Live oral vaccine approaches to typhoid and paratyphoid vaccines

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An ideal vaccine to prevent enteric fever

- Single dose
- Oral
- Protection against *S*. Typhi & *S*. Paratyphi A & B
- Well tolerated and efficacious for all ages, including young infants
- Well tolerated and efficacious in immunocompromised subjects
- Early onset and long duration of protection (at least 7 years)
- “Easy” to manufacture
- “Affordable” (inexpensive cost of goods)

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Why oral?

- Logistically more practical
- Avoids problems of injection safety
- Greater compliance (oral generally preferred)
- Some precedents & encouragements:
  - Trivalent OPV (TOPV) eliminated polio in Latin America and E. Asia
  - TOPV eradicated type 2 poliovirus disease globally
  - Success of bivalent OPV types 1 & 3 vaccine
  - Good record of Ty21a live oral typhoid vaccine
  - Roll out of new oral cholera vaccine
  - Additional option for preventing enteric fever

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Etiologic agents of enteric fever

Salmonella Typhi - typhoid fever

Salmonella Paratyphi A & B - paratyphoid fever

- Similar or identical
  - Clinical syndromes
  - Transmission
  - Pathogenesis
- High degree of homology at the genomic level

- *S.* Paratyphi A & B do not express Vi CPS

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Live oral vaccines to prevent enteric fever

- Lessons learned with Ty21a, the pioneer live oral typhoid vaccine
- “New” single-dose live oral typhoid vaccines
- Live oral typhoid vaccines elicit robust mucosal & cell-mediated immune responses in humans
- A prototype live oral paratyphoid A vaccine is in clinical development

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Salmonella Typhi vaccine strain TY21a

- Derived by chemical mutagenesis of Ty2 & selection
- Vi-negative (R Germanier & E Furer, J Infect Dis 1975)
- Diminished activity of *galE* gene product
  - UDP-4-gal epimerase -- 0%
- **Multiple (~26) other mutations**

Ty21a live oral typhoid vaccine licensed by many national regulatory agencies (including the FDA) in the 1980s

- **Ty21a live oral strain**
  - 3 doses (most countries) or 4 doses (USA & Canada)

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Six controlled field trials of Ty21a live oral typhoid vaccine

	N	Follow-up
Alexandria, Egypt	32,388	3 years
Area Norte, Santiago, Chile	82,543	4 years
Area Occidente, Santiago, Chile	109,594	7 years
Area Sur, Santiago, Chile	216,692	3 years
Area Suroriente, Santiago, Chile	81,621	5 years
Plaju, Indonesia	20,543	3 years

Wahdan et al 1982; Black et al 1990; Levine et al 1987, 1990, 1999; Ferreccio et al 1989, Simanjuntak et al 1991

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Field trial of Ty21a live oral typhoid vaccine in Santiago, Chile

The trials:

- 4 large-scale, randomized controlled field trials
- ~ 490,450 schoolchildren 5-19 years of age
- Followed 3-7 years

The partners:

- Ministry of Health, Chile
- Center for Vaccine Development, U. Maryland
- WHO
- U.S. Department of Defense
- Pan American Health Organization

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Lessons learned with Ty21a

- Attenuated strain can serve as a safe, well tolerated, effective, practical oral vaccine
- Efficacy influenced by:
  - Formulation (liquid > enteric > gelatin/$NaHCO_3$)
  - Number of doses (4 > 3 > 2 > 1)
- Protection mediated by immune responses other than serum Vi antibody (other Abs, **CMI**)
- Confers **long-term protection** (at least 7 years)
- Practicality of school-based immunization
- Evidence of herd immunity
- Evidence of protection (49% VE) against Paratyphi B (Chile) but not A (Indonesia)

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Long-term protection from Ty21a live oral typhoid vaccine

Occidente trial	Ty21a	Plbo	Efficacy
Years 1-3	N=22,170*	N=21,906	
Inc./10^5	104	310	67% (47-97%)$^+$
Years 1-7			
Inc./10^5	226	598	62% (48-73%)
Suroriente trial			
Years 1-3	N=36,623**	N=10,602	
Inc./10^5	63	272	77% (60-87%)
Years 1-5			
Inc./10^5	93	417	78% (65-86%)

