspA.

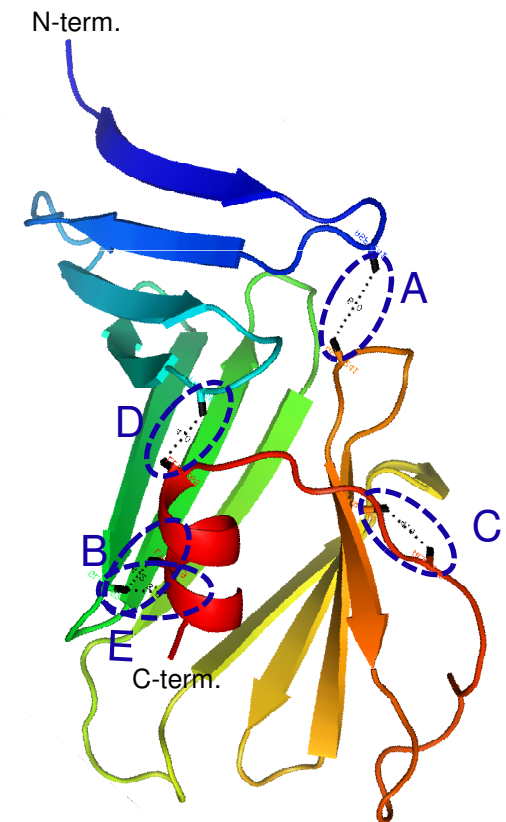
* Li *et al.* PNAS 1997; Ding *et al.* JMB 2000, Makabe *et al.* Prot. Sci. 2006; ** Golde *et al.* Inf. Imm. 1997; Steere *et al.* N Engl J Med 1998; *** Koide *et al.* JMB 2005

The OspA strategy

Stabilizing mutations for C-terminal OspA

- + Disulfide bonds introduced by point mutations
 - › Distance (Å) based on crystal structures (OspA-ST1) with monoclonal antibodies 184.1 and LA-2
 - › Five prioritized stabilized variants of truncated OspA
 - › The final disulfide bond is about 2.1 Å in length

#	Position (aa)	Distance (184.1 or LA-2)
A	141,241*	6.0 Å or 6.2 Å
D	165,265**	4.0 Å or 4.2 Å
B	182,269**	4.2 Å or 4.1 Å
E	182,272**	3.0 Å or 3.9 Å
C	244,259**	4.0 Å or 4.1 Å



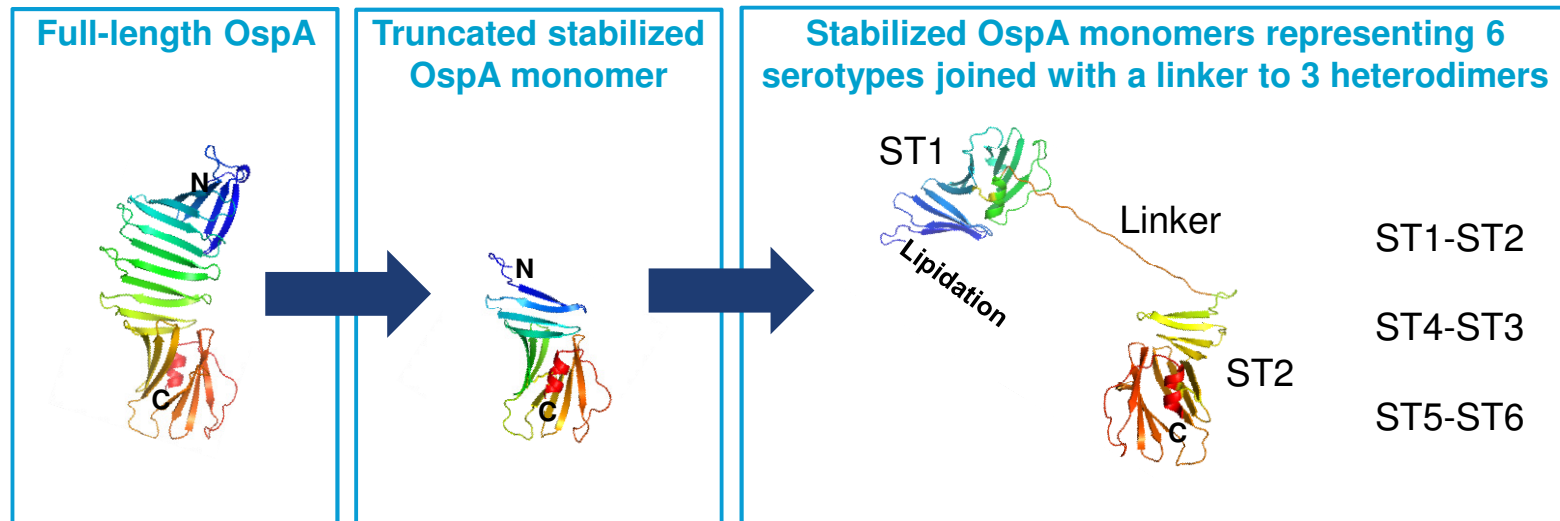
* Proposed by ICLL Bioinformatician, ** Proposed by MODIP (distance and direction/orientation taken into account)

