Subunit vaccine technologies for bacterial enteric diseases: Shigella, ETEC and nontyphoidal Salmonella vaccines

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Bill & Melinda Gates Foundation
Global Vaccine and Immunization Research Forum
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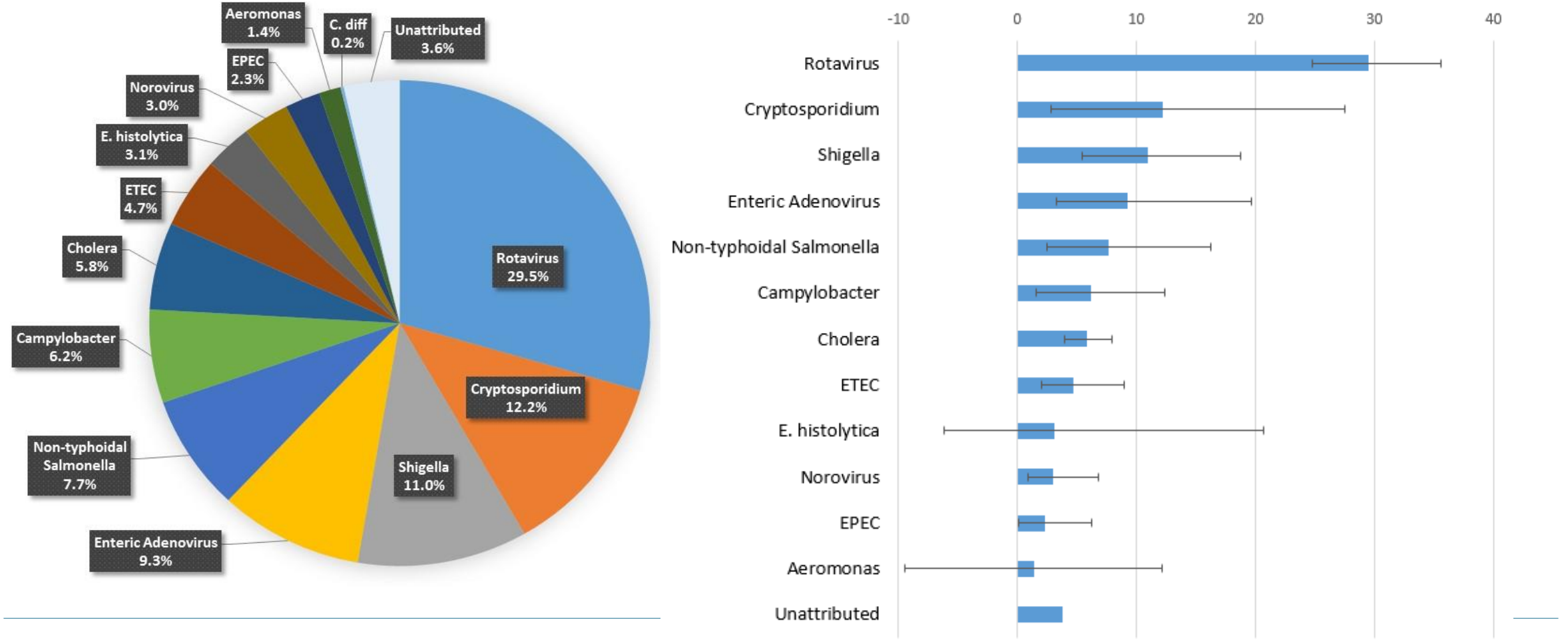
IHME GBD 2015 RESULTS: ETIOLOGY OF GLOBAL U5 DIARRHEAL DEATHS

- 499,000 (95% CI: 447,000 – 558,000) U5 diarrheal deaths

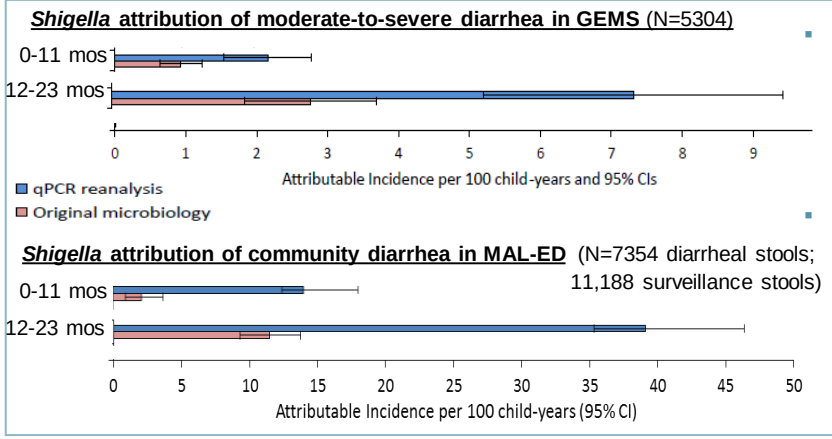
Population Attributable Fraction (%), 2015



SHIGELLA VACCINES

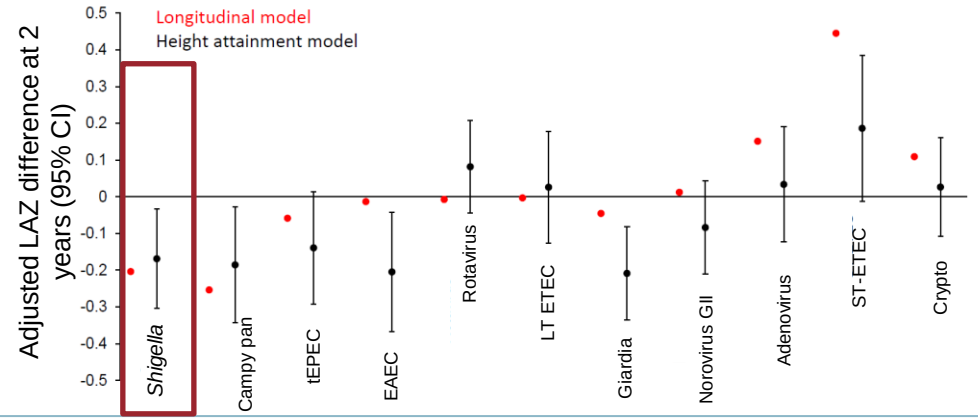
THE CASE FOR A *SHIGELLA* VACCINE

1 *Shigella* burden is greater than we thought…

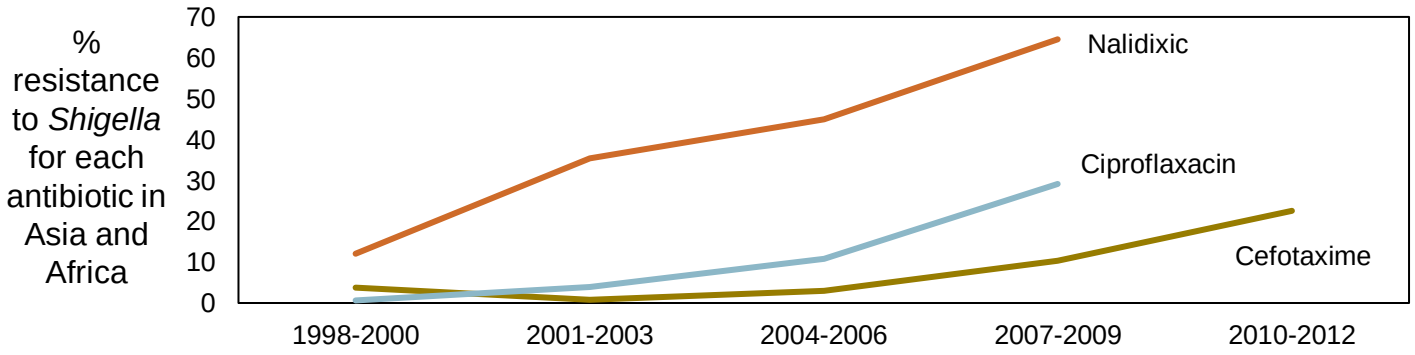


- Quantitative PCR re-analysis of GEMS increased *Shigella* attribution of moderate-to-severe diarrhea by ~2-3X per 100 child-years
- Quantitative PCR re-analysis of MAL-ED increased *Shigella* attribution of community diarrhea by ~7X and ~3X per 100 child-years among infants 0-11m and 12-23m, respectively

2 …its impact on growth faltering is significant…



3 …and the threat of AMR is growing



Adapted from Gu B et al. Int J Antimicrob Agents 2012 and Gu B et al, Epidemiol Infect 2015

From GEMS:

- Only 35% of Indian *Shigella* isolates were sensitive to ciprofloxacin (WHO-recommended antibiotic for *Shigella* dysentery)
- > 80% of African *Shigella* isolates were resistant to cotrimoxazole (most commonly prescribed antibiotic in African sites)

Source: GEMS; MAL-ED; AMR data adapted from Gu et al. *2012 and 2015*

SHIGELLA CANDIDATE PIPELINE

	Phase 1	Phase 2	Phase 3	Licensed	Notes
Live	CVD1208S (S. flex 2a) U Maryland/PATH	SC602 (S. flex 2a) WRAIR		Streptomycin-dep LAV (historic/various) (Yugoslav Army/other)	Historic LAVs no longer in use
		WRSS1 (S. sonnei) WRAIR/PATH			
Killed	TSWC (S. flex 2a) WRAIR/PATH				
Subunit	Oag synthetic conjugate (S. flex 2a) Pasteur Institute	GMMA (S. sonnei) GVGH (GSK)	Oag-TT conjugate (S. sonnei) NIH		NIH vaccine never licensed despite efficacy in phase 3
	Oag Bioconjugate (S. dysenteriae) Limmatech (GSK)	Oag Bioconjugate (S. flex 2a) Limmatech (GSK)			
		Invaplex (S. flex 2a) WRAIR			

BMGF funded

Wellcome funded

DFID funded

EU funded

PHASE 3 EFFICACY WITH SHIGELLA SONNEI CONJUGATE VACCINE IN YOUNG ADULTS

(Cohen D et al. Lancet 1997) Monovalent Shigella sonnei vaccine

Young Israeli military recruits

S. Sonnei LPS O-antigen – rEPA conjugate 25 ug/75 ug – single dose

Overall Vaccine Efficacy **74% (95%CI 28-100)**

Clinical Proof of Concept for O-antigen-based approach

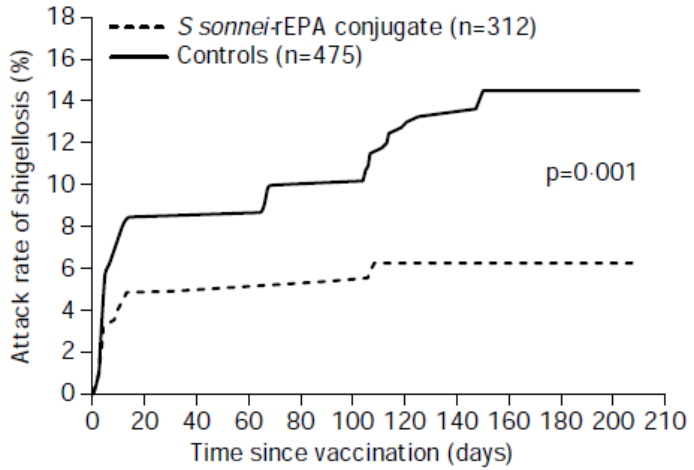


Figure 2: **Attack rates of culture-proven *S sonnei* shigellosis in recipients of *S sonnei* conjugate vaccine and controls in groups A–D**

