Design, production and pre-clinical evaluation of a novel toxin-based vaccine for the prevention of *Clostridium difficile* disease

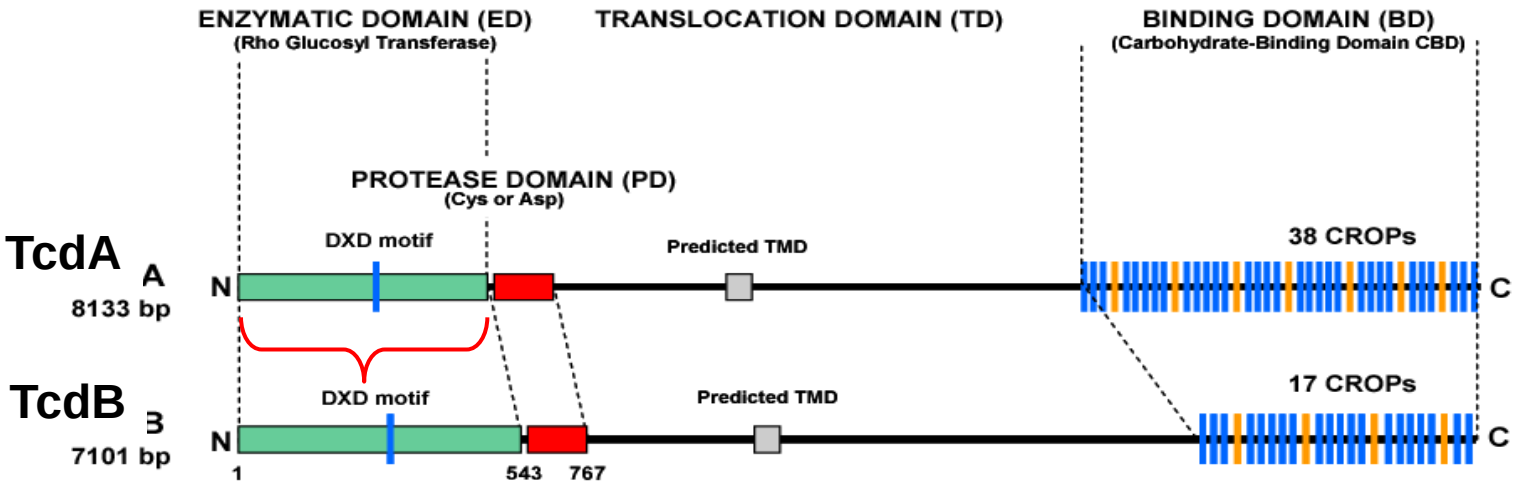
Jon H. Heinrichs, Su Wang, Matt Miezeiewski, Susan Secore, Andy Xie, Julie Zorman, Rachel Xoconostle, Irene Rogers, David Thiriot, Marie-Pierre Gentile, Adam Kristopeit and Aaron R. Goerke