The OspA strategy

Stabilized truncated monomers linked to heterodimers

+ **VLA15-combo**: Lip-D1B2B, Lip-D4Bva3B and Lip-D5B6B

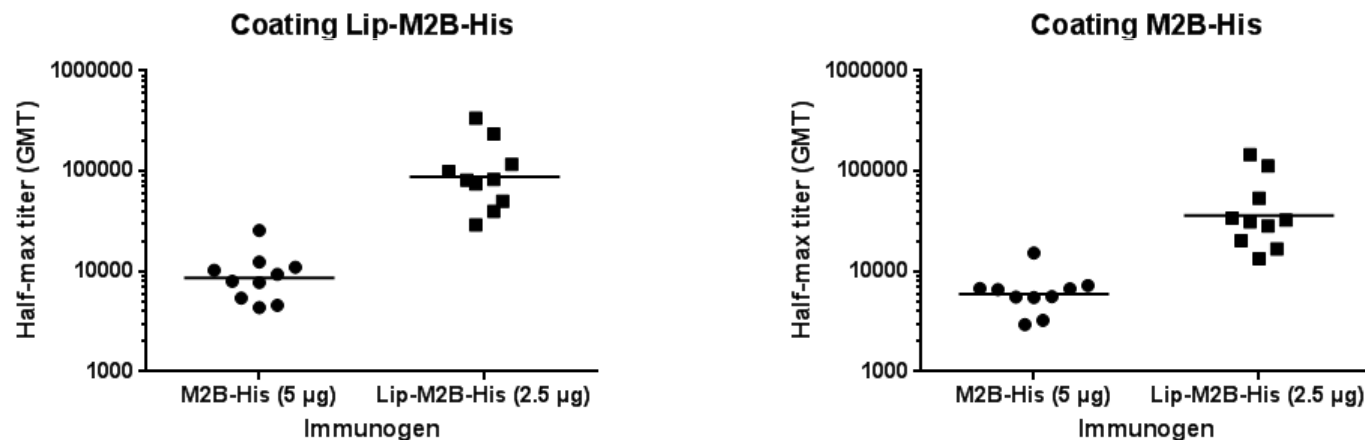
- › Truncated stabilized OspA from 6 different serotypes linked to form 3 heterodimers
- › Lipidation sequence ensures high immunogenicity (*E. coli*, Lpp (major OMP))
- › Monomers linked with a 21 amino acid linker derived from two N-terminal loops of *B. burgdorferi* OspA-ST1 (aa 65-74, aa 42-53)
- › Sequence of heterodimers: Lpp: aa 1 – 23, OspA: aa 126 – 273(274), Linker: 21 aa, OspA: aa 126 – 273(274)



Influence of lipidation on immunogenicity

Assessment of monomers by ELISA

- + Lipidated and non-lipidated OspA monomer ST2 formulated with adjuvant
- + Mice were immunized three times with two weeks interval
- + Dose: 5.0 μg non-lipidated and 2.5 μg lipidated OspA monomer
- + Final bleed was performed one week after the third immunization
- + Sera were analyzed by ELISA with the homologous protein as coating antigen