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* 3 doses of enteric-coated formulation every other day $^+$ (95% CI)

** 3 doses of "liquid" formulation every other day (Levine et al 1987, 1990 & 1999)

LARGE-SCALE EFFECTIVENESS TRIAL OF Ty21a

Area Sur effectiveness: comparison of 2 vs 3 vs 4 doses of Ty21a in enteric-coated capsules

	Number of Doses per Child		
	2	3	4
Per protocol			
No. children	66,615	64,783	58,421
TF Incid./10^5	194[a]	161[b]	98[c]
Intent to treat			
No. children	71,754	77,246	76,998
TF Incid./10^5	208[d]	185[e]	126[f]

[a] vs [b], p = NS; [a] vs [c], p = < .0001; [b] vs [c], p = < .002;
[d] vs [f], p = < .0002; [e] vs [f], p = < .004 (Ferreccio, et al JID, 1989)

Nested case/control study to assess vaccine efficacy, Area Sur & Area Central

Doses	Cases	Ctrls	Odds Ratio	Vaccine Efficacy	p Value
0	38	164	1.0	-	-
1	8	46	0.72	**28%**	0.45
2	87	655	0.56	**44%**	0.008
3	81	692	0.49	**51%**	0.0013
4	28	379	0.31	**69%**	<0.0001

4 neighborhood controls and 4 health center/hospital controls matched for each case of typhoid fever

(Ferreccio et al Am J Epi 1990)

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Placebo-controlled study of 1 vs 2 doses of Ty21a in enteric-coated capsules

AREA NORTE	Year 1	Year 2	Year 3
2 doses (N=27,620)			
TF Incid./10^5	109[a]	40[c]	54
Vaccine Efficacy	52%	71%	22%
One dose (N=27,618)			
TF Incid./10^5	170	91	69
Vaccine Efficacy	25%	35%	0%
Placebo (N=27,305)			
TF Incid./10^5	227[b]	139[d]	70

[a] vs [b], p = 0.001; [c] vs [d], p = < 0.001
(Black et al. Vaccine 1990)

Protection of Ty21a against *S.* Paratyphi B

	Ty21a	Plbo	Efficacy
Years 1-3	N=49,790	N=49,211	
Cases	18	35	
Inc./10^5	36.2 [a]	71.1 [b]	49% (CI, 8 to 73%)

a vs b, p = 0.019

Field trial data from Area Norte and Area Occidente
(Levine et al 1987, Black et al 1990, Levine 2007)

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Single-dose live oral typhoid vaccines under development

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New putative single-dose live oral typhoid vaccines

Vaccine	Mutations	Status
M01ZH09	*aroC,ssaV*	Phase 2 Hindle 2002, Kirkpatrick 2004
CVD 909	*aroC,aroD,htrA,*P_{tac}*-tviA*	Phase 2 Tacket 2004, Wahid 2010

- Well tolerated
- Both much more immunogenic than Ty21a
- CVD 909 constitutively expresses Vi (Wang et al 2000)
- Only CVD 909 stimulates mucosal IgA ASC responses to Vi (Tacket 2004) & B cell memory to Vi (Wahid 2010)
- M01ZH09 was tested in children age 5-14 yrs in Vietnam
- M01ZH09 has been tested in a volunteer challenge study
- Neither live oral vaccine has been evaluated in a large-scale, randomized, controlled Phase 3 field trial of efficacy

