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Typbar TCV™

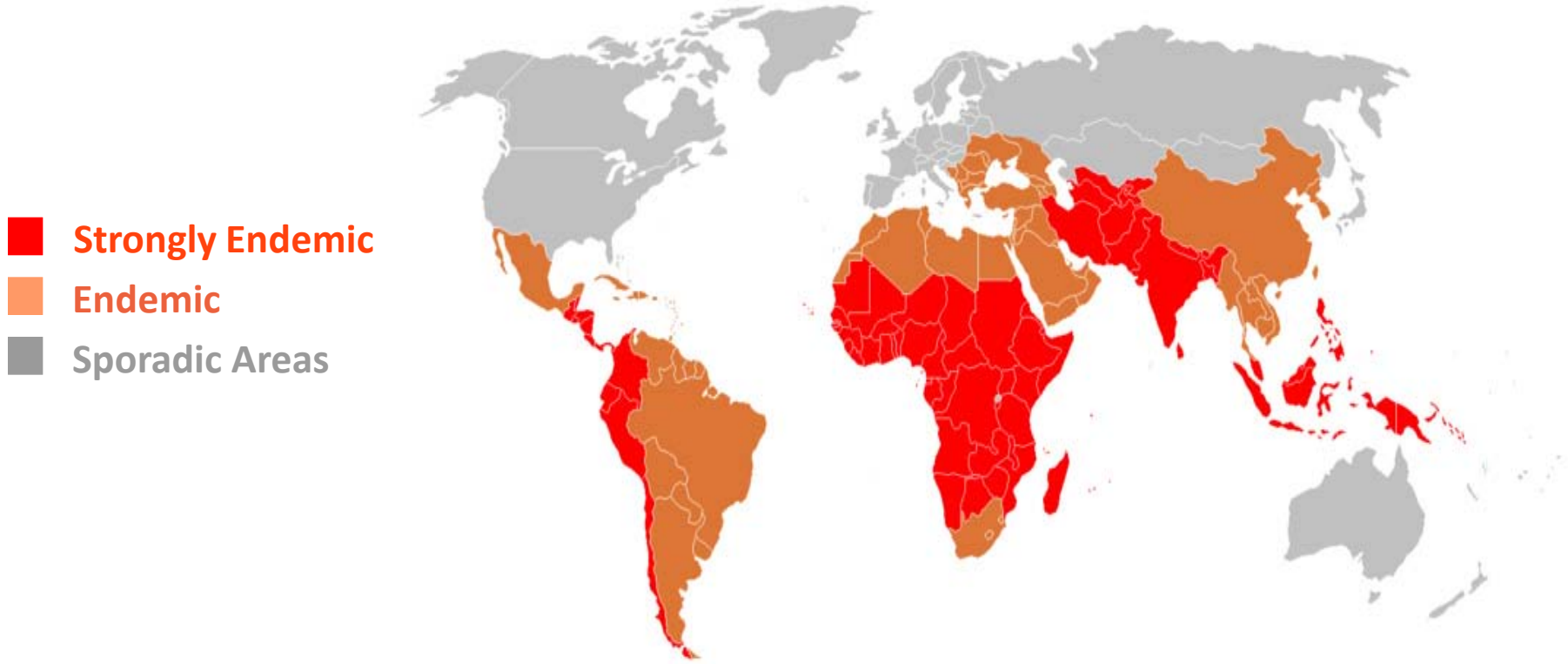
(Typhoid Vi Capsular Polysaccharide-Tetanus Toxoid Conjugate Vaccine)

New generation typhoid conjugate vaccine
for
preventing typhoid disease

TEAM BHARAT

Introduction

This disease is common in many developing countries of **Africa, Asia, The Middle East, The Caribbean, Central and South America.**