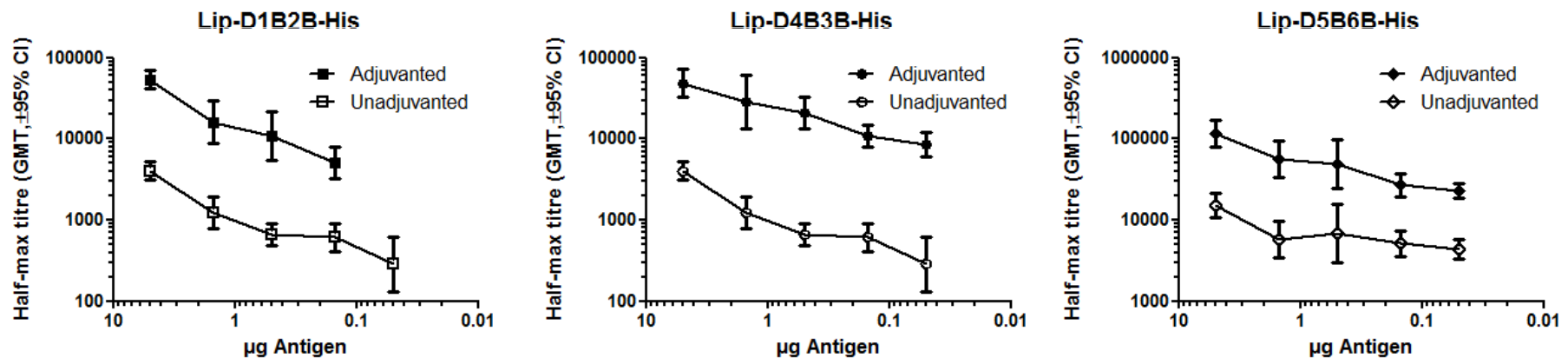
+ **Lipid moiety increases immunogenicity at least 10-fold**

GMT – geometric mean titer

Effect of adjuvant on immunogenicity

Assessment of heterodimers by ELISA

- + Lipidated OspA heterodimers were formulated with or without 0.15% aluminum hydroxide
- + Mice were immunized three times with two weeks interval with five different doses of heterodimer (5.0, 1.5, 0.5, 0.15 and 0.05 μg)
- + Final bleed was performed one week after the third immunization
- + Sera were analyzed by ELISA with the homologous antigen as coating antigen



+ Adjuvanted heterodimer gives approximately 100-fold higher immune response

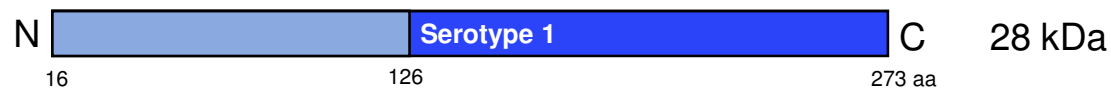
GMT – geometric mean titer, CI – confidence interval



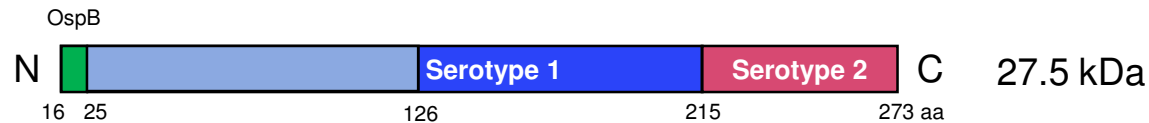
Comparison of *Borrelia* vaccine antigens