Phase 1 trial of CVD 909

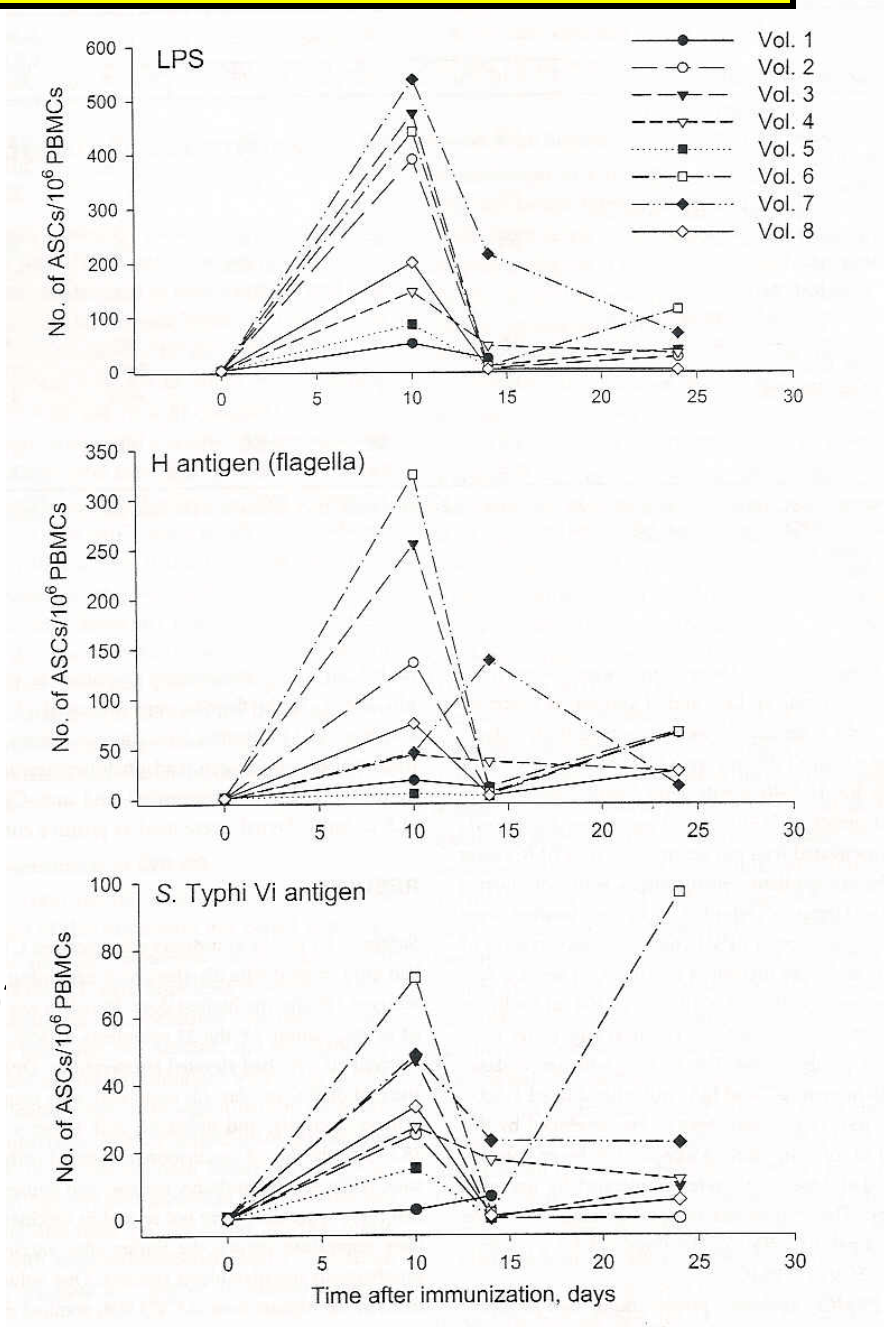
- 24 healthy adult Marylanders got 1 dose of 10^6, 10^7, 10^8 or 10^9 CFU, in step-wise fashion
- 8 got 2 doses of 10^9 CFU, 14 days apart
- Well tolerated
- Increased duration of excretion
- IgA ASC responses following 1dose:

	O	H	Vi
1-dose 10^{6-7}	11/12	3/12	3/12
1-dose 10^{8-9}	18/20*	16/20*	16/20

- Serum IgG Vi antibody in only 2 of 36

* Includes data from 1st dose of 2-dose group

Tacket et al, JID 2004



Live oral typhoid vaccines elicit powerful CMI responses

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Key effector cell-mediated immunity to *S.* Typhi in subjects given live oral vaccines