Different types of Typhoid vaccines

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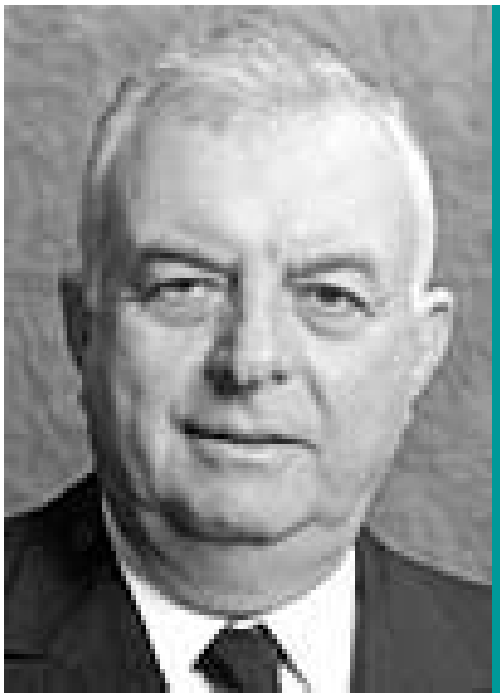
1st	Generation - Inactivated Whole – Cell Vaccine	
2nd	Generation - Cell Vaccine (Live Oral)	
2nd	Generation - Vi Polysaccharide Vaccine	**Typbar**
3rd	Generation - Vi Polysaccharide Conjugate Vaccine	**Typbar-TCV**

Typbar-TCV Development

- Production Vi-Capsular polysaccharide
- Characterization of Vi-polysaccharide bulk
- Conjugation processes evaluation
- Characterization of ViPs-TT Conjugates by NIBSC & BBIL
- Mouse immunogenicity testing by NIBSC & BBIL
- Non-Clinical Toxicity studies in laboratory animals
- Stability studies of ViPs-TT Conjugate vaccine
- Clinical trials Phase II & III
- Further on-going clinical studies

Typbar-TCV development at BBIL



Source for Typhoid Vi Polysaccharide:

Purified Typhoid Vi Polysaccharide Bulk from BBIL. The bacterial strain : *Salmonella typhi* Ty2 given to BBIL by

Dr. John Robbins, *NIH,USA*.
Source for Tetanus Toxoid: P.T. Biofarma (WHO Prequalified)

Used as Carrier Protein

Why conjugate vaccine

Polysaccharide Vaccine	Conjugate Vaccine
T-independent Immunogens.	T-cell dependent immune response to the saccharide.
Induce mainly IgM / IgG2 (in humans) of low avidity.	Affinity maturation, isotype switching and memory effects.
No immunological memory, isotype switching or affinity maturation.	Antibody response boosted by repeated immunization.
IgM levels drop rapidly: IgG down to 25% after 5 years.	IgG levels maintain for long time.
Are not effective in infants under two years.	Are effective in infants.
Immunogenicity depends on molecular weight.	Small glycan hapten can make effective vaccine

Conjugate Vaccines

- The covalent attachment of the polysaccharide to a protein carrier can create a conjugate molecule
- This conjugate molecules can convert the T-independent Polysaccharides (TI) to T-dependent (TD) by creating an enhanced immune responses
- Several highly immunogenic proteins have been proposed as the protein component, but mainly four have been used Diphtheria, Tetanus toxoid, CRM197, Outer Membrane protein, N. Meningitidis.
- NIH Scientists also used Recombinant Pseudomonas aeruginosa exotoxin A (rEPA) .

Characterization of Typbar-TCV

S. No.	Name of the Test	Specifications
1	Description	A clear colorless liquid free from visible particles
2	Identification	Clear precipitation arc should be observed
3	pH	6.5 - 7.5
4	O- acetyl content (Hestrin)	NLT 0.085µmol / 0.5 ml
5	Conjugate Vi Content	NLT 25µg / 0.5 ml
6	Free Vi Ps	NMT 20 %
7	Sterility	Should comply the test
8.	Pyrogen Test	Should comply the test
9.	Abnormal Toxicity test	Should comply the test
10.	Potency test	NLT of 50 % of test animals should sero conversion