Rationale for increased immune response

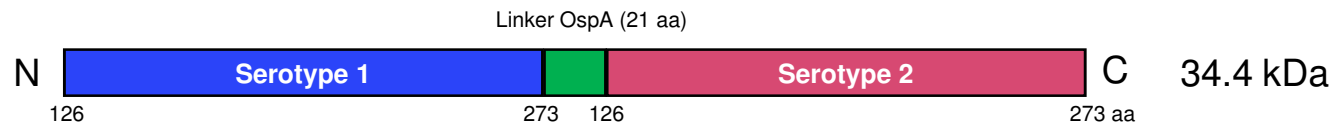
wt OspA



Baxter OspA



VLA15 OspA

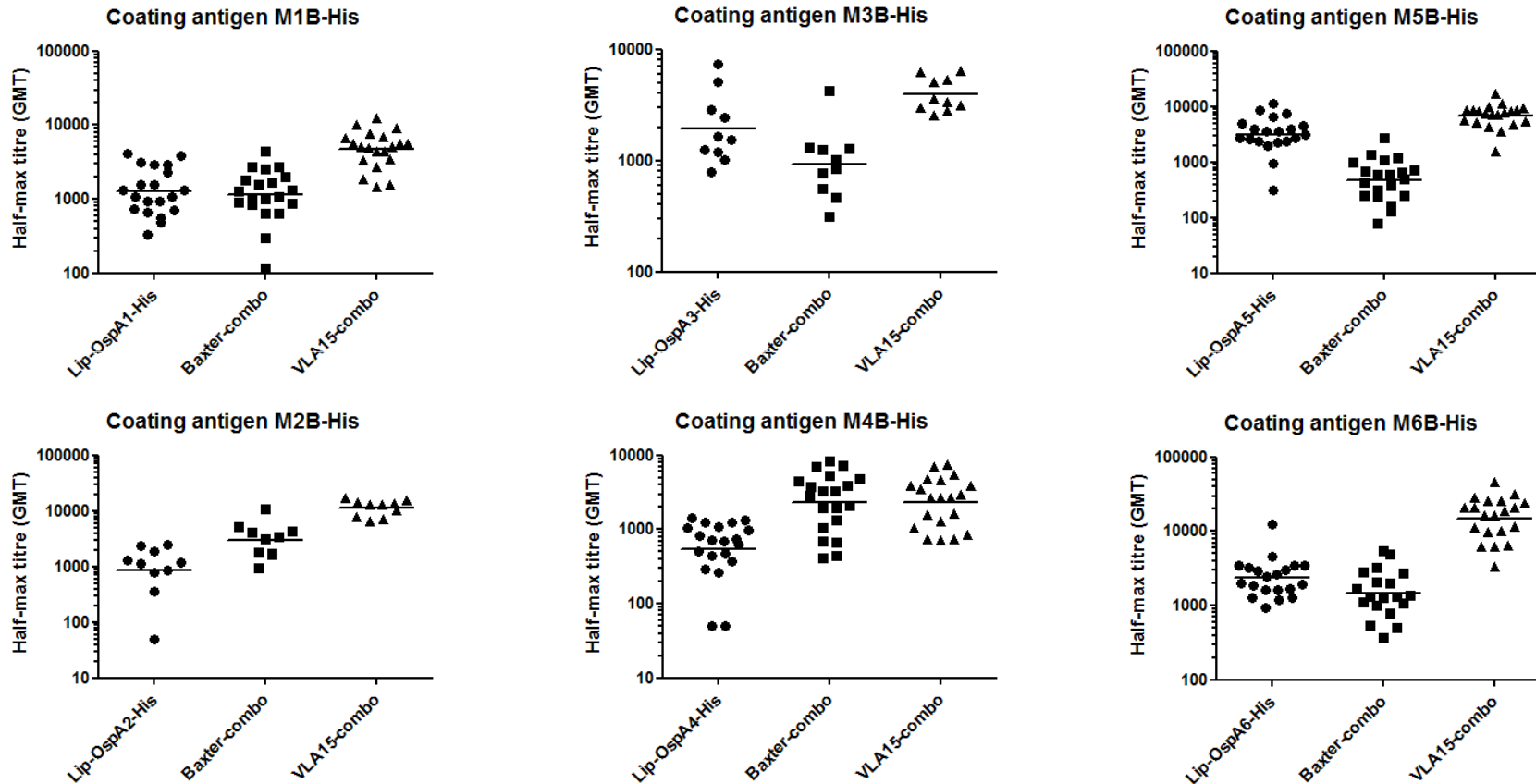


+ VLA15-combo

- › Similar dose of VLA15 contains almost 2x the amount of C-terminal region of OspA (aa 126 - 273) than Baxter OspA
- + Comparison does not calculate for cross-reactivities between serotypes

Immunogenicity of VLA15 in mice

ELISA for C-terminal OspA



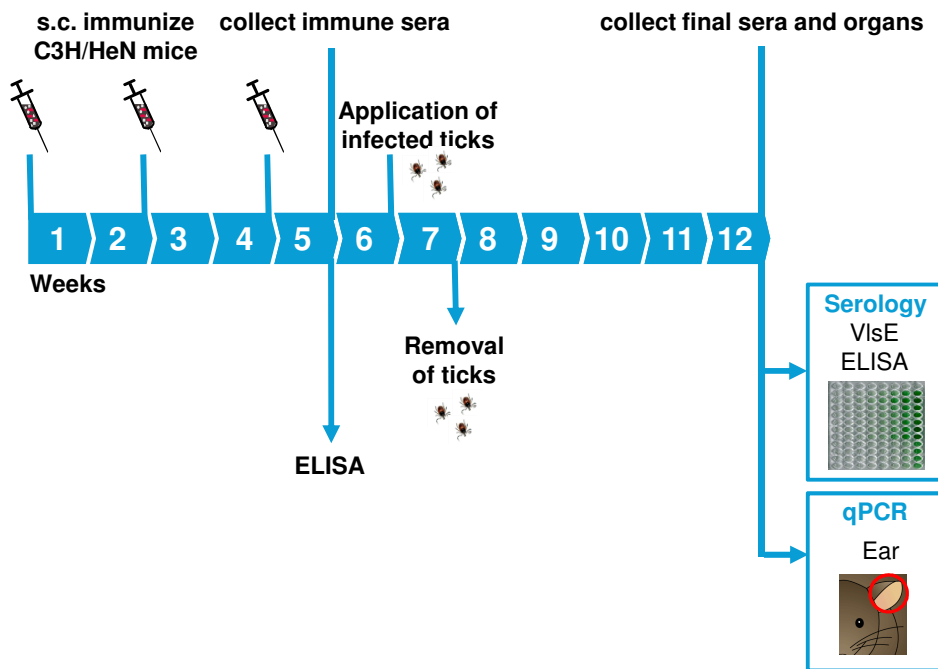
+ The immune responses of VLA15-combo as measured by ELISA for all serotypes are non-inferior or superior to Baxter-like vaccine