- **Proliferation** and predominant **type-1 cytokine responses** to soluble *S.* Typhi antigens
- **Effector responses** to *S.* Typhi-infected targets:
  - Cytotoxic T lymphocytes (CTL) activity
  - IFNγ production (TNF-α, others)
  - Mediated by both $CD8^+$ (dominant) and $CD4^+$ cells
- **Homing to mucosal and non-mucosal tissues**
- Long-term T & B memory cell responses
- **Long-term multifunctional** $CD8^+$ cells

Marcelo Sztein

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CVD 1902

A live oral vaccine candidate to prevent S. Paratyphi A disease

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Candidate live oral *Salmonella* Paratyphi A vaccine strain CVD 1902

- 2 independently attenuating deletion mutations:
  - *guaBA* - impedes guanine nucleotide biosynthesis (J Wang et al Infect Immun 2001)
  - *clpX* - encodes a multifunctional chaperone ATPase with regulatory properties (H Matsui et al 2003)
- Pre-clinical animal model data completed
  - Safety, immunogenicity, efficacy
- IND approved by FDA
- Phase 1 dose-ranging study to assess safety, preliminary immunogenicity & transmissibility completed

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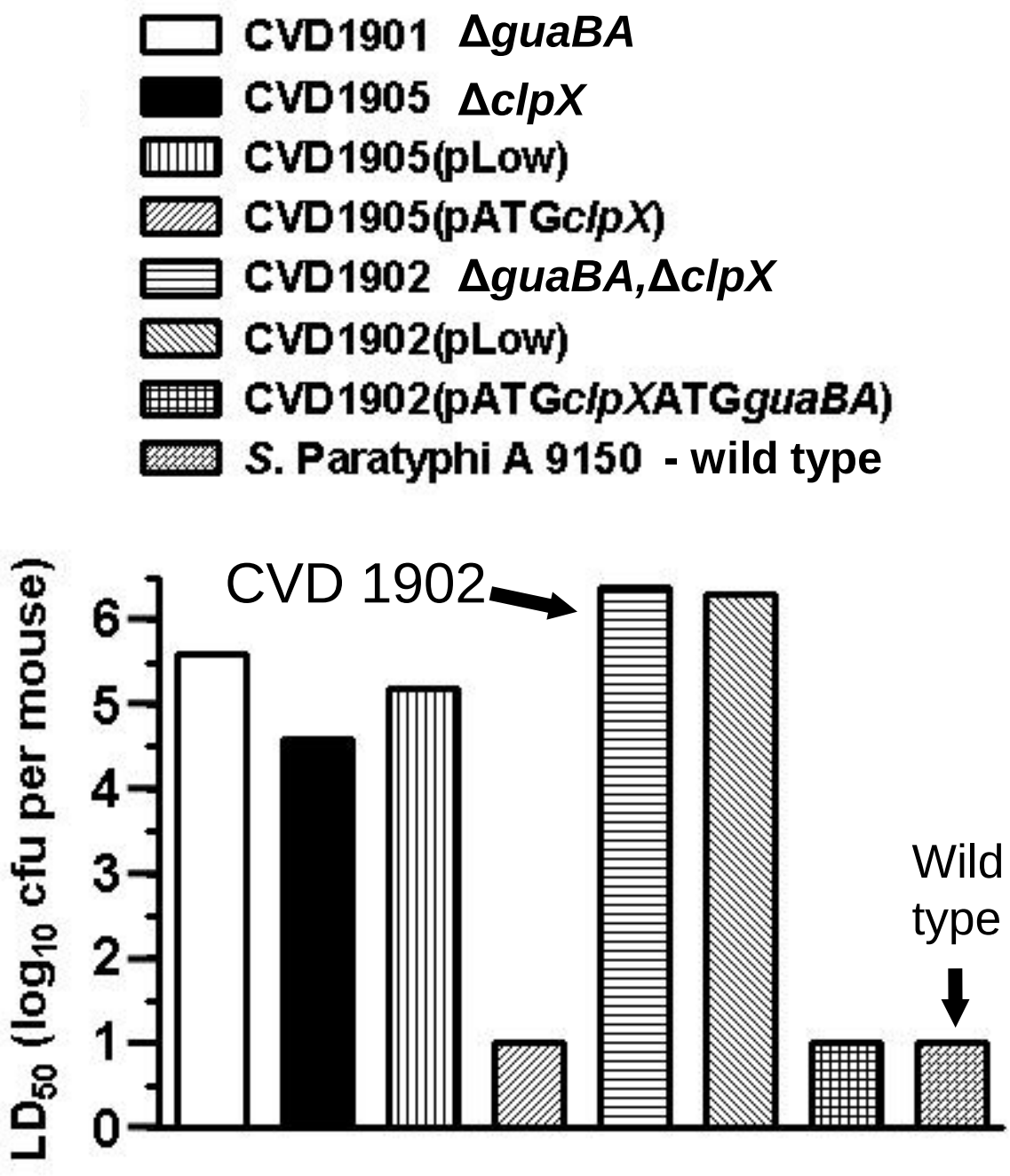