VIPs–TT Conjugates Analysis Report: of NIBSC – 1

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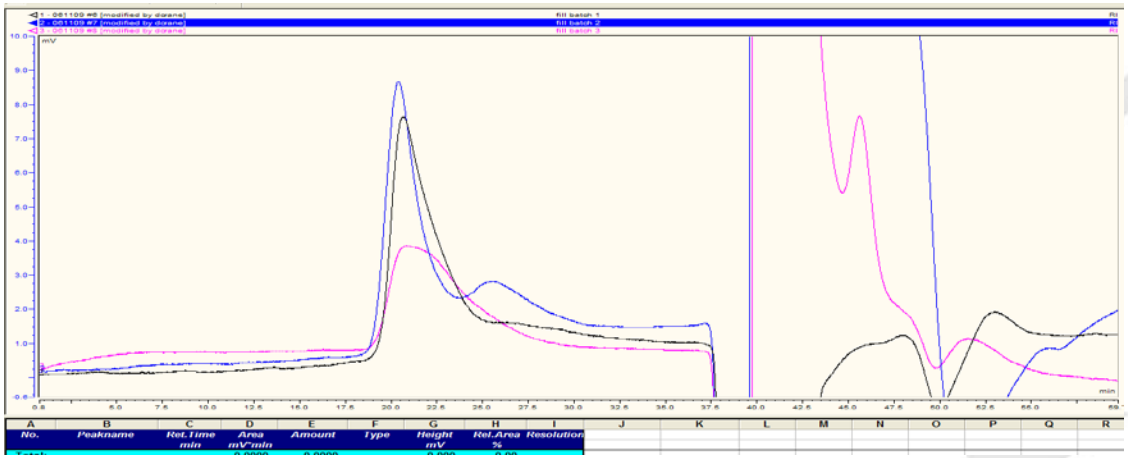
1. Physico-Chemical Studies:

a) LAL Assay

Endotoxin content of final formulated vaccine batches ranged from 300-1200 IU/mL, i.e., below EP requirements for ViPs (less than 3750 IU per dose).

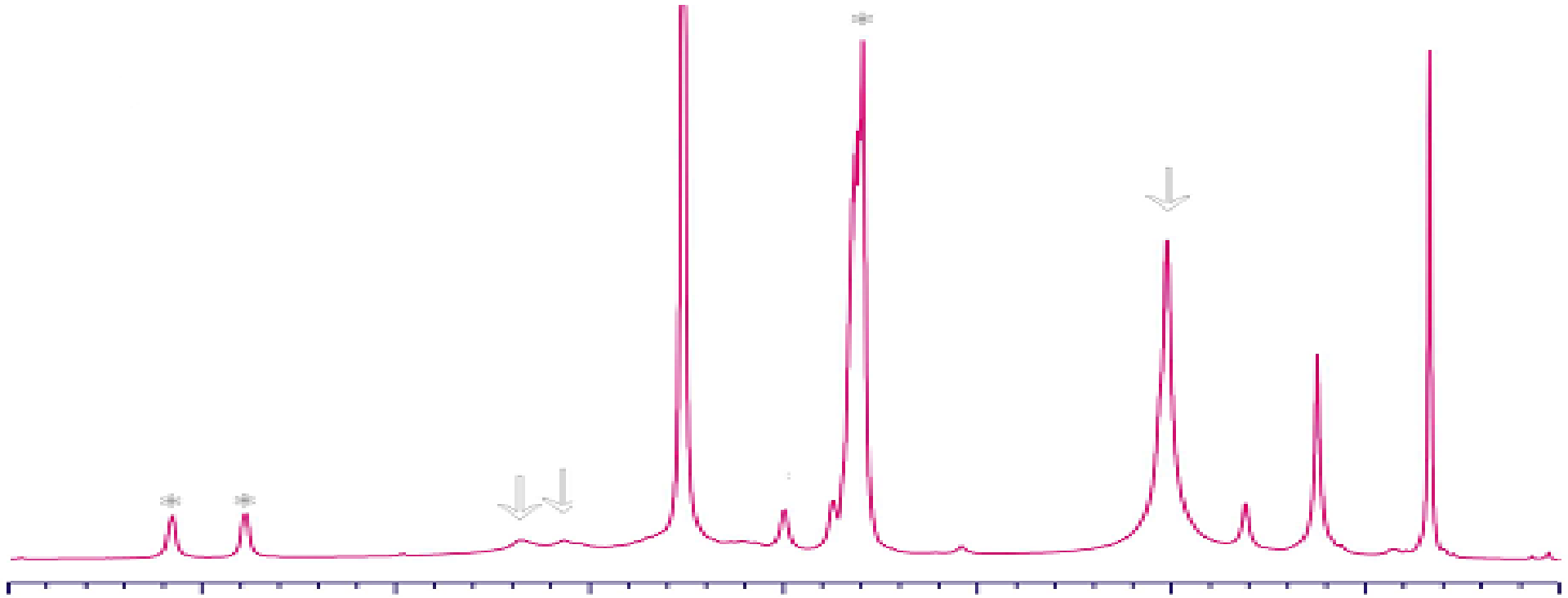
b) Size Exclusion Chromatography

The final Conjugated high molecular weight molecules in formulated material were identified as that of ViPs–TT conjugates.