GMT – geometric mean titer, CI – confidence interval, LL – lower limit, UL – upper limit

Mouse challenge models

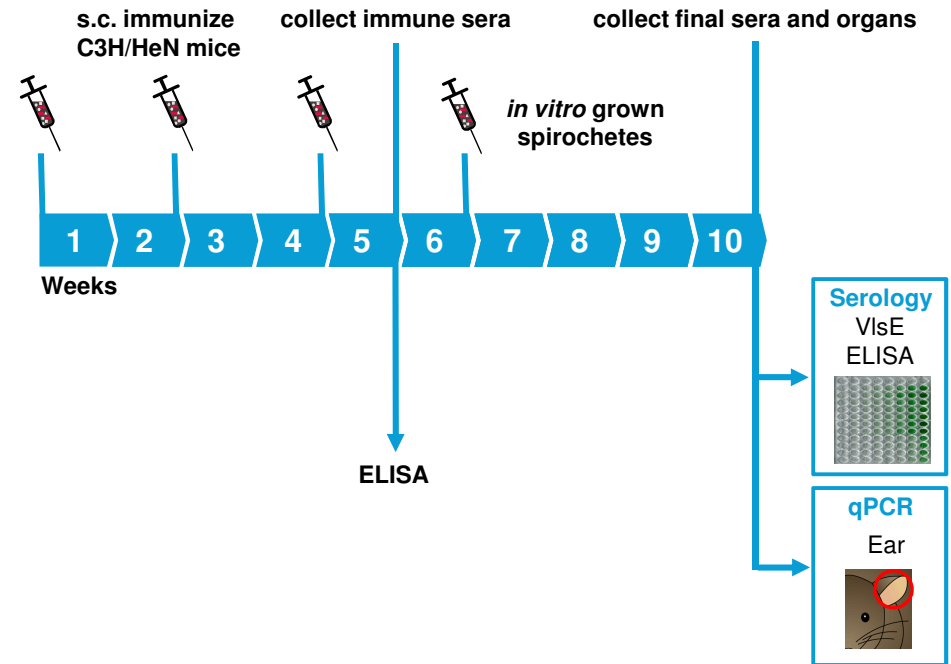
Layout and readout of the two challenge models

MOUSE TICK CHALLENGE MODEL



- + Mice are challenged with ticks feeding for 3-5 days
- + Mice are sacrificed 6 weeks after tick application
- + Readout based on VisE ELISA and qPCR

MOUSE NEEDLE CHALLENGE MODEL



- + Mice are challenged with *in vitro* grown bacteria
- + Mice are sacrificed 4 weeks after challenge
- + Readout based on VisE ELISA and qPCR

VisE: Variable major protein [VMP]-like sequence E

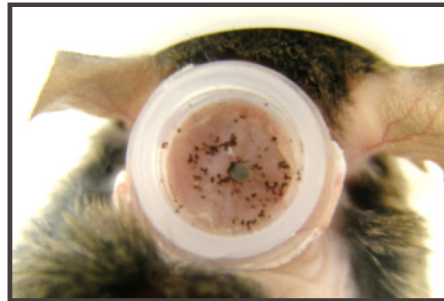
Mouse tick challenge model

Application of ticks

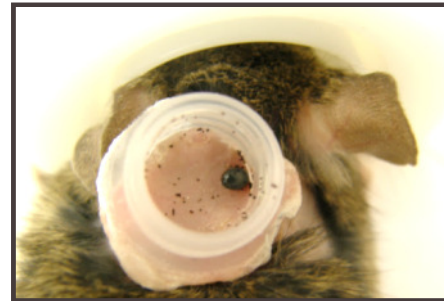
1. The hair of the back is removed with epilation cream
2. Ventilated containers are glued to the naked skin with super glue
3. 1-2 ticks infected with *B. afzelii* (OspA-ST2) are put in each container
4. The feeding is monitored for 5 days and non-feeding ticks are replaced
5. Only mice with at least 1 fully fed tick are included in the final readout



container mounted
(day 0)



tick feeding
(day 2)



tick feeding
(day 3-4)



fully fed tick
(day 4-5)