LD_{50} determinations (i.p. challenge with hog gastric mucin) of *Salmonella* Paratyphi A strains, and complementation

C Vindurampulle, EM Barry, JE Galen, MM Levine

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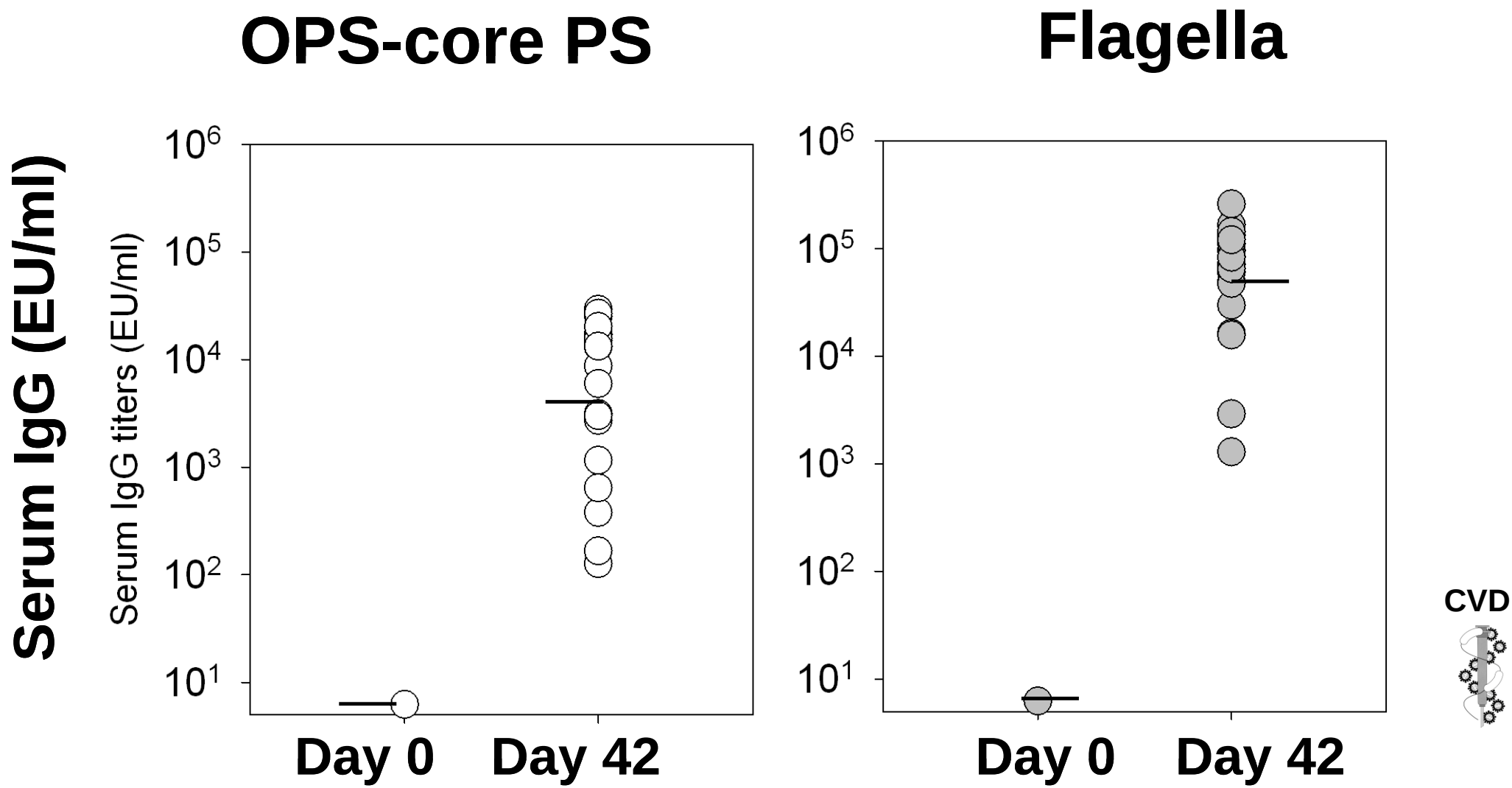
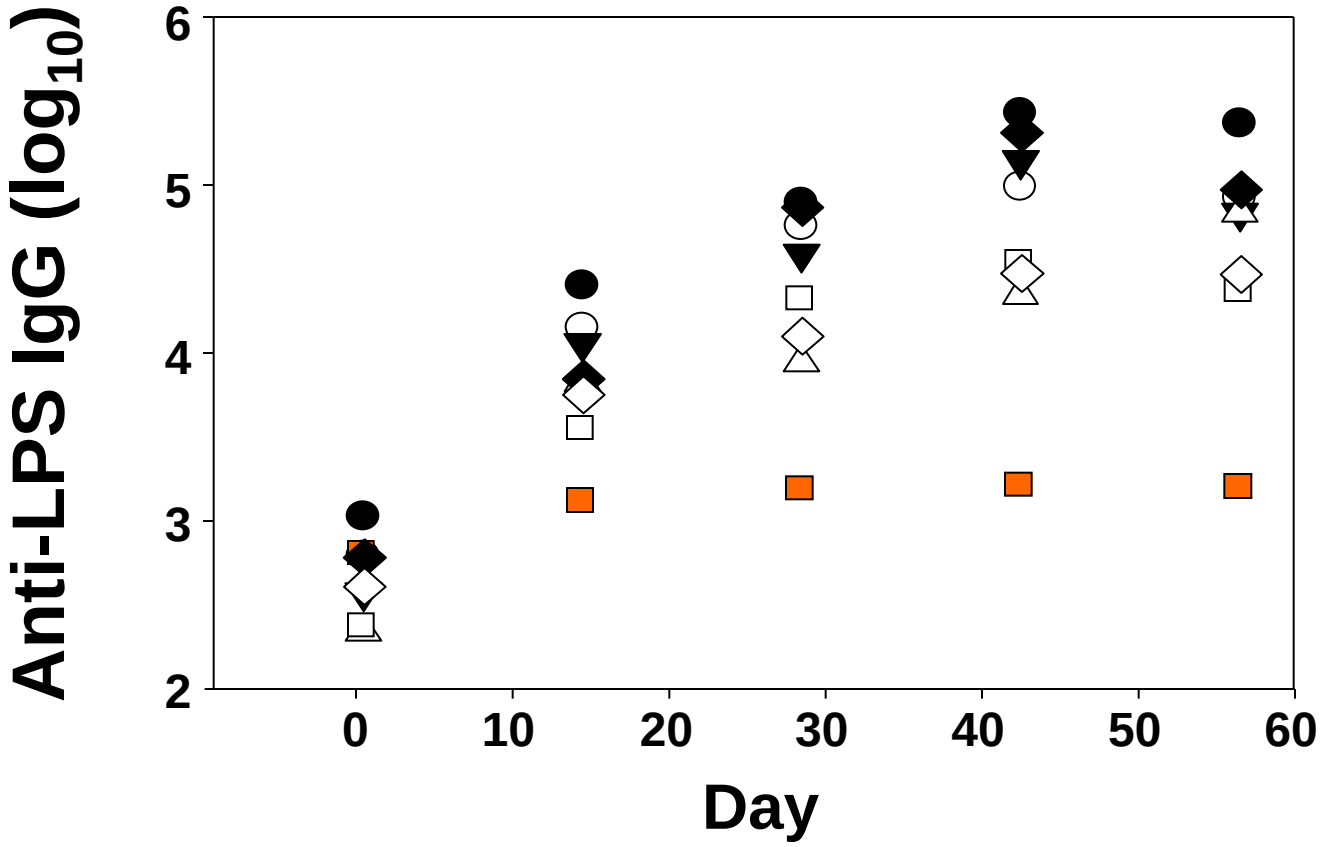
Serum antibody response to purified *S.* Paratyphi A LPS and H:a flagella following i.n. immunization of Balb/c mice on days 0, 21 & 28 with CVD 1902



