Preclinical development of a trivalent typhoid/non-typhoidal *Salmonella* glycoconjugate vaccine for sub-Saharan Africa

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A comprehensive typhoid/iNTS vaccine for use in sub-Saharan Africa

  - $\geq 33\%$ of all bacteremias, and multidrug resistance ($S. \text{Typhi} = 47\%; \text{iNTS} = 48\%$)

- Regional variability in typhoid fever/iNTS incidence, and heterogeneity in iNTS burden over time.

- Antibodies recognizing surface polysaccharides correlate with protection in children.
  (Klugman, 1987; MacLennan, 2008; Nyirenda, 2014; de Alwis, 2018).

- **An effective vaccine against invasive *Salmonella* disease should target all three.**
Trivalent typhoid-iNTS glycoconjugate formulation
(S. Enteritidis COPS:FliC + S. Typhimurium COPS:FliC + Typbar-TCV™)

S. Typhimurium (B), S. Enteritidis (D)

conjugate vaccines: STm COPS:FliC SE COPS:FliC

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S. Typhi (D)


Baliban, PloS NTD, 2017
Simon, Infect Immun, 2011
Mohan, Clin Infect Dis, 2015
Overview of study

**Immunogen**
- SE COPS:FliC (5 µg) +
- STm COPS:FliC (5 µg) +
- Typbar-TCV™ (5 µg)

**NZW**
- $n = 5 \text{ m/f}$

**Immunize**
- 0

**Bleed**
- 14

**IP Transfer**
- 28
- 70
- -4h

**Challenge**
- 6-8 wk CD-1
- $n = 18 \text{ f/group}$
- STm D65
- SE R11
Serum anti-polysaccharide IgG: Impact of conjugate valency in rabbits

Baliban, 2018
Serum anti-FliC IgG: Impact of conjugate valency in rabbits

S. Enteritidis FliC

S. Typhimurium FliC

IgG titer (EU/mL)

$P = 0.03$

S. Enteritidis COPS:FliC

S. Enteritidis COPS:FliC + S. Typhimurium COPS:FliC

S. Enteritidis COPS:FliC + S. Typhimurium COPS:FliC + Typbar-TCV™

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Trivalent-induced anti-OPS IgG is primarily serotype (serogroup)-specific.
Passive transfer of trivalent vaccine-induced sera protects mice against fatal iNTS challenge

- Mice (n = 18/group) transferred PBS, or diluted pre- or post-immune sera
- Challenged 4 hours later with virulent iNTS isolate (STm D65, SE R11)
Trivalent post-immune sera mediates robust opsonophagocytosis (OPA) of both *S. Typhimurium* and *S. Enteritidis*

- Heat-inactivated sera
- Viable CFU measured after incubation with J774 murine macrophages and cell lysis
Trivalent post-immune sera mediates strong complement-dependent serum bactericidal activity (SBA) against S. Typhimurium

- Heat-inactivated sera + baby rabbit complement
- Viable CFU measured after incubation

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Summary

• Immunization with the trivalent typhoid-iNTS conjugate formulation elicited robust IgG responses to all three polysaccharide antigens.

• Anti-COPS IgG antibodies directed primarily against serogroup-specific OPS epitopes.

• Post-vaccination rabbit sera mediated functional OPA (STm + SE) and SBA (STm) in vitro.

• Passive transfer of post-vaccination sera protected against challenge with virulent STm or SE Malian blood isolates (88-100% efficacy).
Partnership with Bharat Biotech

- UK Wellcome Trust Strategic Translational funding to UMSOM-CVD with Bharat Biotech (Hyderabad, India)
- Phase 1 & 2 clinical trials with trivalent S. Enteritidis COPS:FliC + S. Typhimurium COPS:FliC + Typbar-TCV™ formulation
Team & Funding

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Extra slides
Trivalent glycoconjugate vaccine formulation to prevent invasive *Salmonella* disease in sub-Saharan Africa

**S. Typhimurium (STm)**
- **Antigens:** STm COPS & FliC
- **Architecture:** Sun-type (end-link)
- **Chemistry:** thioether
- **Linkers:** GMBS (FliC lysines), aminooxy-thiol (COPS-KDO)
- **Linkage:** COPS-KDO -> FliC amines

**S. Enteritidis (SE)**
- **Antigens:** SE COPS & FliC
- **Architecture:** Lattice (multi-point linkage)
- **Chemistry:** CDAP cyanylation
- **Linkers:** Adipic acid dihydrazide (FliC carboxyls)
- **Linkage:** COPS hydroxyls -> FliC amines & carboxyls

**S. Typhi (ST, Typbar-TCV™)**
- **Antigens:** ST Vi CPS & TT
- **Architecture:** Bead-on-string
- **Chemistry:** carbodiimide
- **Linkers:** Adipic acid dihydrazide (Vi-TT carboxyls)
- **Linkage:** Vi-carboxyls -> TT carboxyls
Conjugation ablates TLR5 bioactivity in SE COPS:FliC conjugates

- HEK-293 cells stably expressing luciferase reporter under control of Nf-kB promoter
- Cells treated 4 hours, activation measured by luciferase assay
Functional activity of antibodies directed against S. Enteritidis flagellin

- Complement mediated killing
- Baby rabbit complement
- Polyclonal mouse anti-FliC vs. normal (NI) sera

Immunogold EM with anti-SE flagellin antisera & S. Enteritidis

Protective efficacy after passive transfer of monoclonal anti-FliC in mice against S. Typhimurim D65 challenge

Mice passively administered anti-SE or anti-STm FliC Mab, challenged IP with STm D65