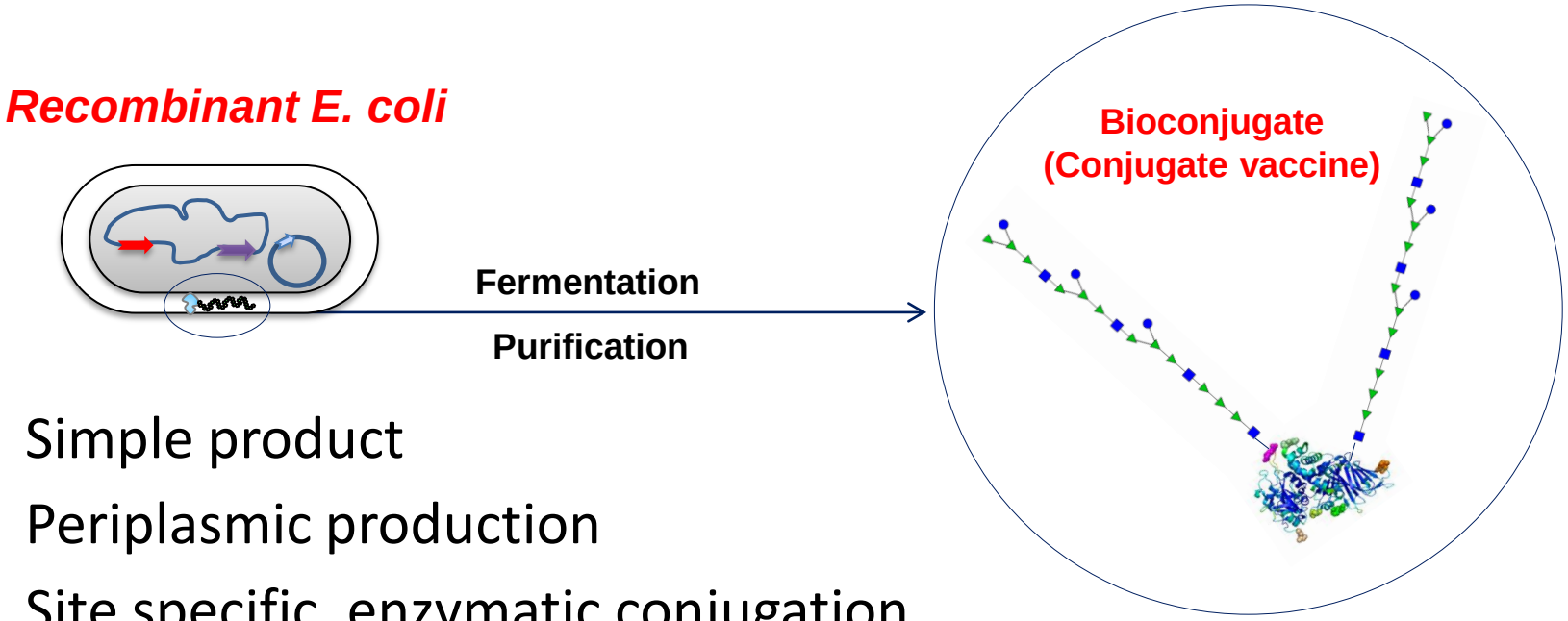
(Passwell JH et al. Vaccine 2010) Bivalent vaccine S. sonnei/S. flexneri 2a

2 doses 6 weeks apart. 2 year follow up

71.1% vaccine efficacy against S. sonnei diarrhea at 3-4 yrs age, but not < 3yrs

Loss of efficacy with reduced IgG LPS O-antigen titer

Bioconjugation: Simplified manufacturing of a safe and efficacious vaccine



- Simple product
- Periplasmic production
- Site specific, enzymatic conjugation

✓ *S. flexneri* 2a bioconjugate Flexyn2a: phase 1 and 2b clinical PoC

➢ Multivalent Shigella bioconjugate (manufacturing ongoing):
S. flexneri 2a, S. sonnei, S. flexneri 3a and 6

Flexyn2a vaccine: human challenge data

	Attack Rate N(%)		Vaccine Efficacy	
	Flexyn2a N=30	Placebo N=29	(%)	p-value
Shigellosis (primary endpoint)	13 (43.3)	18 (62.1)	**30.2**	0.11
More Severe Shigellosis* (post hoc analysis)	8 (27.6)	16 (53.3)	**51.7**	0.015
More Severe Diarrhea(secondary endpoint)**	2 (6.7)	7 (24.1)	**72.4**	0.07

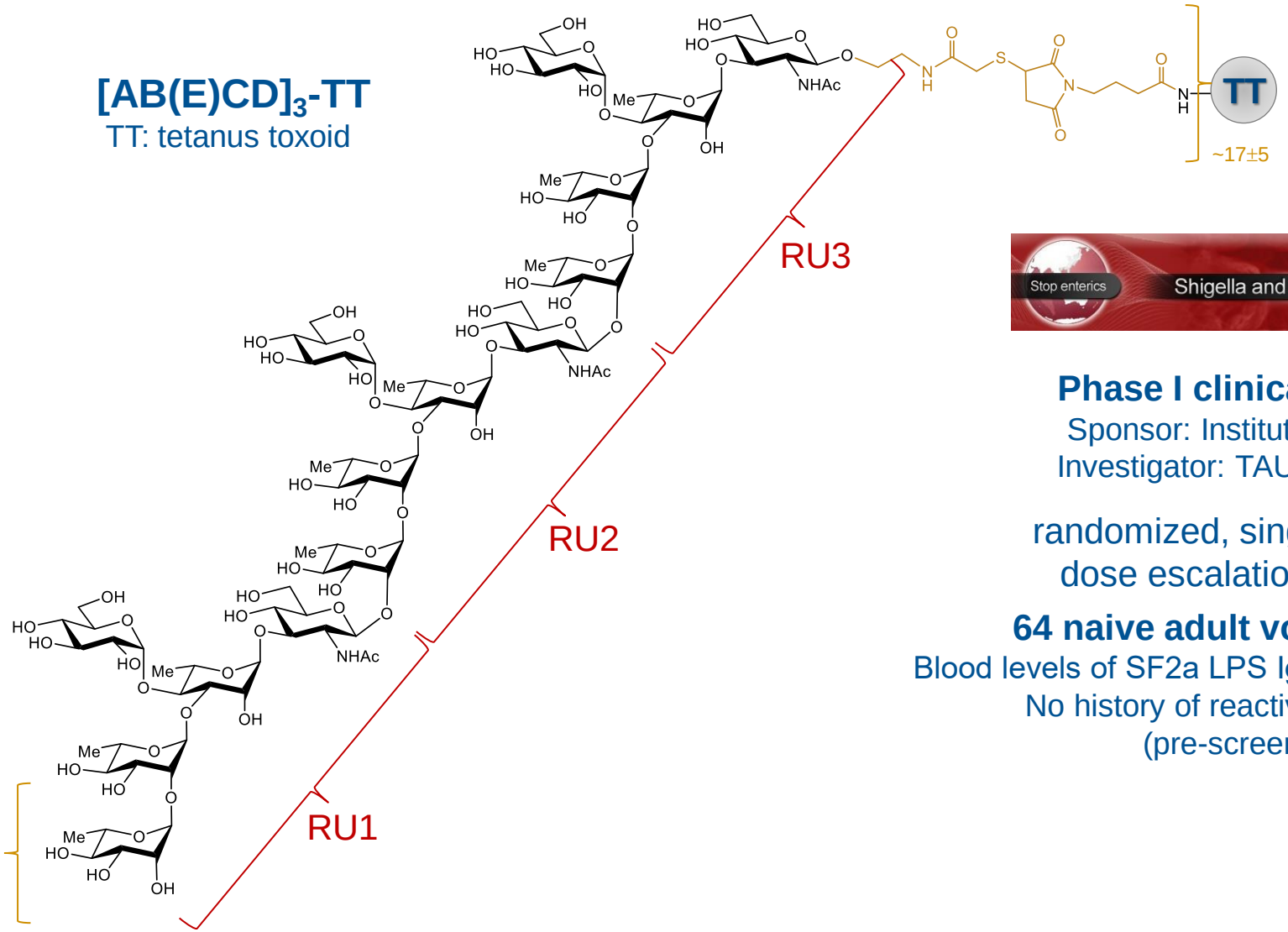
*severe enteric symptoms included (no mild or moderate); ** ≥10 loose stools (or ≥ 1kg) in 24h

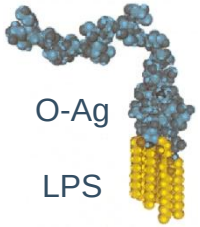
Correlate of protection

Significant correlation between **serum IgG anti Sf2a-LPS** and protection against Shigellosis (p=0.0061)

A synthetic carbohydrate-based vaccine candidate against *S. flexneri* 2a produced for a phase I clinical trial

$[AB(E)CD]_3$-TT
TT: tetanus toxoid





Phase I clinical study
Sponsor: Institut Pasteur
Investigator: TAU, TASMC

randomized, single-blind
dose escalation study

64 naive adult volunteers
Blood levels of SF2a LPS IgG ≤ percentile 80
No history of reactive arthritis
(pre-screen)

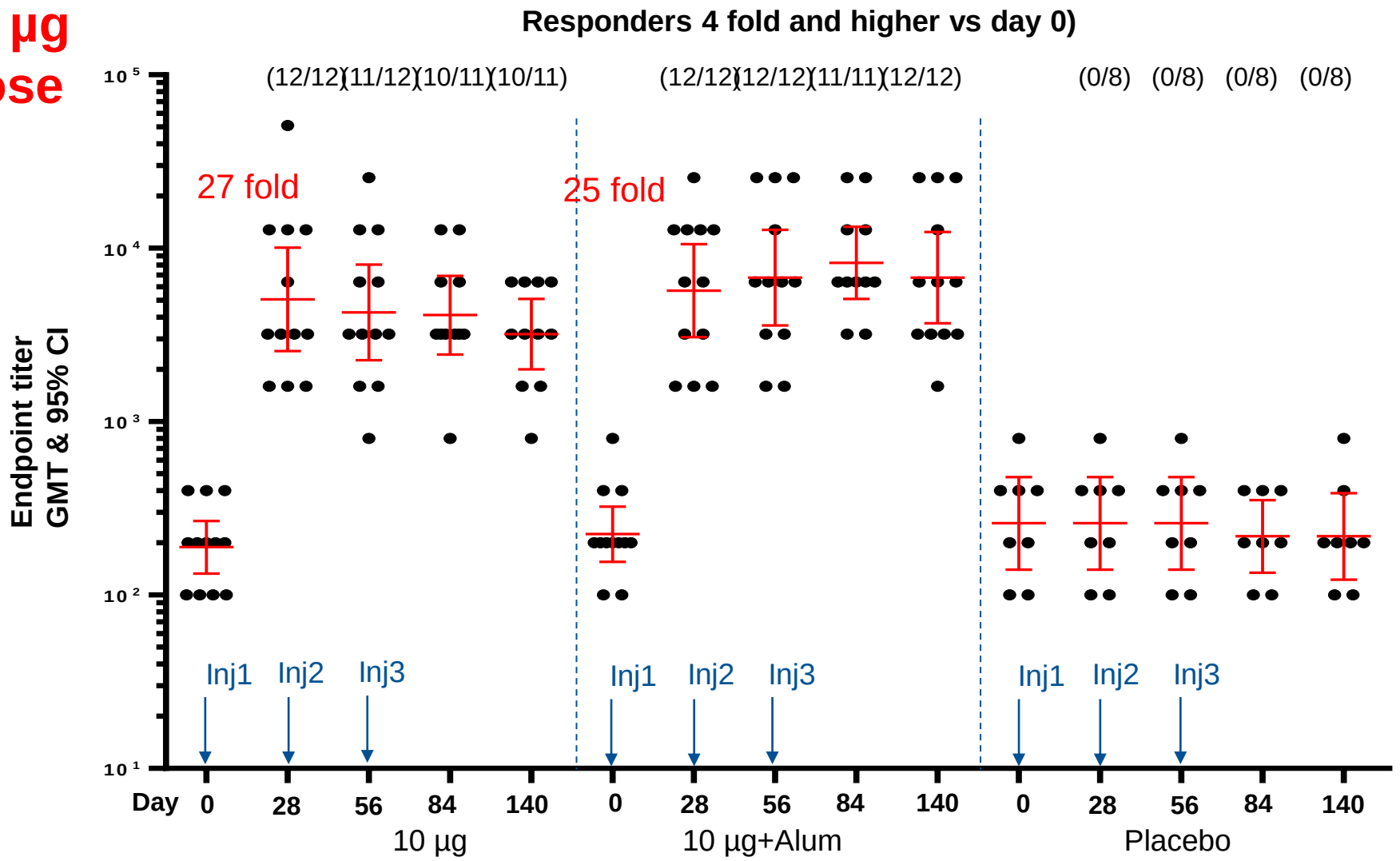
https://clinicaltrials.gov/ct2/show/NCT02797236