Pictures above illustrate the feeding progress on different mice

Protection by VLA15-combo

Active immunization and tick challenge: *B. afzelii* (ST2)

- + Three immunizations with two weeks interval, adjuvant 0.15% Al(OH)₃
 - › Controls: Lip-OspA2-His, Baxter combo
- + Tick challenge: Nymphs infected with *B. afzelii* (ST2) strain IS1 two weeks after third immunization
- + Final bleed and organ collection four weeks after challenge
 - › Infection read-out: VlsE ELISA and qPCR

Immunogen	Dose	Infected/Total		
		Experiment 1	Experiment 2	Experiment 3
Lip-OspA2-His	1 µg	0/8**	0/9***	0/7**
Baxter-combo	3 µg	0/7**	0/9***	0/7**
VLA15-combo	3 µg	0/9***	0/7***	0/4*
Placebo	-	5/5	8/8	7/8

- + All vaccines resulted in significant protection in three separate experiments

Only mice with at least one fully fed tick were included in the readout

P-values were calculated with Fisher's exact test (two tailed); * <0.05, ** <0.01, *** <0.001 and ^{ns} not significant

Protection by VLA15-combo

Active immunization and tick challenge: *B. burgdorferi* (ST1)

- + Three immunizations with two weeks interval, adjuvant 0.15% Al(OH)₃
 - › Controls: Lip-OspA1-His, Baxter-combo
- + Tick challenge: Nymphs infected with either *B. burgdorferi* (ST1) strain Pra1 or Pra4 two weeks after third immunization
- + Final bleed and organ collection four to six weeks after challenge
 - › Infection read-out: VlsE ELISA and qPCR

Immunogen	Dose	Infected/Total		
		Experiment 1 (Pra4)	Experiment 2 (Pra1)	Experiment 3 (Pra1)
Lip-OspA1-His	1 µg	0/4*	1/8 ^{ns}	1/9*
Baxter-combo	3 µg	0/8**	0/9*	0/7**
VLA15-combo	3 µg	0/5*	0/10*	0/7**
Placebo	-	5/6	4/7	5/6

- + **VLA15-combo and Baxter-combo provide significant protection using ticks infected with different strains for challenge**

Only mice with at least two fully or almost fully (≥48 hours) fed ticks were included in the readout, P-values were calculated with Fisher's exact test (two tailed); * <0.05, ** <0.01, *** <0.001 and ^{ns} not significant



Status of *Borrelia* animal model

OspA serotype specific mouse challenge models

Serotype	Challenge model (strain)	Statistical significant protection*		
		Full-length OspA (1 µg)	VLA15-combo (3 x 1 µg)	Baxter-combo (3 x 1 µg)
1	Needle (ZS7)	0.001, <0.001, <0.001	<0.001, <0.001, <0.001	<0.001, <0.001, <0.001
	Tick (Pra1)	0.048, 0.187, 0.022	0.026, 0.029, 0.009	0.006, 0.039, 0.009
2	Tick (IS1)	0.002, <0.001, 0.003	0.001, <0.001, 0.020	0.003, <0.001, 0.003
5	Needle (Phei)	<0.001, <0.001, <0.001	<0.001, <0.001, <0.001	<0.001, <0.001, <0.001
6	Needle (Ma)	0.011, 0.488, 0.033	<0.001, <0.001, 0.003	<0.001, <0.001, <0.001