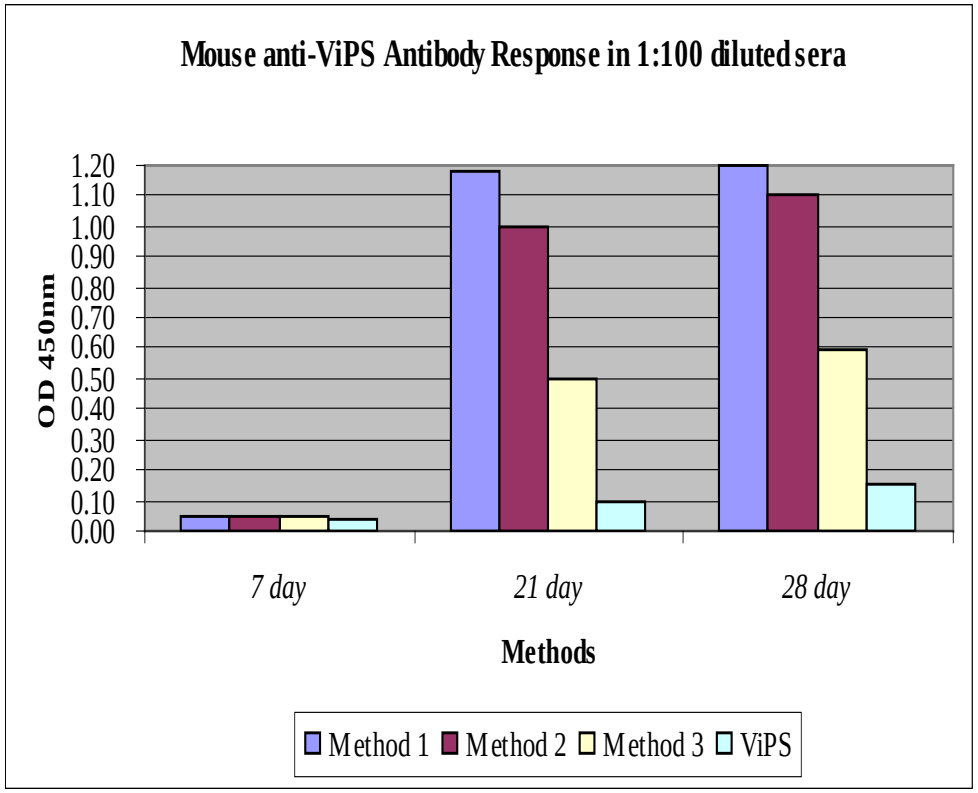
ViPs–TT Conjugates Analysis: Report of NIBSC – 2

c) NMR: The NMR spectrum of the ViPs revealed good levels of *O*-acetylation



Immunogenicity studies in Mice

3 different Conjugation methods attempted



The anti-ViPs antibody response of the conjugate polysaccharide vaccine (Method 1) is showing a good response when compared with the plain polysaccharide vaccine and other conjugate methods.

Pre Clinical Toxicity Studies

Mice and Rabbits were used in these studies. Studies done as per Schedule Y guidelines.

1. Single-Dose Toxicity of Typhoid Vi Polysaccharide – TT Conjugate Vaccine in Mice and Rabbits

No premature deaths and no clinical observations of acute toxicity seen

Body weight gain was normal and satisfactory.

No abnormalities were detected.

2. 28 Day Repeated Dose Toxicity Study of Typhoid Vi Polysaccharide – TT Conjugate Vaccine in Mice and Rabbits

No treatment related effects on Mortality, Clinical observations, Body weights, Food consumption, Water consumption, Coagulation, Hematology or Clinical chemistry analysis of both Mice and Rabbits.

Typbar-TCV development

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Special Thanks to

Dr. Szu

for providing the Conjugate Reference Standards for analysis

used for Phase II clinical trial samples

Clinical Trial Phase-IIa/IIb

Open label active controlled Phase IIa / IIb study to evaluate the safety and immunogenicity of BBIL's Typhoid VIPs – TT Conjugate Vaccine *Vs* Reference Typhoid Vi Capsular Polysaccharide Vaccine in healthy teenagers (13–17 yrs) and children (2–12 yrs old).