Bioconjugate Chem 27 883 (2016)

Institut Pasteur

A synthetic carbohydrate-based vaccine candidate against *S. flexneri* 2a is strongly immunogenic in naive adults

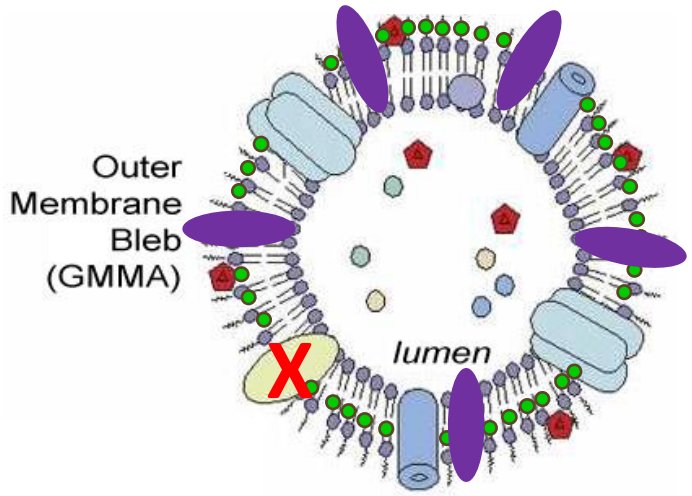
10 µg dose

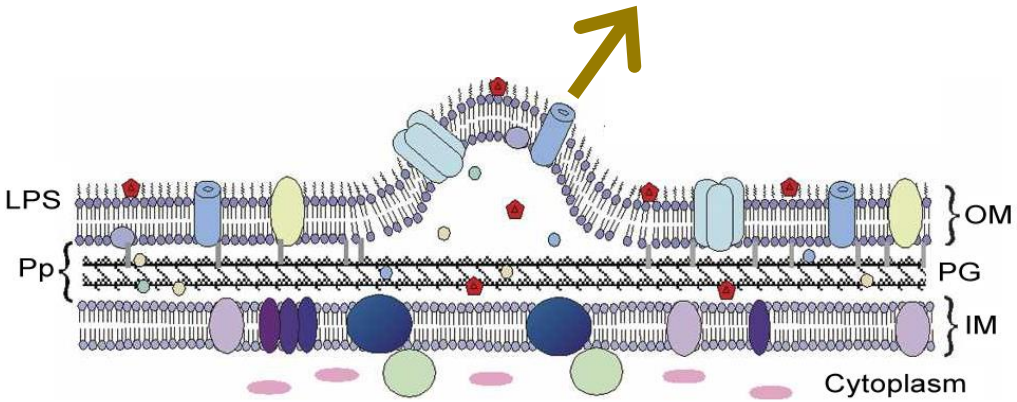


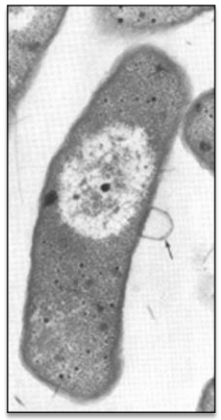
https://clinicaltrials.gov/ct2/show/NCT02797236

Institut Pasteur

GENERALIZED MODULE FOR MEMBRANE ANTIGENS (GMMA)
GSK VACCINES INSTITUTE FOR GLOBAL HEALTH (GVGH)







Genetic modifications

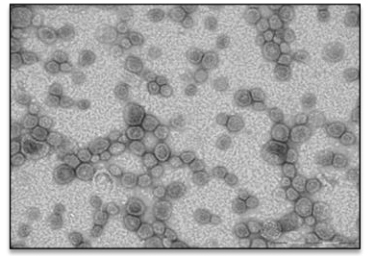
1. **Increase GMMA production**
2. **Reduce toxicity**

Purification

Micro-filtration
Collect 0.22 µm permeate

Ultra-filtration
Collect 300 kDa retentate

Purified GMMA



- Outer membrane vesicles
- Multiple antigens (O-antigen, outer membrane proteins)
- Straightforward production
- Potential low cost of good

GVGH (GSK) GMMA – PHASE 1 & 2

Phase 1 - French adults (Launay O et al EBioMedicine 2017)

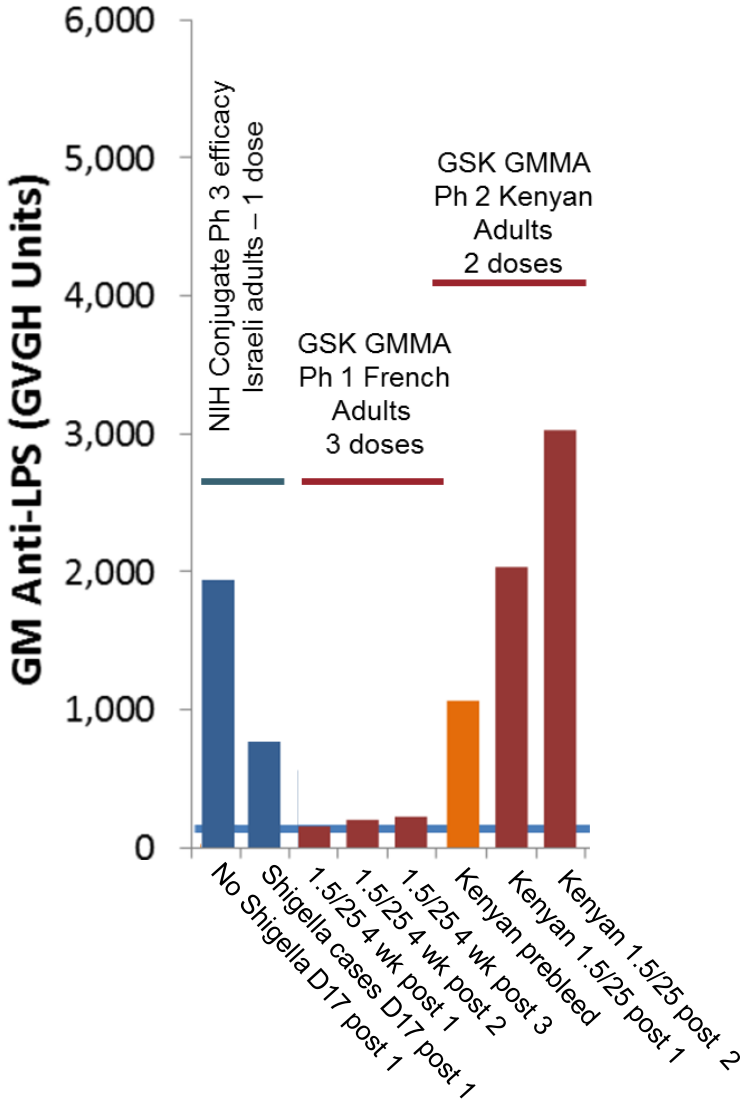
Dose-escalating Phase 1 study with monovalent *S*. sonnei GMMA

O-antigen/protein 0.059/1, 0.29/5, 1.5/25, 2.9/50 & 5.9/100 µg

3 vaccinations, 4 weeks apart

Phase 2 - Kenyan adults (Obiero CW et al Front Immunol 2017)

– high pre-existing antibody titers make interpretation of findings difficult



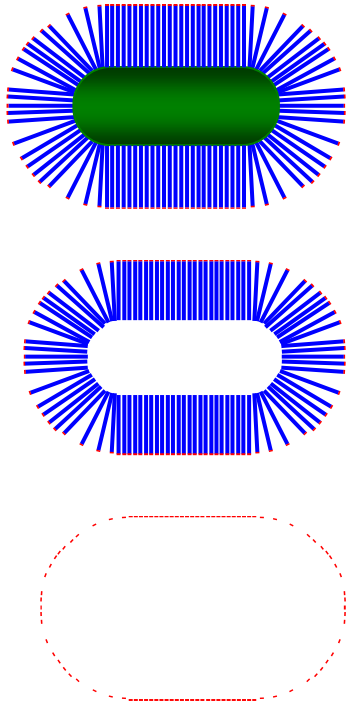
Low IgG titers due to low O-antigen content of *S*. sonnei GMMA?

Potential protection through other non-O-antigen related mechanisms

- 2018 CHIM with current monovalent *S*. sonnei GMMA – to assess efficacy of O-antigen-sparse GMMA
- 2019 matched 4-valent studies with Limmatech bioconjugate into target population

ETEC VACCINES

ETEC COLONIZATION FACTORS AND TOXINS



Whole cell

Fimbriae

Fimbrial tip adhesins

<u>Fimbriae</u> ***Intestinal adherence***

CFA*/I

CFA/II CS1, CS2, CS3**

CFA/IV CS4, CS5, CS6

Others (CS17, CS14, PCF071)