Merck Research Labs, West Point, PA

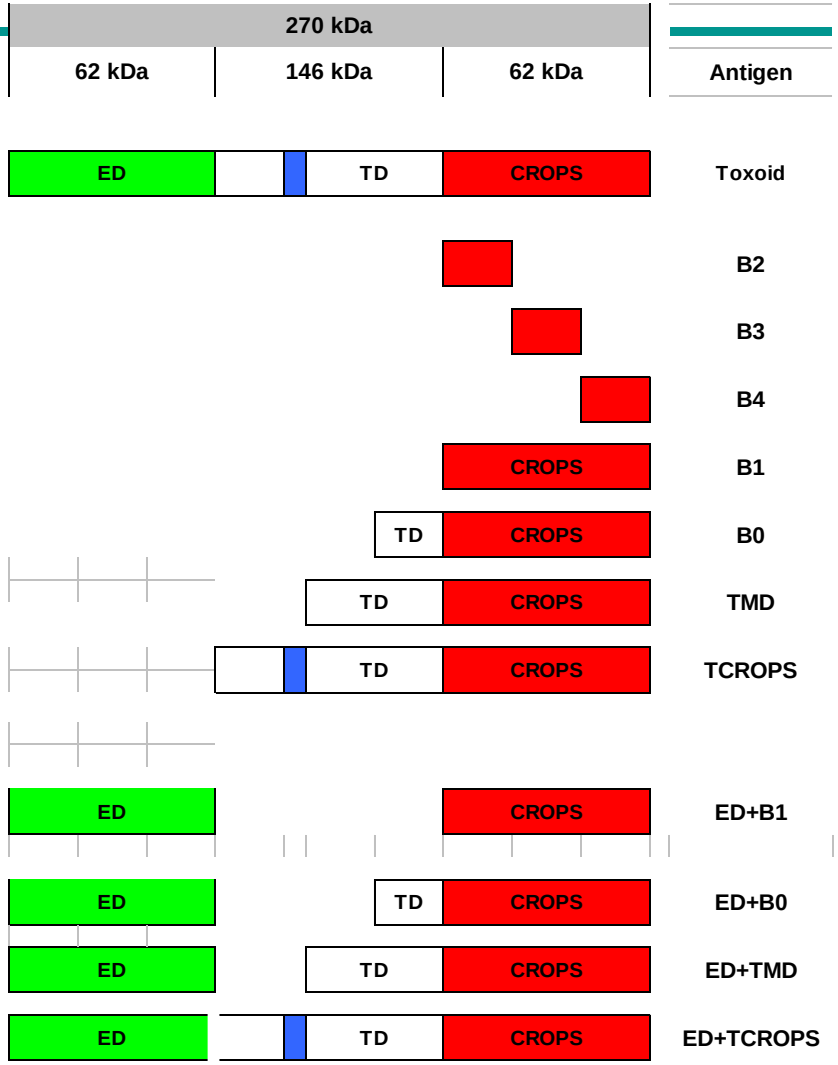
Outline of talk

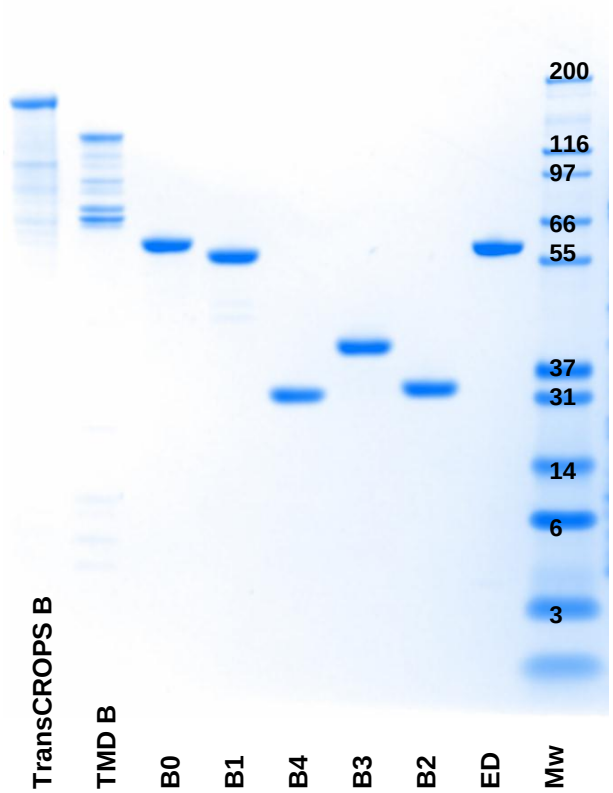
- **Evaluation of recombinant fragment vaccines**
- **Development of rapid, semi-automated assays for evaluation of neutralizing antibody responses**
- **Correlation of neutralizing antibody responses and protection from lethal challenge in hamsters**
- **Identification of importance of binary toxin in pathogenicity and protection against NAP1 strains in animal models**
- **Recombinant production of large clostridial toxins and binary toxin in various host species**
- **Characterization of microbiome changes in hamsters following antimicrobial treatment**