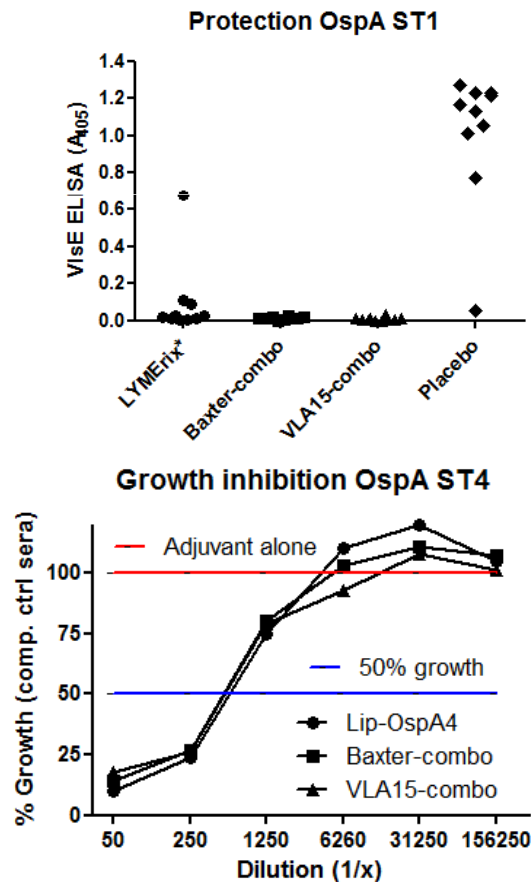
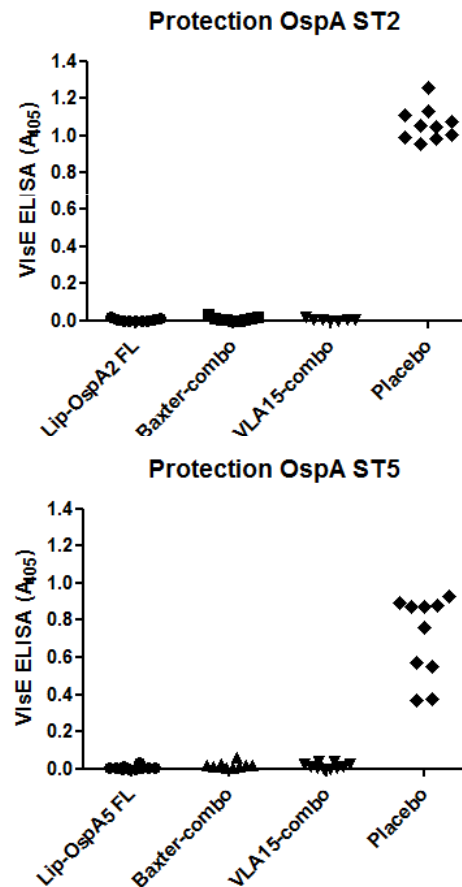
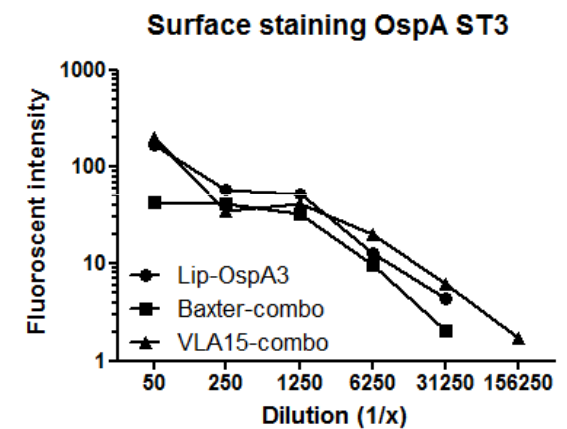
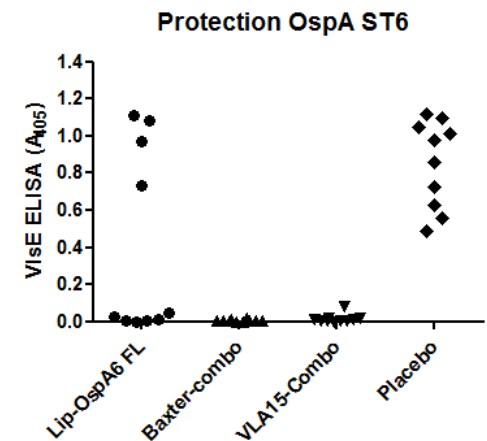
- + Challenge experiments have been performed two or three times, further experiments with additional doses
- + Significant protection seen in all five tick or needle challenge models

* Three independent experiments, Protection compared to the placebo group, Fisher exact test, two-tailed

Pre-clinical data for all six OspA serotypes

In vivo protection & in vitro assays

- + Mice were immunized three times (s.c.) with two weeks interval (1 μ g/protein)
 - › Sera analyzed one week after third immunization for growth inhibition & surface binding
 - › Mice challenged two weeks after third immunization and analyzed for infection 4 (needle challenge) or 6 weeks (tick challenge) later by VisE ELISA and qPCR