Efficacy of CVD 1902 in preventing mortality in mucosally immunized mice (days 0, 14 & 28) vs controls challenged i.p. (day 49) with $3x10^5$ CFU of wild type *S. Paratyphi A* in hog gastric mucin

Group	Mortality Rate	Vaccine Efficacy	p value
PBS controls	8/8	-	-
CVD 1902	4/10	60%	p=0.013

Serum IgG O antibodies in rabbits immunized orally on days 0, 14 & 28 with 10^{10} CFU of *Salmonella* Paratyphi A live vaccine CVD 1902



PBS; n=1;

CVD 1902; n=7; open and **black** symbols

J Galen et al

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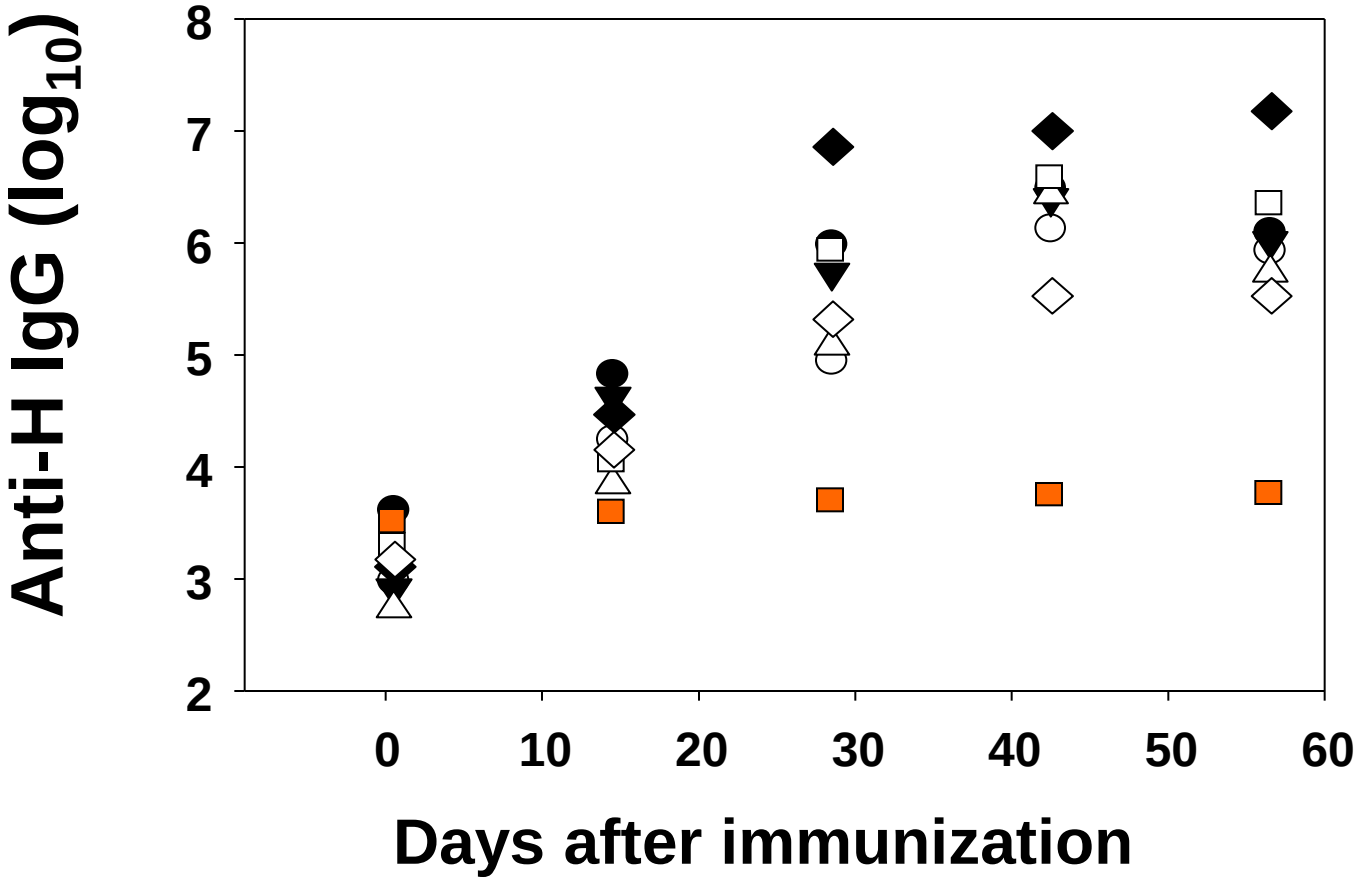
Serum IgG H antibodies in rabbits immunized orally on days 0, 14 & 28 with 10^{10} CFU of *Salmonella* Paratyphi A live vaccine CVD 1902