Structure of *C. difficile* large-clostridial toxins



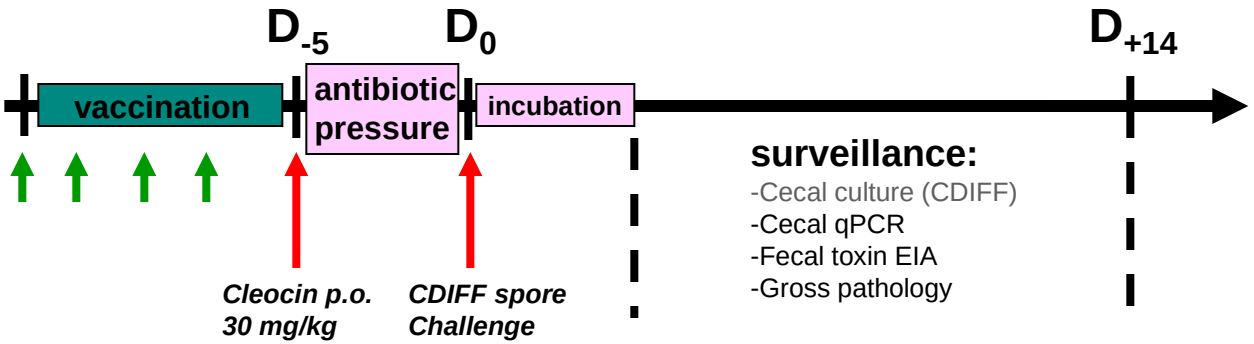
Design of recombinant large-clostridial-toxin fragments





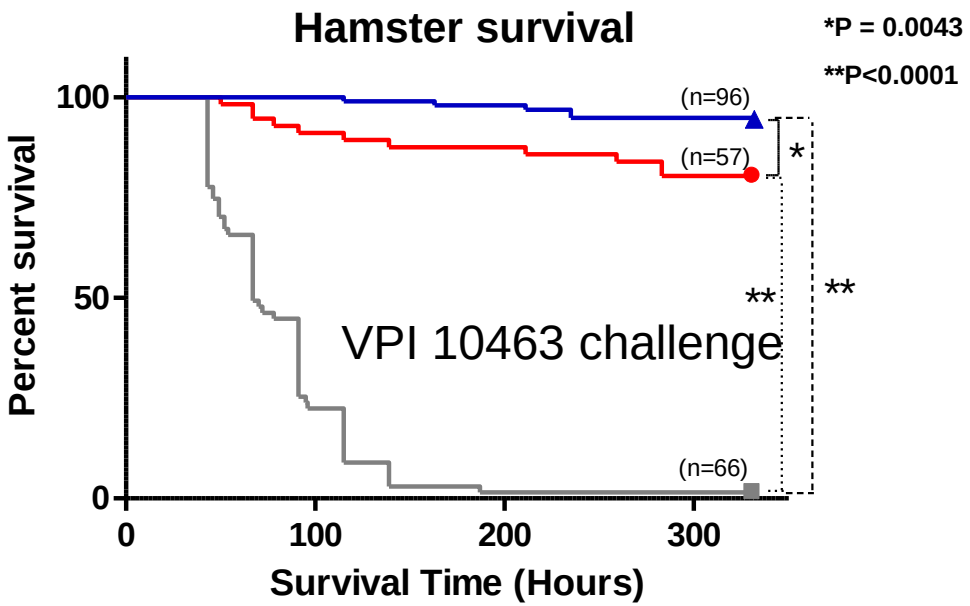
All antigens were purified from *E. coli* using Ni-affinity followed by anion exchange chromatography