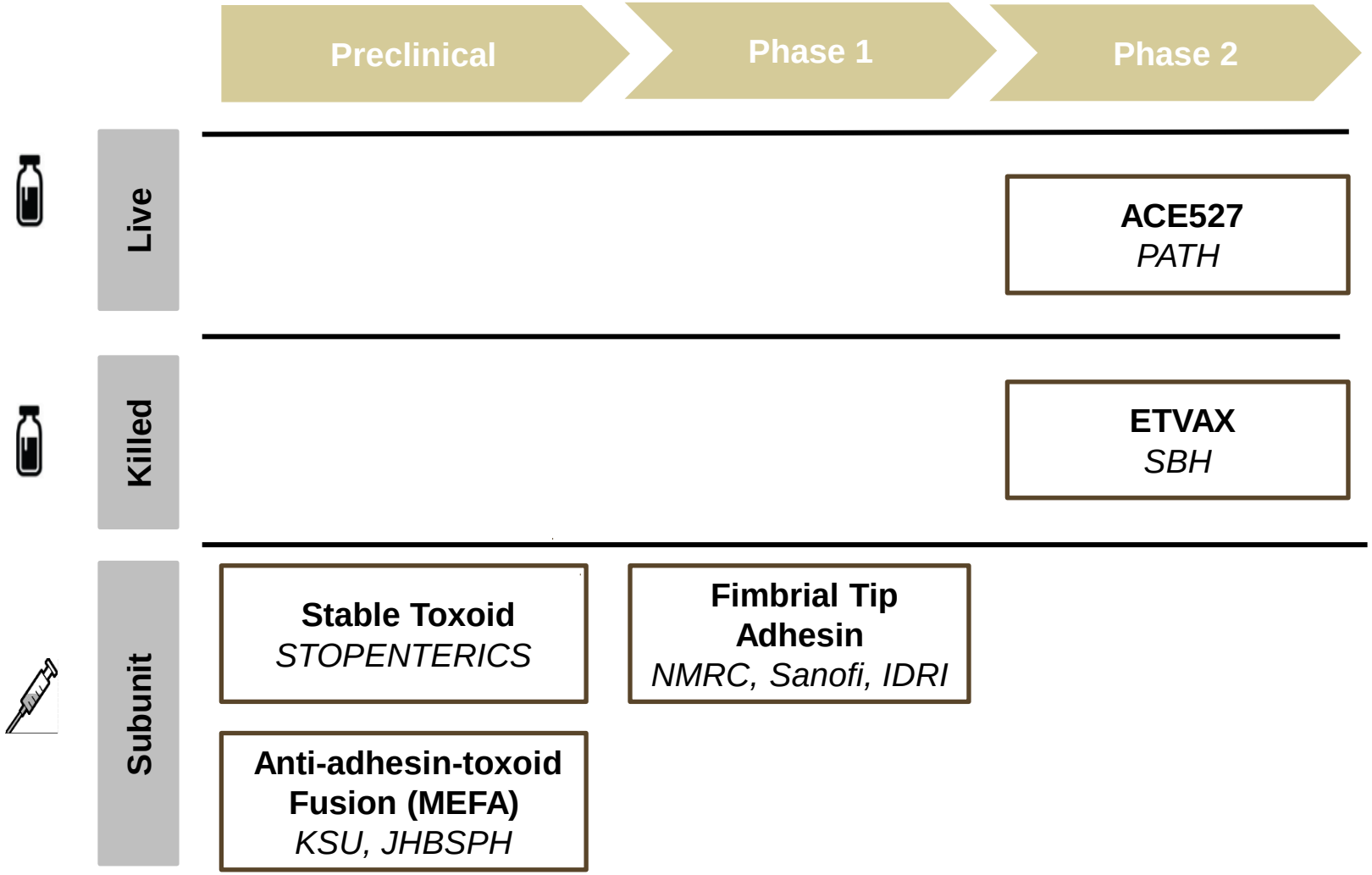
<u>Toxins</u> ***Cause diarrhea***

LT (Thermal labile)

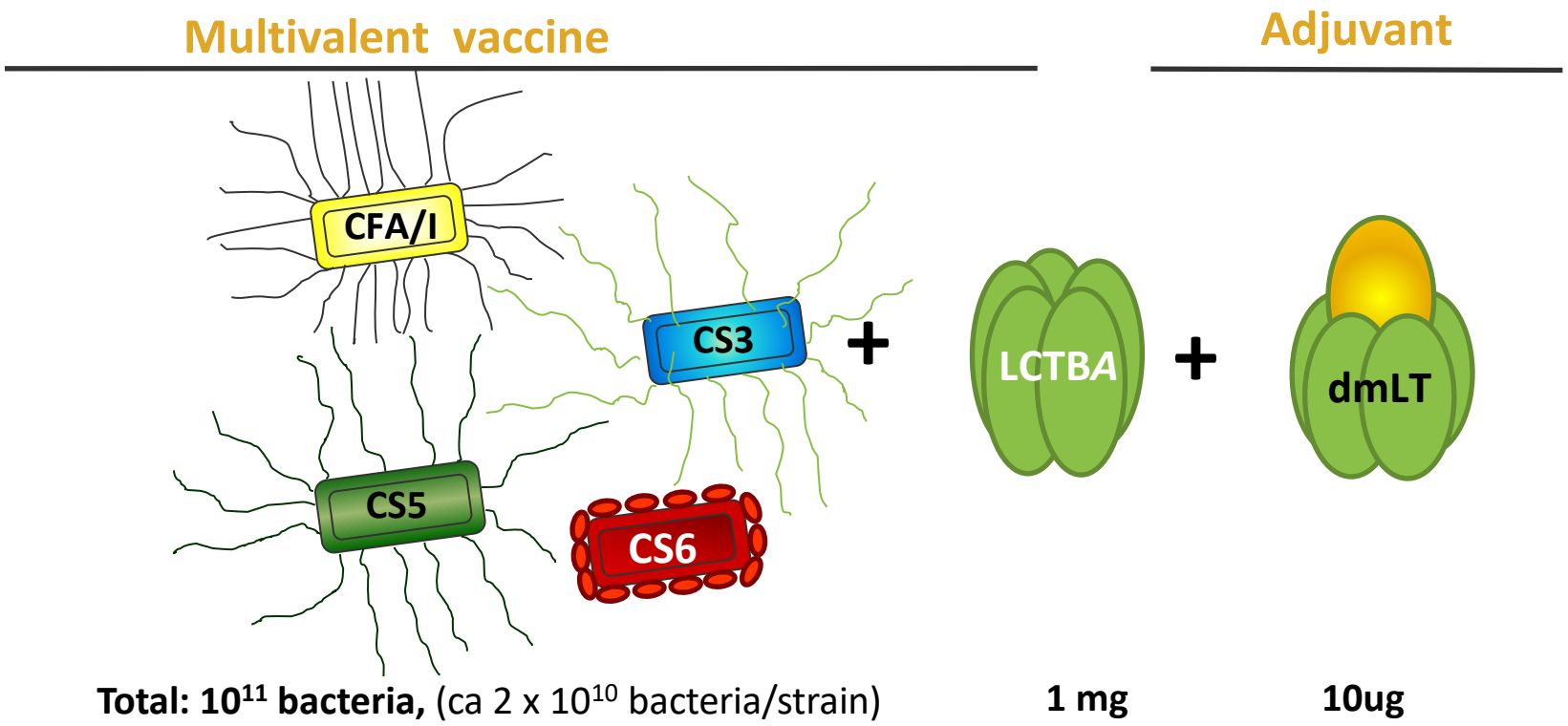
ST (Thermal stable)

LT/ST

CURRENT PIPELINE OF ETEC VACCINE DEVELOPMENT

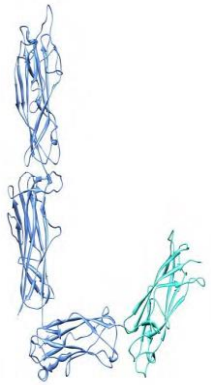


ETVAX – SCANDINAVIAN BIOPHARMA



- 1st generation: efficacy against severe travelers' diarrhea, but not in Egyptian infants
- WHO recommended increased CF/CS content; add CS6; assess adding adjuvant (dmLT)
- 2nd generation: immunogenic in Ph1 trial in Swedish adults; then descending age study in Bangladesh; now in a travelers study

ETEC FIMBRIAL TIP ADHESIN (FTA) VACCINE

Antigen	CfaEB	CsbDA-CooA	CotDA	CssBA
Structure				
Estimated coverage	Class 5a CFA/I, CS14, CS4	Class 5b CS1, PCF071, CS17, CS19	Class 5C CS2	CS6

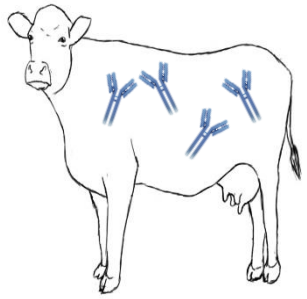
- Concept: Antibodies to adhesive fimbrial subunits abrogate intestinal adherence, preventing disease
- A multivalent vaccine approach covering four of the most prevalent colonization factors plus and LT
- Estimated to provide 75-80% coverage for most common ETEC strains.

PASSIVE OR ACTIVE IMMUNIZATION WITH CF/CS ANTIGENS CAN PROTECT IN CHIMS AND NHP CHALLENGE MODELS

CHIMS - Volunteer anti-CfaE bovine IgG passive immunoprophylaxis trial

End point	PE (%)	95% CL
All diarrhea	63	2, 86
Moderate-severe diarrhea	84	-6, 98

Anti – CFA/I and CS17 bovine Ab also protective in passive immunization model



Protective efficacy of Class 5 adhesin-pilin fusions in *Aotus nancymaae* model of ETEC diarrhea

Class	Vaccine	Route	PE(%)[1]	P-value[2]
5a	CfaE, LTB	ID	84	0.015
5b	CsbDA-CooA, LTB	ID	70	0.05
5c	CotDA, LTB	ID	45	0.07
CS6	CssBA, dmLT	ID	100	0.03

[1]Protective efficacy against CF-homologous ETEC Strain

[2]Fisher's exact test, one-tailed

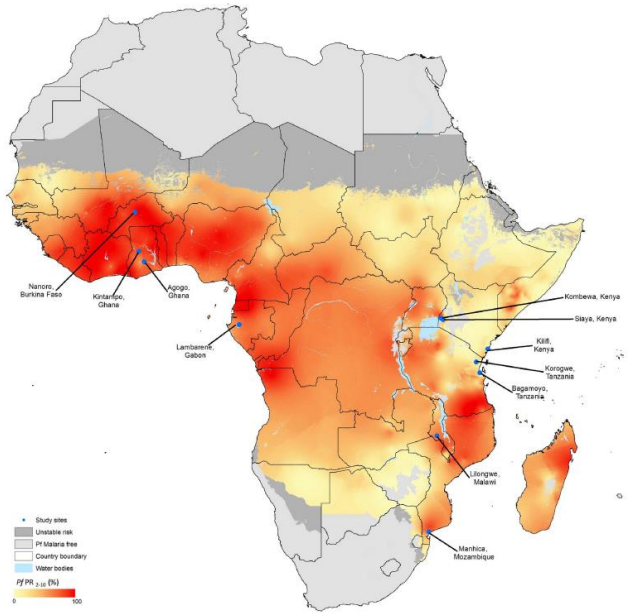
- CssBA+dmLT, phase 1 trial (IM route) currently underway

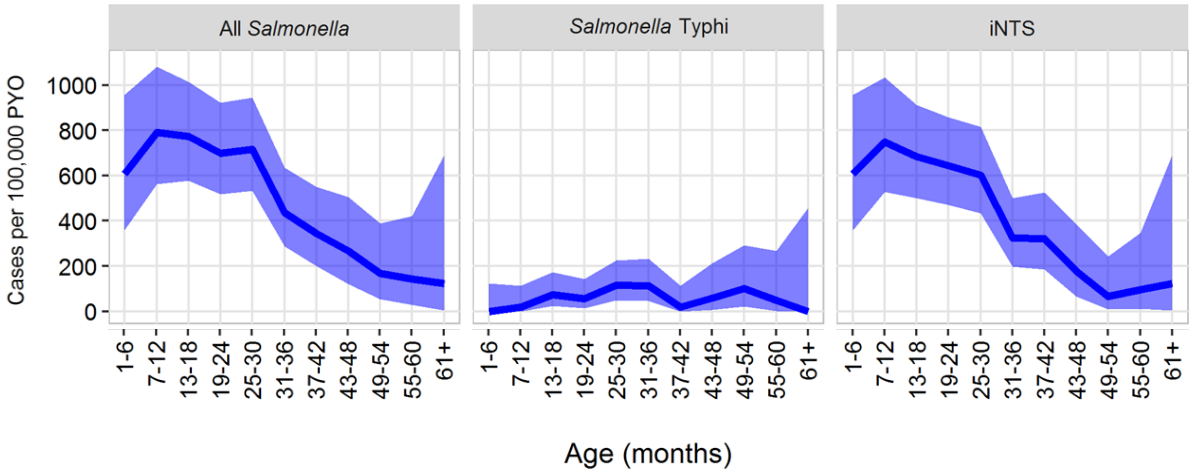
NONTYPHOIDAL SALMONELLA VACCINES

Invasive nontyphoidal *Salmonella* (iNTS) disease in Africa: RTS,S-AS01 phase 3 malaria vaccine trial, 2009 to 2014

11 sites across sub-Saharan Africa. Children < 5yrs. *Salmonella* responsible for **60%** of all bacterial bloodstream infections (bacteremias).