- **Sample size:** Number of subjects enrolled: **100,** Number of subjects completed study and analyzed: **95** Number of subjects dropped out: **5**

Clinical Trial Phase-IIa/IIb

- Single & two dose of 25µg/0.5mL and two doses of 15µg/0.5mL were tested in Phase IIa/IIb study.
- Single dose of 25µg/0.5mL Typhoid-TT conjugate vaccine showed excellent immune response (100% Seroconversion).
- Based on several considerations, Bharat Biotech carried out a large diverse, multi-centric Phase III Clinical Trial with 25mcg/0.5mL as single dose.

Phase-III Clinical Trial

A Phase III, randomized, multicentric, controlled study to evaluate the immunogenicity and safety of BBIL's Typhoid Vi Capsular Polysaccharide Tetanus Toxoid Protein Conjugate Vaccine (Typbar – TCV) vs. Reference Vaccine (Typbar) in healthy subjects.

Study Rationale

- The comparator vaccine is a polysaccharide vaccine which has T-cell independent nature of immune response and is not effective below 2 years and hence not approved for use in this age group.

- Also we examined other vaccines which could be used as comparator vaccine in below 2 years age group.

- The other vaccines which could be given in the control arm of this group would be different in the various stratified age range based on the Expanded Program on Immunization (EPI)/Indian Academy of Pediatrics (IAP) vaccine schedule leading to complexity of the study design and also statistical analysis.

Cont..

- As per the EPI schedule recommendations the Measles, Mumps and Rubella should be administered at 9 to 12 months of age.

- Recommended age for Hepatitis A vaccine administration is 12 to 23 months. Considering all these issues this group (Cohort-1) would be single arm, an open labeled study.

Study Design & Study Population

Study Design: randomized, multicentric, controlled Phase III study

Study Population: The healthy subjects divided in to two groups of ≥6 months to ≤2 years, and >2 years to ≤45 years

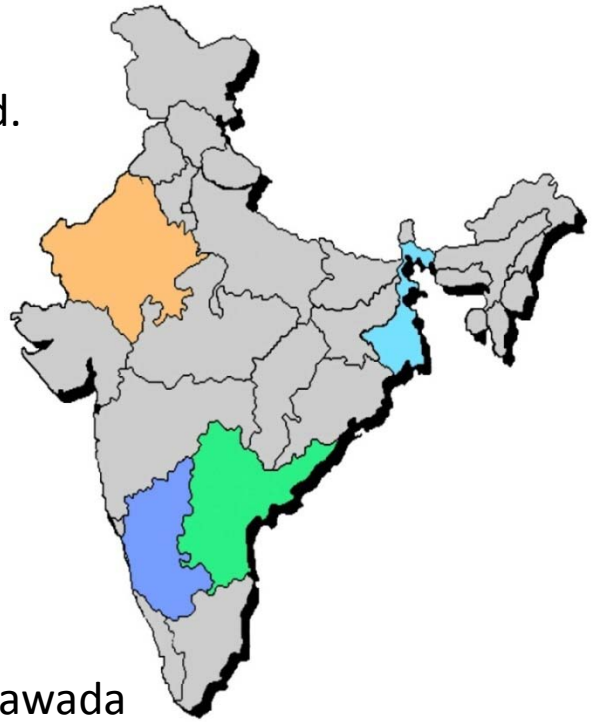
Randomization and labeling done by third party

A total of 981 subjects allocated at 2:1 ratio to test vaccine and reference vaccine across all the centers.

Cohort	Age group	Treatment Groups		Enrolled	Completed	Drop outs
		Test	Ref			
1	≥6 months to ≤2 years (Single Arm – Open label)	327	-	327	307	20
2	>2 years to ≤45 years (Two arm – Double blind)	340	314	654	637	17

Study investigators and sites

- **Dr. Monjori Mitra**, Institute of Child Health, Kolkata
- **Dr. G. Sampath**, Institute of Preventive Medicine, Hyderabad.
- **Dr. P. Venugopal**, King George Hospital, Visakhapatnam.
- **Dr. Mukesh Gupta**, Soumya Child Clinic, Jaipur
- **Dr. Sudhakar**, Priya Children's Hospital, Vijayawada
- **Dr. S.N. Mahantashetti**, JNMC-WMKS, Belgaum
- **Dr. Sri Krishna**, Mahavir Hospital, Hyderabad
- **Dr. Bhuvaneswar Rao**, Sri Srinivasa Children's Hospital, Vijayawada



Dose, Dosage and ROA

Test Vaccine:

- Typbar-TCV vaccine
- 25mcg/0.5 ml single dose by IM Route.