Clindamycin-induced colitis in Syrian hamsters, a lethal challenge model of CDI

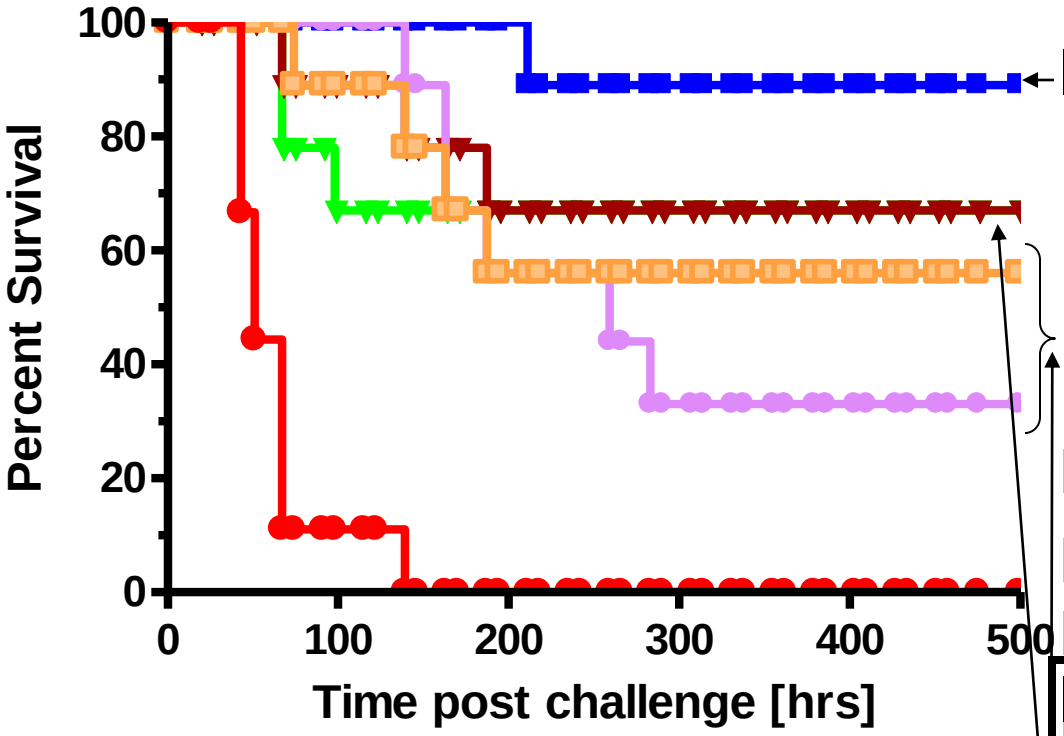


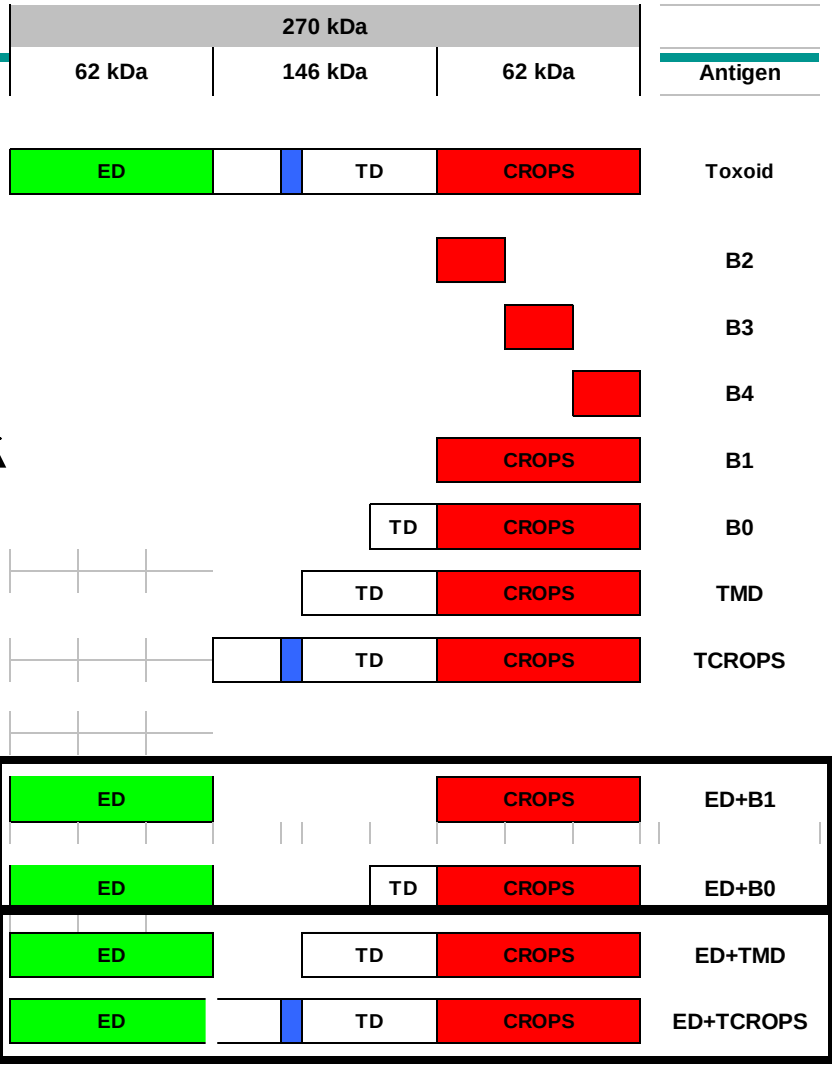
control

C. difficile - infected



Protection of hamsters in *C. difficile* challenge model



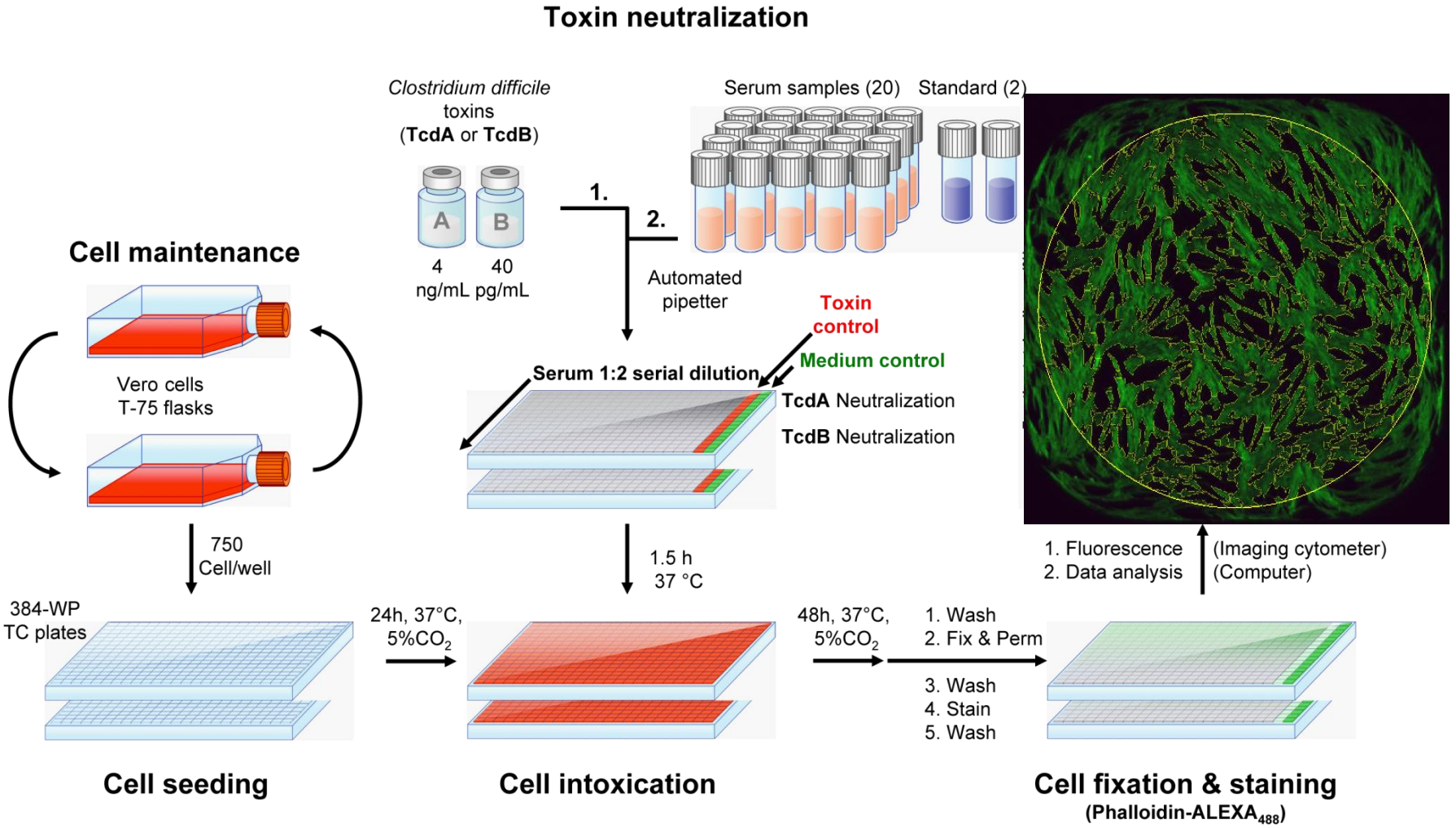


- Hamsters (n=10) immunized with a combination of native Toxoid A and recombinant