Incidence	per 100,000 PYO (95% CIs)
All *Salmonella*	**534** (471, 604)
S. Typhi	**66.5** (45.5, 93.9)
NTS	**461** (402, 526):
• *S*. Typhimurium	283 (237, 334)
• *S*. Enteritidis	133 (102, 170)





A **monovalent typhoid vaccine** could have prevented **12.5%** of *Salmonella* bacteremias.

A **trivalent *Salmonella* vaccine** could have prevented **90.3%** of *Salmonella* bacteremias.

(MacLennan CA, Wiegand R, Westercamp N, Kariuki S, et al unpublished)

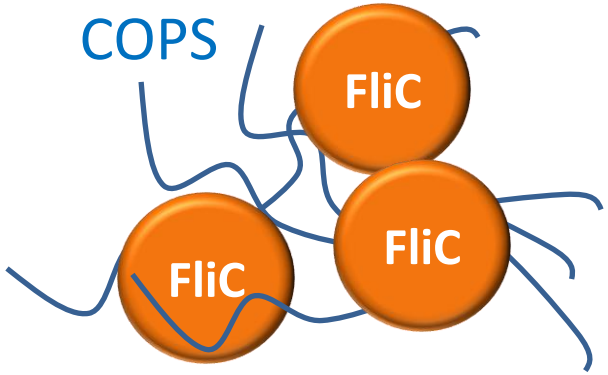
University of Maryland/Bharat Biotechnology Trivalent NTS/Typhi conjugates

S. Typhimurium

Architicture: Sun-type (end-link)
Chemistry: thioether
Linkers: GMBS (FliC lysines), aminooxy-thiol (COPS-KDO)
Linkage: COPS-KDO -> protein amines
Conjugation pH: 5-7

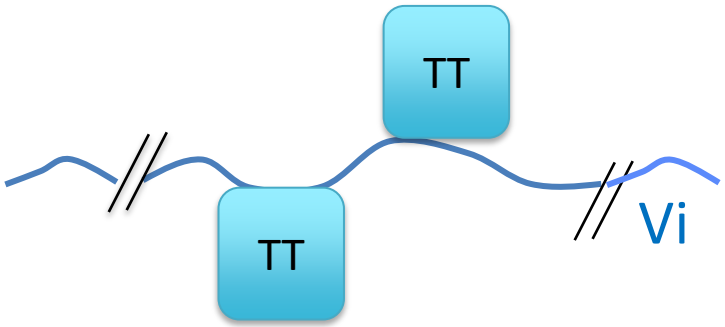
S. Enteritidis

Architecture: Lattice (multi-point linkage)
Chemistry: CDAP cyanylation
Linkers: Adipic acid dihydrazide (FliC carboxyls)
Linkage: COPS hydroxyls -> protein amines and carboxyls
Conjugation pH: 9-10



S. Typhi (Typbar-TCV)

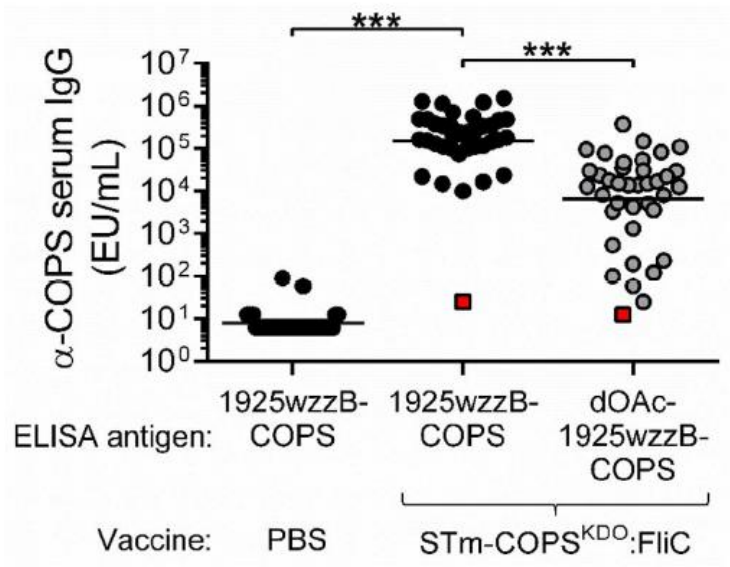
Architecture: Bead-on-string
Chemistry: ADH/EDC
Linkers: Adipic acid dihydrazide (Vi-TT carboxyls)
Linkage: Vi-carboxyls-> TT carboxyls
Conjugation pH: 6-7

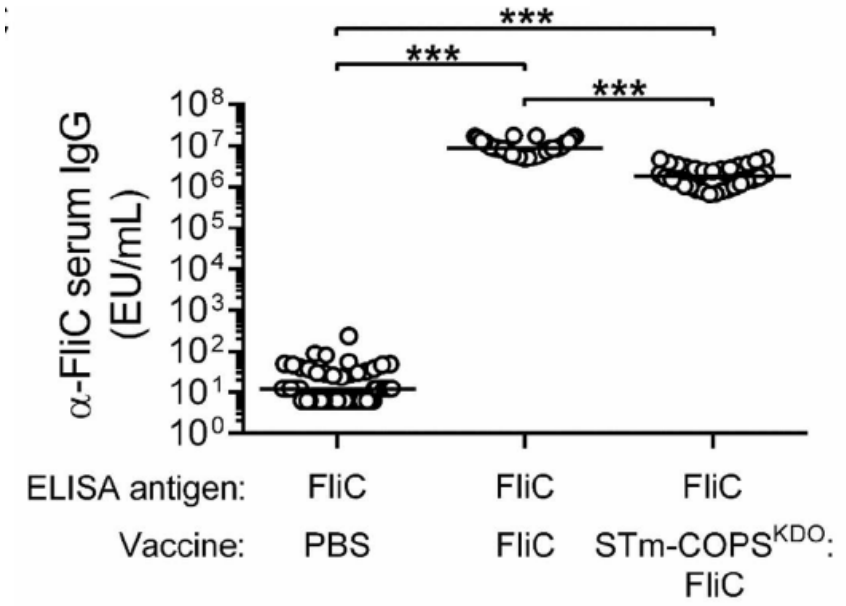


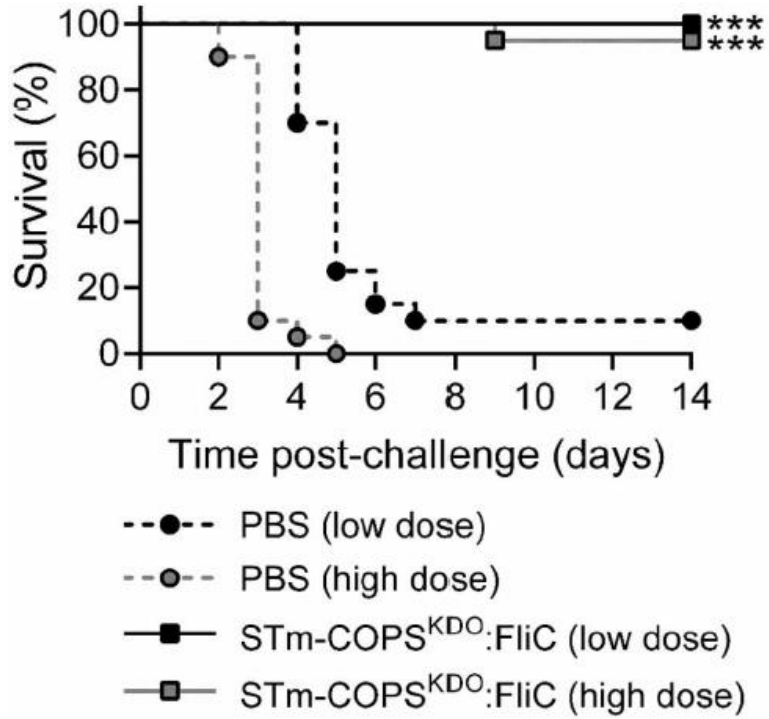
Development of a glycoconjugate vaccine to prevent invasive *Salmonella* Typhimurium infections in sub-Saharan Africa

(PLoS Negl Trop Dis 2017)

Scott M. Baliban[1,2], Mingjun Yang[3], Girish Ramachandran[1,2], Brittany Curtis[1,2], Surekha Shridhar[1,2], Rachel S. Laufer[1,2], Jin Y. Wang[1,2], John Van Druff[4], Ellen E. Higginson[1,2], Nicolas Hegerle[1,2], Kristen M. Varney[5], James E. Galen[1,2], Sharon M. Tennant[1,2], Andrew Lees[4], Alexander D. MacKerell, Jr.[3], Myron M. Levine[1,2,6], Raphael Simon[1,2]*





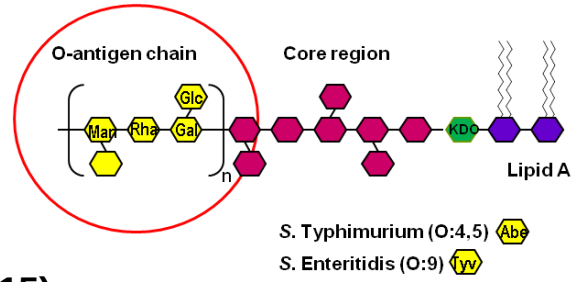


Glycoconjugates and GMMA

- GSK Vaccines Institute for Global Health vaccine approaches

1- *Bivalent conjugate vaccine against iNTS(S. Typhimurium and S. Enteritidis)*

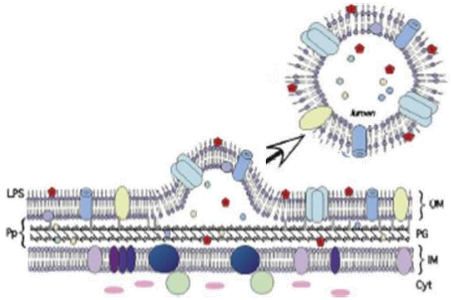
O:4,5-CRM_{197} + O:9-CRM_{197}



(Rondini S et al, Infect Immunity 2015)

2- Bivalent GMMA vaccine against iNTS (S. Typhimurium and S. Enteritidis)