Reference Vaccine:

- A commercially approved Typhoid Vi capsular polysaccharide vaccine (TYPBAR) for children > 2 years..
- 25mcg/0.5 ml single dose by IM Route.

Sera Analysis : Different, though validated, ELISA systems used; steps being taken to “use” one technique for all sera samples.

End Points

Primary end points:

The comparative assessment of the immunogenicity of the test vaccine versus reference by estimation of:

- 4-fold Seroconversion rates in each treatment group at 6 weeks post-vaccination.

Secondary Endpoints:

The clinical safety evaluation

- Comparative assessment of safety and tolerability of the vaccine in all subjects up to 12 weeks post vaccination.
- Based on the Geometric Mean Titers of the primary objectives 1 & 2 the superiority of test over reference vaccine.

Safety results

Cohort - I

Events Occurred	AEs (%)
Fever	10.0
Pain at Injection site	3.7
Redness at injection site	0.3
Vomiting	0.3
Cough	0.6
Cold	0.6
Itching	0.3
Lower respiratory tract Infection	0.3
Diarrhoea	0.3
Malaise	0.3
Myalgia	0.3

Safety results

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Cohort - II

Events Occurred	Test Group AEs %	Reference Group AEs %	p-value
Fever	4.28	2.75	0.5245
Arthralgia	0.3	0.3	1.0000
Itching	0.3	0	
Tenderness	0.61	0	
Nausea	0	0.6	
Weakness	0	0.3	
Cold	0	0.3	
Myalgia	0	0.3	
Pain at Injection site	3.6	2.6	0.5057
Swelling	1.53	0.3	0.2194

Immunogenicity Results – Cohort I

Serum samples of Day 0 & 42 were tested by validated Enzyme Immunoassay Kit.

Day	Group	GMT (EU/mL) (CI)	Seroconversion (%) (>4 fold)
Day 0	Test vaccine	9.44 (8.66,10.31)	98.05%
Day 42		1952.03 (1795.48, 2122.23)	

Immunogenicity Results - summary

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Serum samples of Day 0 & 42 were tested by validated method of Enzyme Immunoassay by an independent accredited Laboratory.

Cohort	Response	Test N= 639	Reference N=305	p- Value
I	SC (4-Fold Rise) Day 0 - 42	98.05%	NA	NA
II		97.29%	93.11%	0.01

Immunogenicity Results

Batch Consistency :

Batch consistency established by using three different batches from Single dose (0.5mL) and Multi dose (2.5mL) vial presentations in the clinical trial. (Data on file)

Literature review

- *The effect of immunogenicity upon the selective dosage of Vi conjugates witnessed that 25 microgram/dose is efficient in raising high levels anti-Vi IgG antibodies in 2-5 year children(Lin & Szu etal.,2004. Infection and Immunity)*
- *NIH estimated the protective level of Vi antibody is 7 Elisa unit/mL (Lin and Szu et al., 2001 NEJM)*
- *NIH estimated 3.52 Elisa unit/mL (Lin & Szu etal.,2004.Infection and Immunity)*
- *The Vi conjugate prepared using ADH as a linker proven to raise efficient immunogenicity with administration of a single dose containing 25 microgram/dose., Kossaczka & Szu etal., 1999. (Infection and immunity)*

Typbar-TCV development

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Special Thanks to

Dr. Szu

for providing the Conjugate Reference Standard for Phase III clinical trial sera samples analysis (under progress).

Discussion & Conclusion

- ✓ The Typbar-TCV vaccine is safe and more effective than Typbar vaccine.
- ✓ There is statistically significant difference between Typbar-TCV vaccine and Typbar vaccine in terms of GMT and four fold seroconversion ($p<0.05$).
- ✓ Typbar-TCV has found to induce high Ab titres in children less than 2 years age.
- ✓ The Superiority of Typbar-TCV vaccine over Typbar vaccine with respect to GMT post vaccination in both the groups with respect to estimated GMT of Typbar-TCV vaccine is ~ 3 - 4 times larger than the Typbar vaccine GMT.

Future Studies

- ✓ **3- year follow-up underway for seroconversion data of Phase III subjects (18 months data would soon be available).**
- ✓ **Measles Interference Study with subjects at 9 months in preparatory phase.**
- ✓ **Post licensure : plan to evaluate safety in around 3000 subjects.**
- ✓ **Study the effect of a booster dose on antibody titres.**

Thank You