PBS; n=1;

CVD 1902; n=7; open and **black** symbols

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Phase 1 clinical trial of the safety and & immunogenicity of CVD 1902 (K Kotloff 2012)

Cohort	Vaccine Inoculum Size	No. of subjects	
		CVD 1902 vaccine	Placebo
√ 1	10^6 CFU	6	2
√ 2	10^7 CFU	6	2
√ 3	10^8 CFU	6	2
√ 4	10^9 CFU	6	2
√ 5	10^{10} CFU	6	2

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ASC responses (still blinded)

Cohort 4 (10^9 CFU; 2 placebo & 6 vaccinees)

IgA anti-O ASC responders: 4/8 total

IgA anti-H ASC responders: 5/8 total

IFN-gamma production by PBMC stimulated with:

S. Paratyphi A homogenate: 4/8 total

H:a flagellin: 5/8 total

Cohort 5 (10^{10} CFU; 2 placebo & 6 vaccinees)

IgA anti-O ASC responders: 4/8 total

IgA anti-H ASC responders: 4/8 total

IFN-gamma production by PBMC stimulated with:

S. Paratyphi A homogenate: 4/8 total

H:a flagellin: 5/8 total

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Conclusions & next step

- A single dose of CVD 1902 was well tolerated and immunogenic in Phase 1 clinical evaluation

Next steps:

- Future cohorts to receive ~ 5 x 10^9 CFU
- Compare 2 spaced doses vs single dose
- Bharat Biotech will make Phase 2 GMP pilot lots
- Phase 2 trial to assess safety & immunogenicity of CVD 909/CVD 1902 combination vaccine versus each monovalent alone versus placebo
- Studies in endemic populations

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Bivalent oral vaccine to prevent enteric fever caused by *Salmonella* Typhi, *S.* Paratyphi A and *S.* Paratyphi B

- CVD 909
  - to protect against *S.* Typhi and *S.* Paratyphi B disease
- CVD 1902
  - to protect against *S.* Paratyphi A disease

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