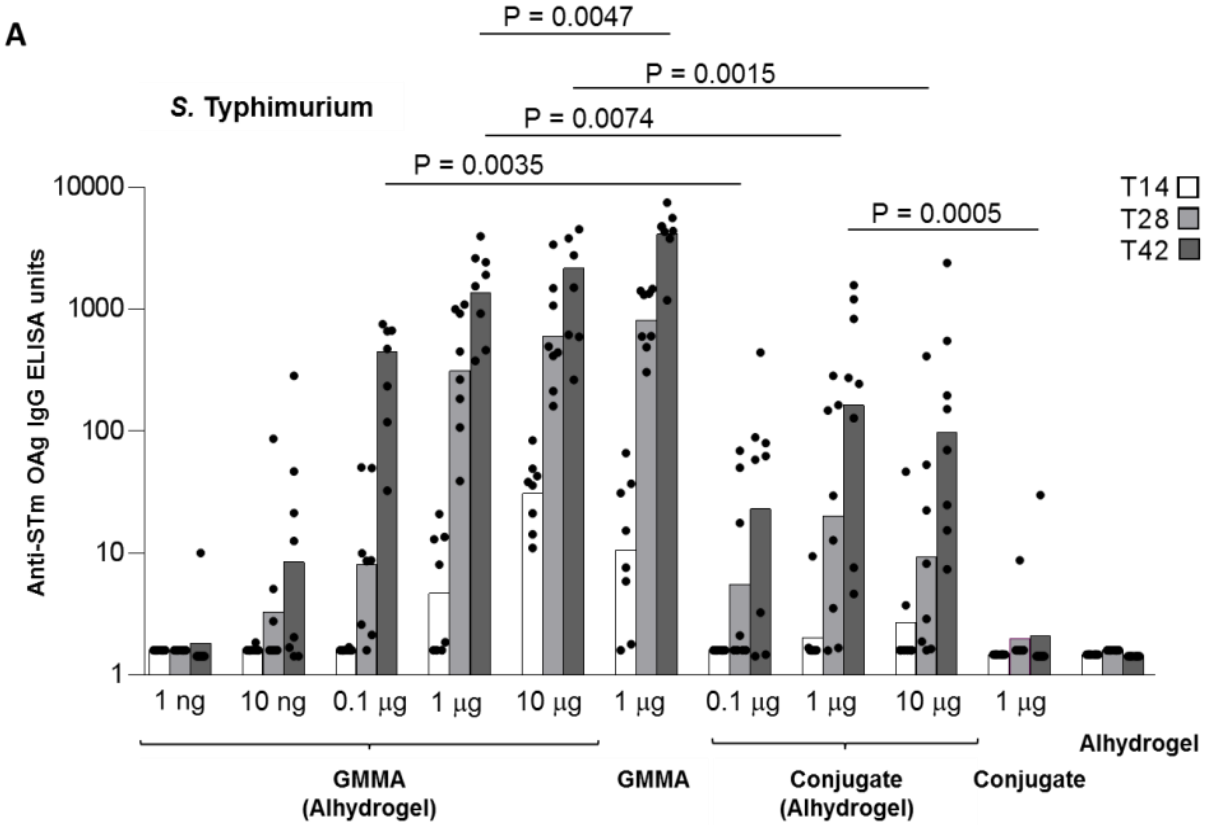
STmGMMA* + SEnGMMA†

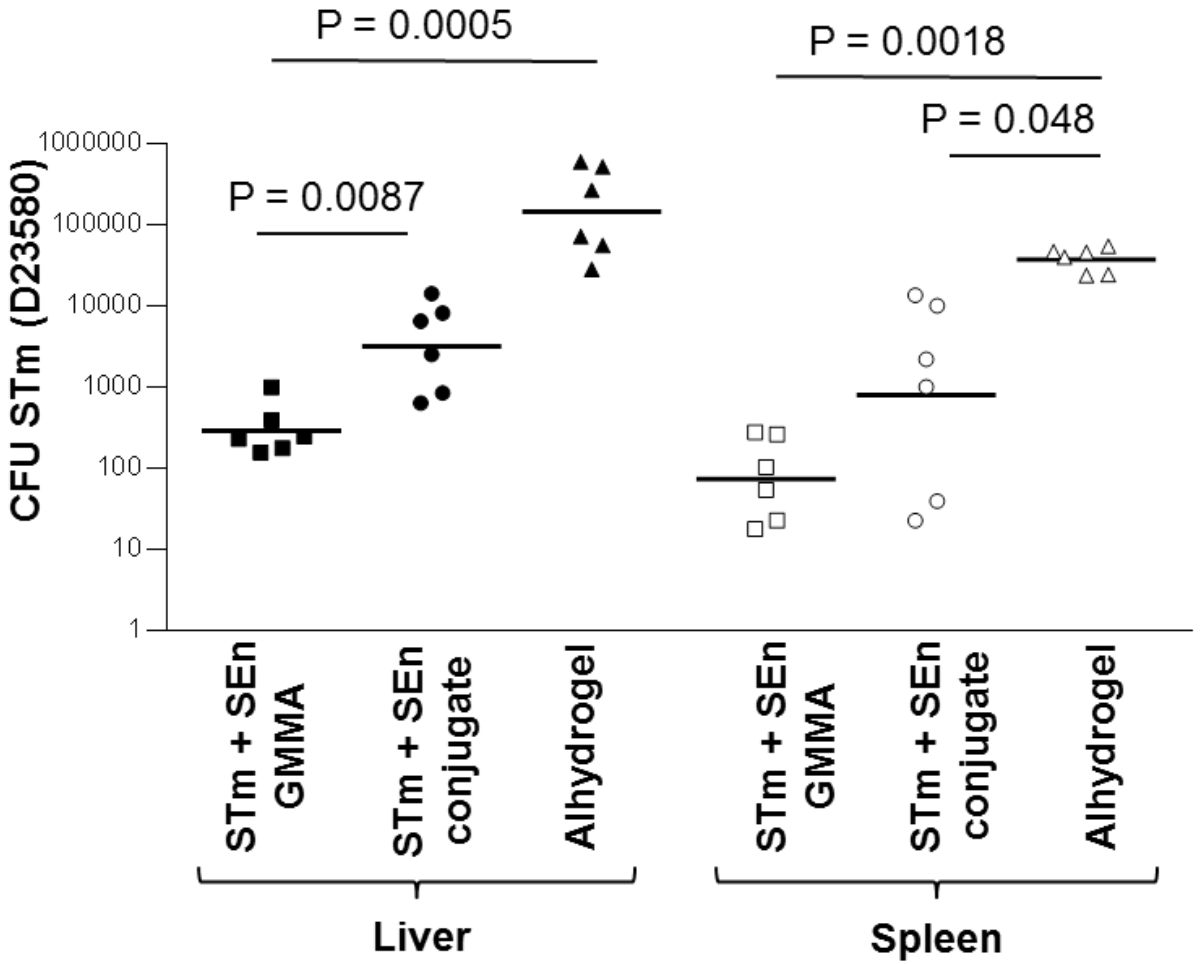


**S*. Typhimurium GMMA

†*S*. Enteritidis GMMA

Equivalent *S.* Typhimurium GMMA and glycoconjugate vaccines compared in mice





(Micoli F, Rondini S, Alfini R, Lanzilao L, Necchi F, Rossi O, Clare S, Mastroeni R, Saul A, MacLennan CA, unpublished)

(Supported by a BMGF Grand Challenges Explorations Award: 'A novel approach to manufacture of highly immunogenic and affordable polysaccharide vaccines for Global Health priority diseases.')

SUMMARY

- New subunit vaccine technologies in clinical trials for ETEC and Shigella
  - Shigella O-antigen-based approaches, building on 1st generation NIH conjugate vaccine efficacy
  - ETEC optimal approach less clear – fimbrial tip adhesins as lead approach
- Nontyphoidal Salmonella O-antigen-based subunit vaccines to enter clinical trials in 2019

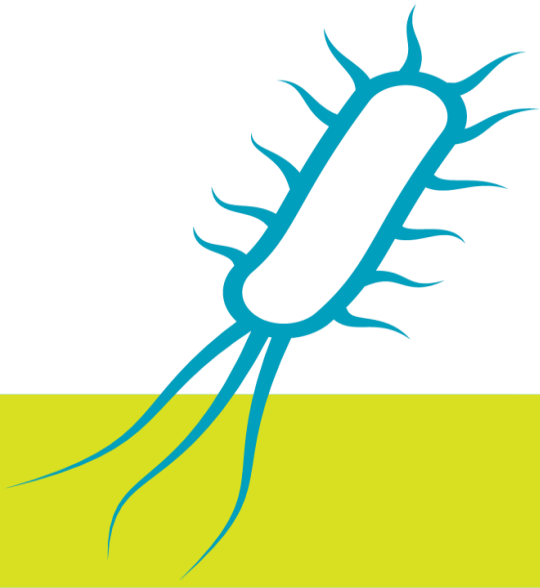
- Challenge of inducing sufficient immunity (and protection) in target populations – children <1 year in LMICs
- Need to age-descend earlier to assess immunogenicity in target population
- Need to maximize information from controlled human infection models – protection/correlates

- Correlates of protection lacking apart from IgG to Shigella O-antigen
- Assay standardization and qualification required for all three disease areas
- Need for clarity on regulatory pathways to licensure and implementation

BactiVac bacterial vaccinology network

- Part of the MRC UK's GCRF (Global Challenges Research Fund) Networks in Vaccines Research and Development initiative
- Promote and accelerate the development of vaccines against bacterial infections relevant to low and middle-income countries
- Encourage cross-collaboration between academic and industrial partners in developed and developing nations
- Catalyst projects and training awards
- Please join:

http://www.birmingham.ac.uk/research/activity/immunology-immunotherapy/research/bactivac/index.aspx



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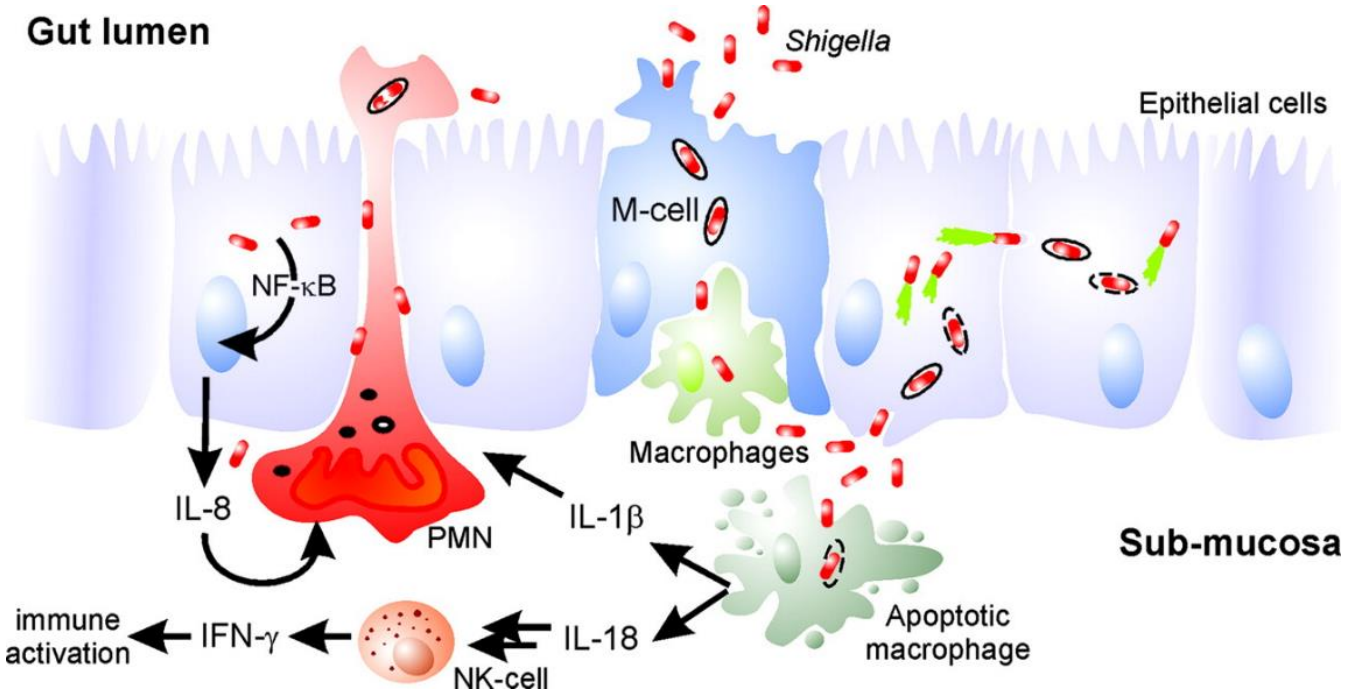
- Francesca Micoli – GSK Vaccines Institute for Global Health
- Michael Prouty – Naval Medical Research Center
- Lou Bourgeois – PATH
- Mike Levine – University of Maryland
- Laura Lamberti - BMGF

BACK UP

SHIGELLA VACCINES

Four serogroups of *Shigella*

- Flexneri (6 serotypes)
- Sonnei (1 serotype)
- Bodyii (19 serotypes)
- Dysenteriae (15 serotypes)