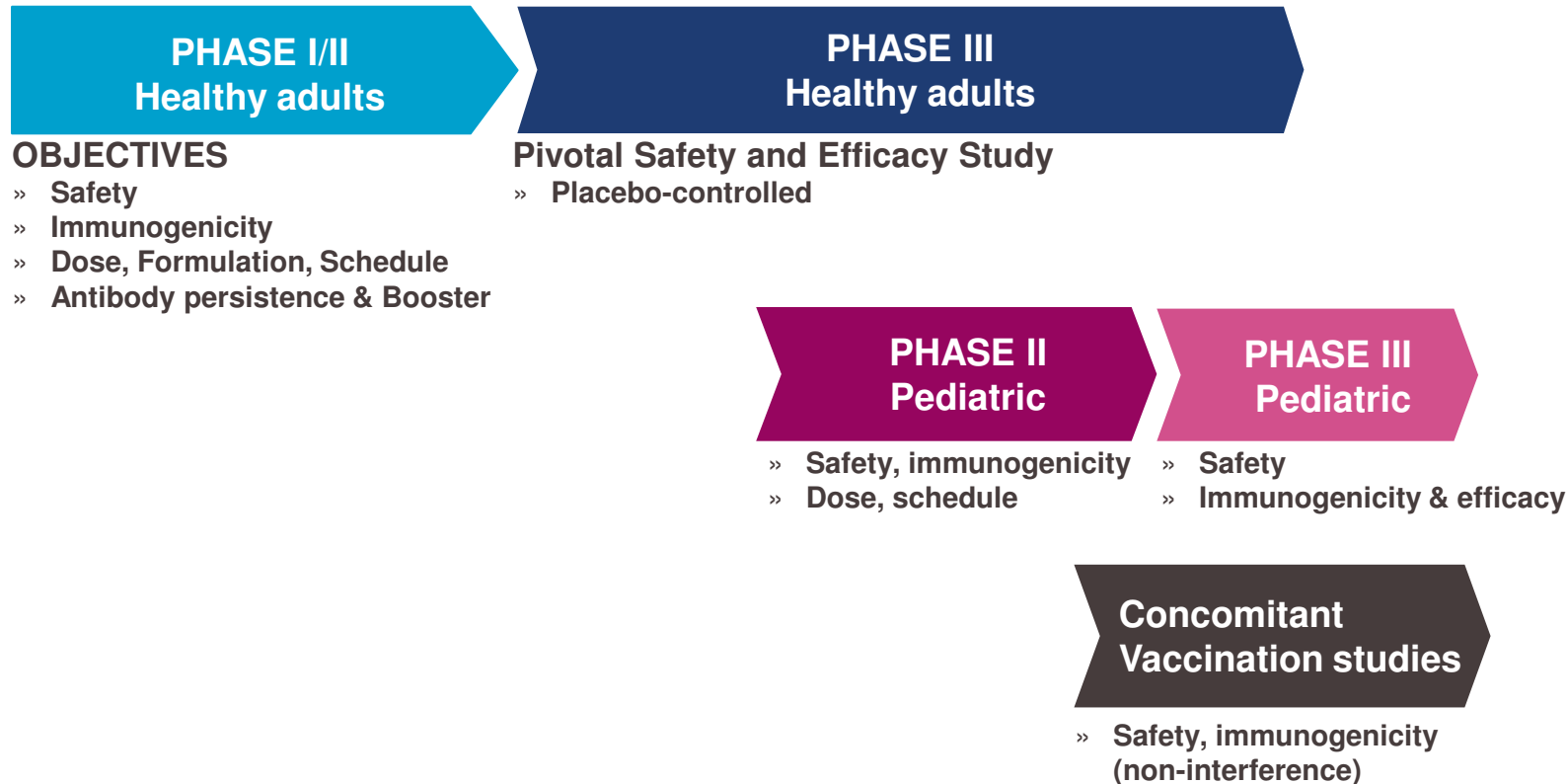




Clinical development of LB vaccine

Draft clinical development strategy

LICENSURE



VLA15 – Summary



- + Protein-based vaccine protective against the 6 major OspA serotype Borrelia species causing disease in EU & US
- + Pre-clinical protection shown in 5 different animal models (4 OspA serotypes)
- + VLA15 drug substance of excellent quality produced in E. coli in scalable fermentation process (fed-batch) in defined synthetic medium, yielding app. 1.2 g/L of mature lipoprotein
- + Semi-generic, scalable production process for all 3 proteins, resulting in high purity and low amount of residual impurities
- + Project ready to move into clinical testing within 12- 15 months

VLA15 – Acknowledgements



- + Pre-clinical Research at Valneva: Urban Lundberg, Pär Comstedt, Wolfgang Schüler, Robert Schlegl, Markus Hanner, Sandra Jost, Ana Kremers, Christina Satke and many others



- + Collaborators: Sven Bergström, Hans Dautel, Volker Fingerle

Merci
Danke
Thank you