- **Invasive pathogen**
- **Complex multivalent vaccine construct likely required**

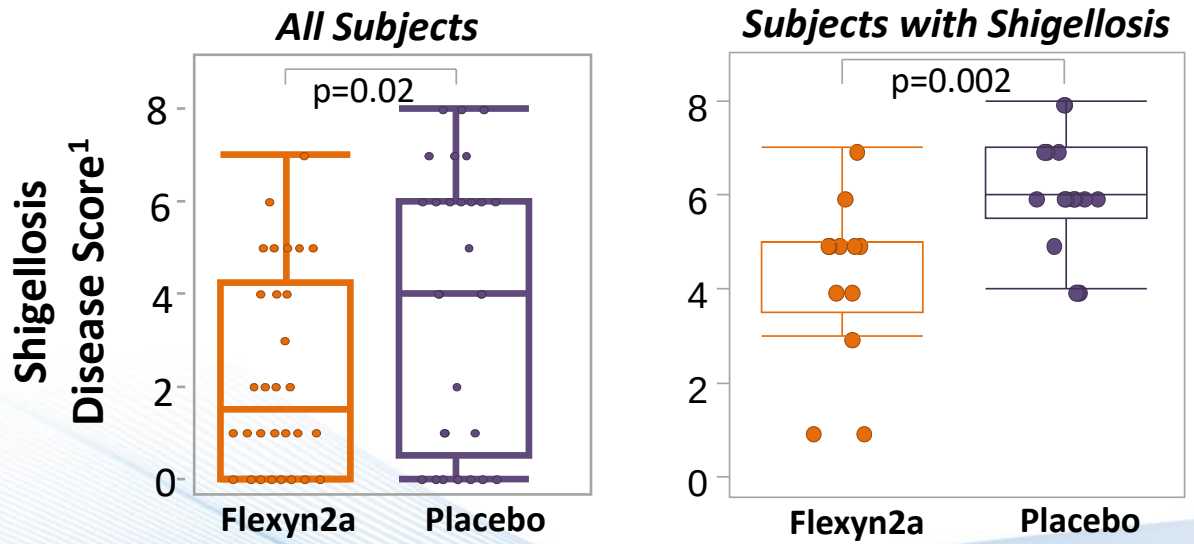
Bioconjugation proven success of Shigella Vaccines

- ✓ Clinical safety and immunogenicity demonstrated with *S. dysenteriae* vaccine and *S. flexneri* 2a
- ✓ Clinical Proof of Concept (cPOC) shown for the monovalent Shigella-bioconjugate Flexyn2a (WT-project 2013-2016), with efficacy data in CHIM
- ✓ Positive correlation identified for Flexyn2a between immune-response and protection
- ➢ In addition bioconjugate uses same Shigella antigens which were protective in the field with the chemical conjugate

Flexyn2a vaccine: human challenge data

	Attack Rate N(%)		Vaccine Efficacy	
	Flexyn2a N=30	Placebo N=29	(%)	p-value
Shigellosis (primary endpoint)	13 (43.3)	18 (62.1)	**30.2**	0.11
More Severe Shigellosis* (post hoc analysis)	8 (27.6)	16 (53.3)	**51.7**	0.015
More Severe Diarrhea(secondary endpoint)**	2 (6.7)	7 (24.1)	**72.4**	0.07

*severe enteric symptoms included (no mild or moderate); ** ≥10 loose stools (or ≥ 1kg) in 24h



[1] C. Porter VED 2017

Correlate of protection

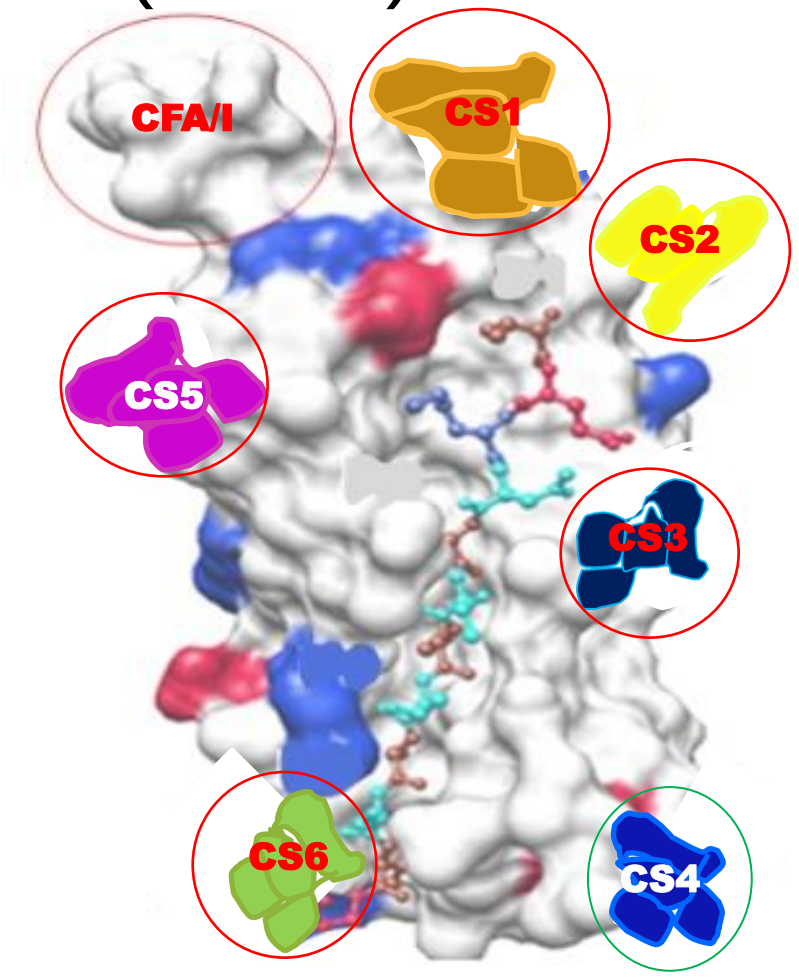
Significant correlation between **serum IgG anti Sf2a-LPS** and protection against Shigellosis (p=0.0061)

LimmaTech™ Biologics
better technology for better health

ETEC MULTI-EPITOPE FUSION ANTIGEN (MEFA) VACCINE

- One MEFA expresses the dominant epitopes of CFAs in a single protein
- One MEFA expresses non-toxic LTA LTB and ST in a single protein
- MEFA vaccines stimulate neutralizing antibodies against each of these virulence antigens
- 28 out 28 piglets acquired toxin MEFA-induced antibodies were protected against STa+ ETEC challenge, whereas 26 out 32 control piglets developed watery diarrhea.
- Quantitative culture of piglet ileum showed reduced colonization following immunization

  Immunized group: 3.2 x10^8 CFU **Control group**: 1.6 x 10^9 CFU



CFA/I – grey, CS1 – brown, CS2 – yellow, CS3 – ocean blue, CS4 – blue, CS5 – purple, CS6 - green ; CfaB backbone - grey

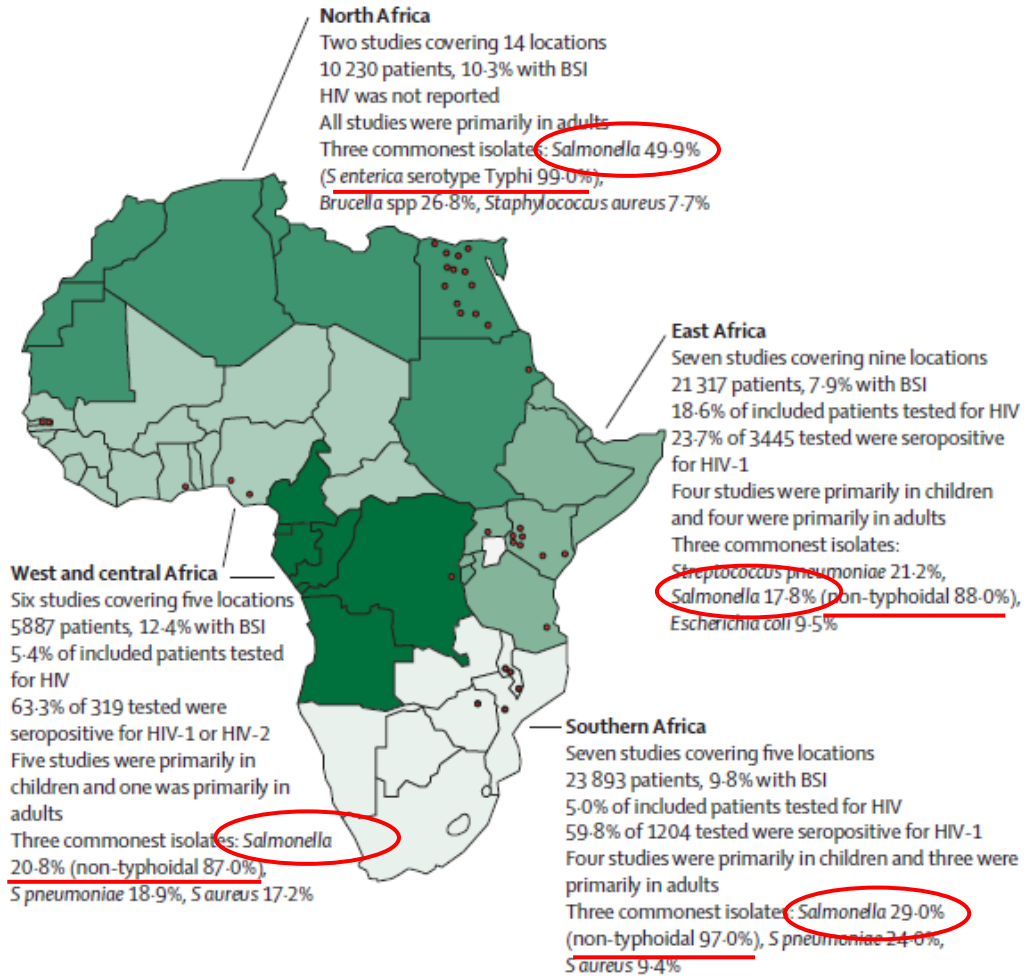
Data courtesy of Weiping Zhang, KSU

Invasive nontyphoidal Salmonella (iNTS) disease

a major neglected disease in Africa

- Commonest cause of bacteraemia in much of Sub-Saharan Africa
- High case fatality rate (~20%)
- High levels of AMR
- Diagnostic conundrum
- Conclusion: key target disease for vaccine development

- Affected groups:
  - Young immune-naïve children
  - HIV-infected individuals
  - Malaria-infected children



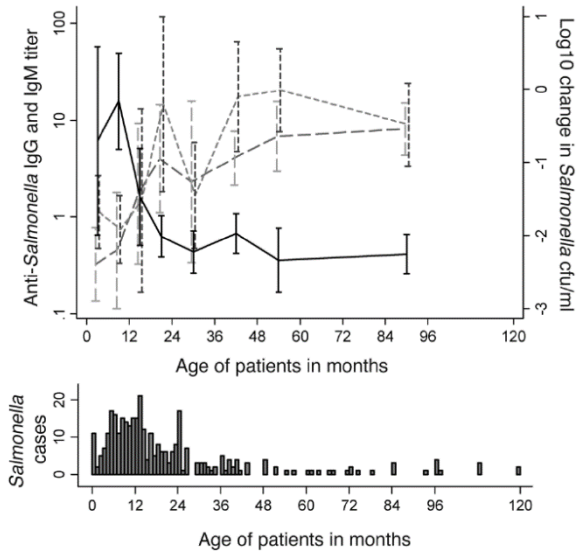
(Reddy EA, et al, Lancet Infect Dis 2010)

A role for antibodies in immunity to invasive nontyphoidal *Salmonella* disease

Research article

The neglected role of antibody in protection against bacteremia caused by nontyphoidal strains of *Salmonella* in African children

(MacLennan CA, et al J Clin Invest 2008)



nature Vol 452|3 April 2008

RESEARCH HIGHLIGHTS

Acquisition of bactericidal antibodies inversely corresponds to age at which African children are susceptible to iNTS

IMMUNOLOGY

Antibiotic antibodies

J. Clin. Invest. doi:10.1172/JCI33998 (2008)

The discovery of functional antibodies against strains of *Salmonella* that do not cause typhoid raises hopes that a vaccine can be developed. In Africa, such strains kill up to 24% of infected children in communities in which appropriate antibiotics and blood-culture facilities are available.

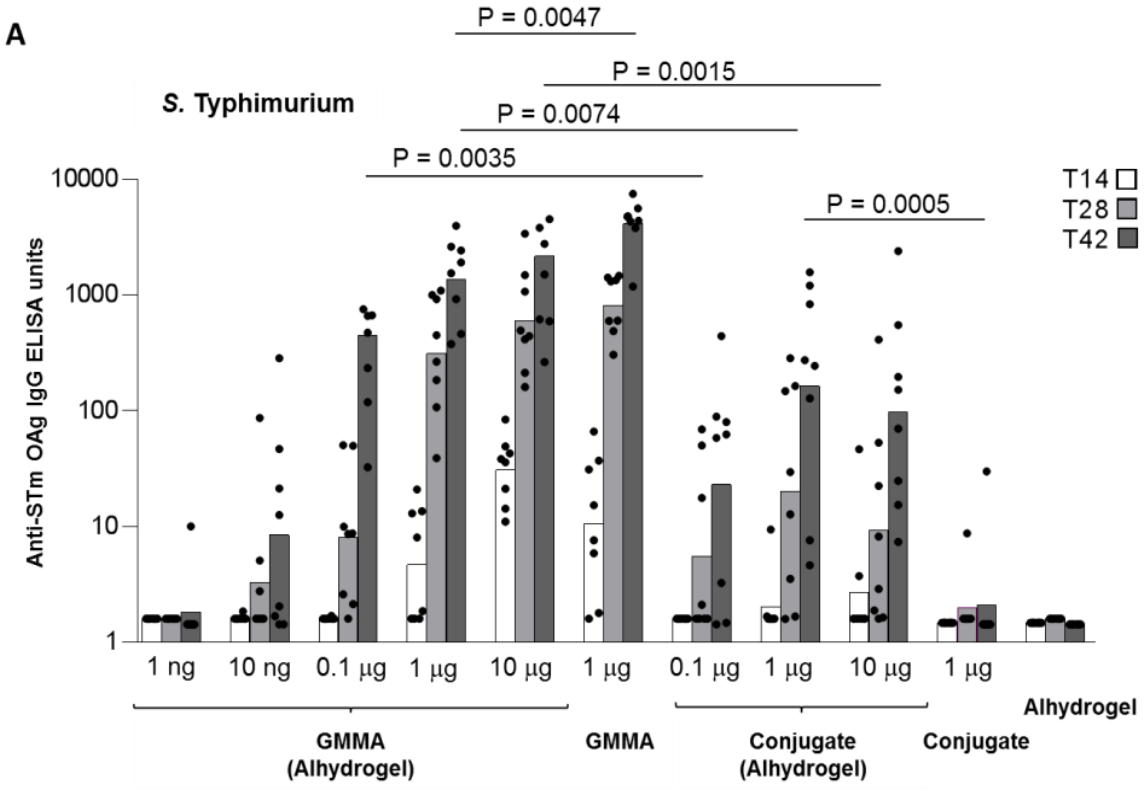
Vaccines in development against iNTS

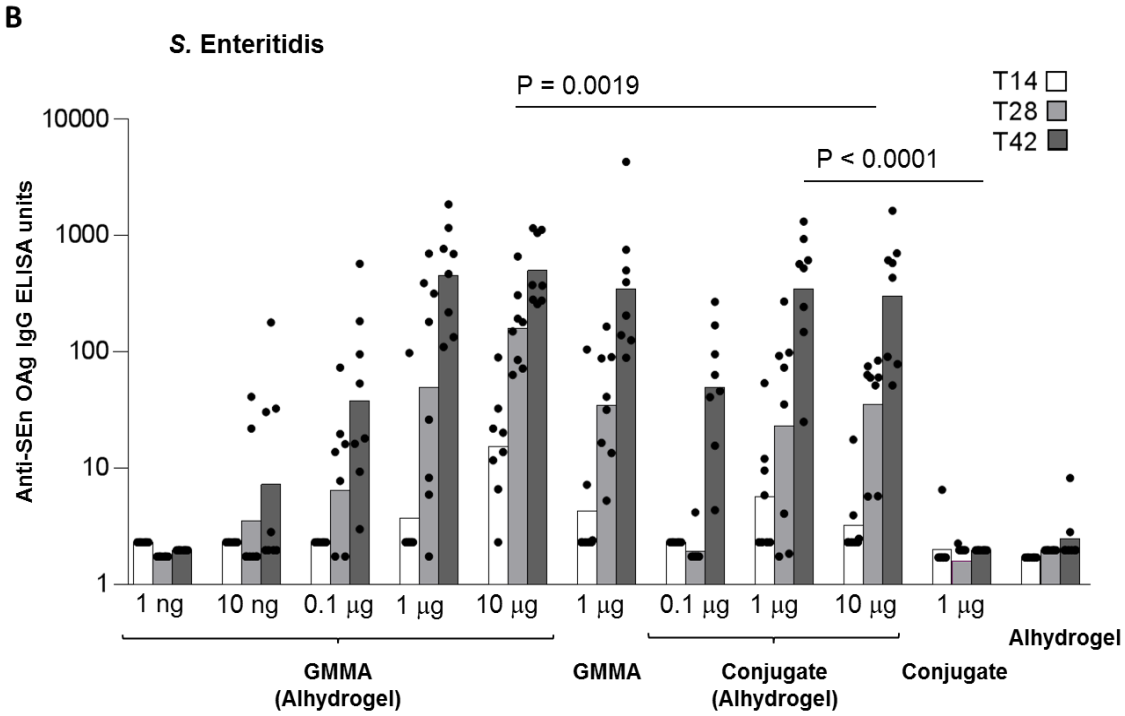
Name	Description	Developer	Stage of development	References
O:4,5/O:9-flagellin	O:4,5/O:9 Conjugate	University of Maryland	Preclinical	50,69
O:4,12-TT	O:4-TT Conjugate	NIH	Preclinical	51
Os-po	O:4-porin Conjugate	National Bacteriology Laboratory, Stockholm	Preclinical	146
O:4,5/O:9-CRM_{197}	O:4,5/O:9 Conjugate	NVGH	Preclinical	145
WT05	Live attenuated	Microscience, Wokingham Berkshire	Phase 1	147
CVD 1921 and CVD 1941	Live attenuated	University of Maryland	Preclinical	148
S. Typhimuirum ruvB mutant	Live attenuated	Seoul National University	Preclinical	149
Salmonella hfq deletion mutant	Live attenuated	Indian Institute of Science Bangalore	Preclinical	150
SA186	Live attenuated	Istituto Superiore di Sanità Roma	Preclinical	151
MT13	Live attenuated	KIIT University Odisha	Preclinical	152
Various	Live attenuated, DNA adenine methylase mutants	University of California, Santa Barbara	Preclinical	153,154
Various	Live attenuated, regulated delayed attenuation	Arizona State University	Preclinical	155-157
Porins	*S.* Typhimurium porins	National Bacteriology Laboratory, Stockholm	Preclinical	146
OmpD	Outer membrane protein	University of Birmingham, UK	Preclinical	73
S. Typhimurium and *S.* Enteritidis GMMA	Generalized Modules for Membrane Antigens	NVGH	Preclinical	65,158,159

*an exhaustive list, particularly of all candidate vaccines in preclinical studies, is beyond the scope of this review

(MacLennan CA, et al Hum Vaccin Immunother 2014)

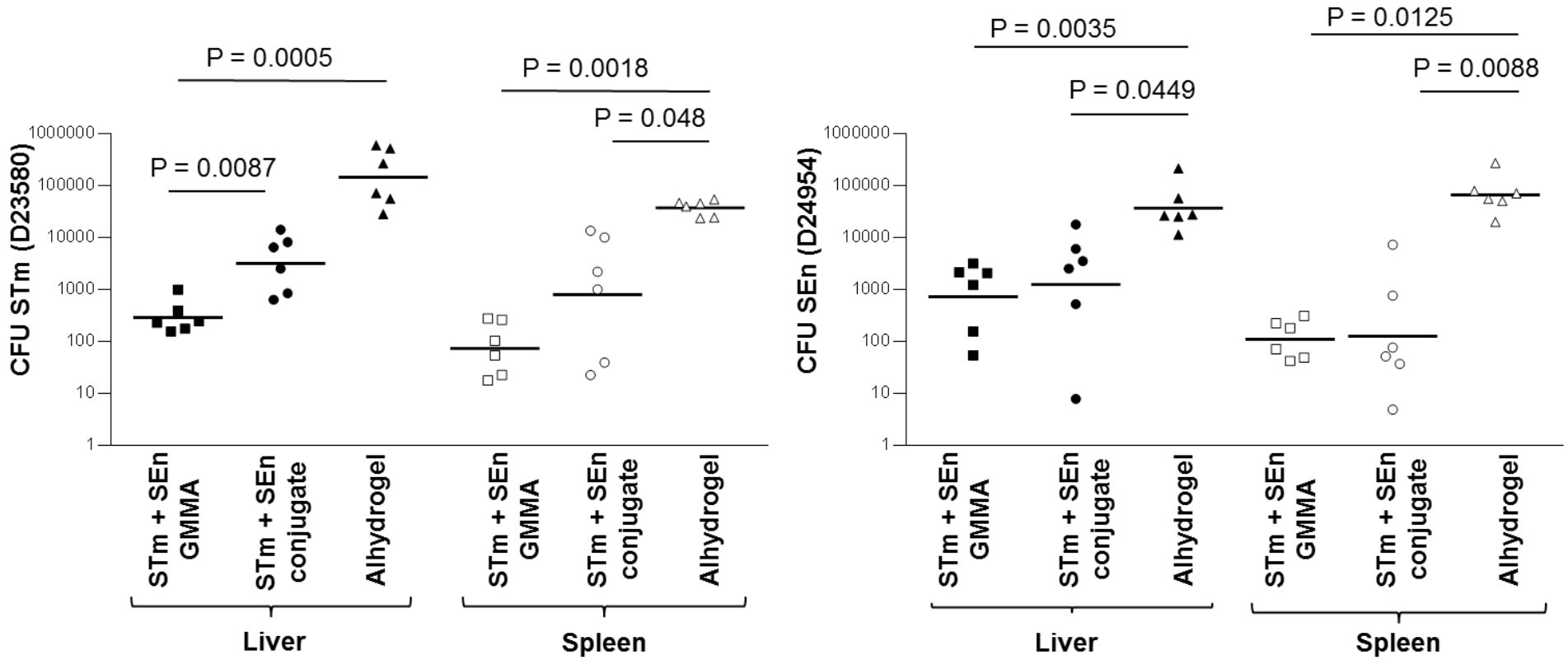
S. Typhimurium and *S.* Enteritidis GMMA and glycoconjugate vaccines compared in mice as monovalent formulations





Supported by a BMGF Grand Challenges Explorations Award: ‘A novel approach to manufacture of highly immunogenic and affordable polysaccharide vaccines for Global Health priority diseases.’

In vivo infection study in mice immunized with GMMA and conjugate in bivalent formulation



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