fragments of TcdB were challenged with *C. difficile* VPI 10463 spores
- Larger fragments were more protective

Summary of results obtained using recombinant toxin fragments

- **Larger fragments (greater in size than CROPs fragments) were difficult to express in *E. coli***
  - Especially true for TcdA
- **Short fragments produced potent antibody responses but sub-optimal neutralizing antibodies and protection in hamsters**
- **Full length TcdA and TcdB, either produced recombinantly or purified from *C. difficile*, consistently protected hamsters from VPI10463 challenge**

Neutralizing antibody titers as a measure of vaccine efficacy

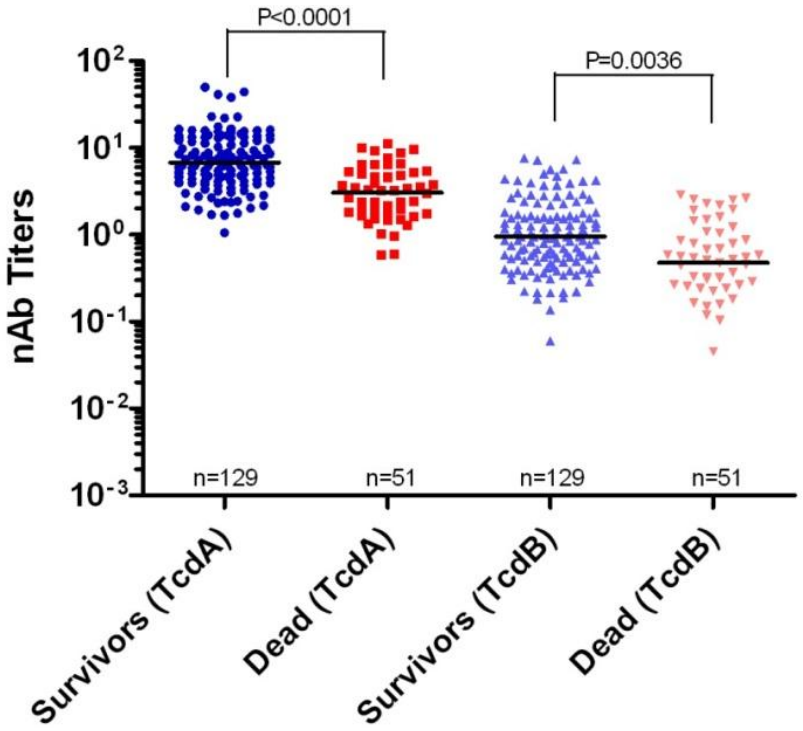


MERCK Vaccine R&D

Neutralizing antisera prevents toxin-induced actin depolymerization

MERCK

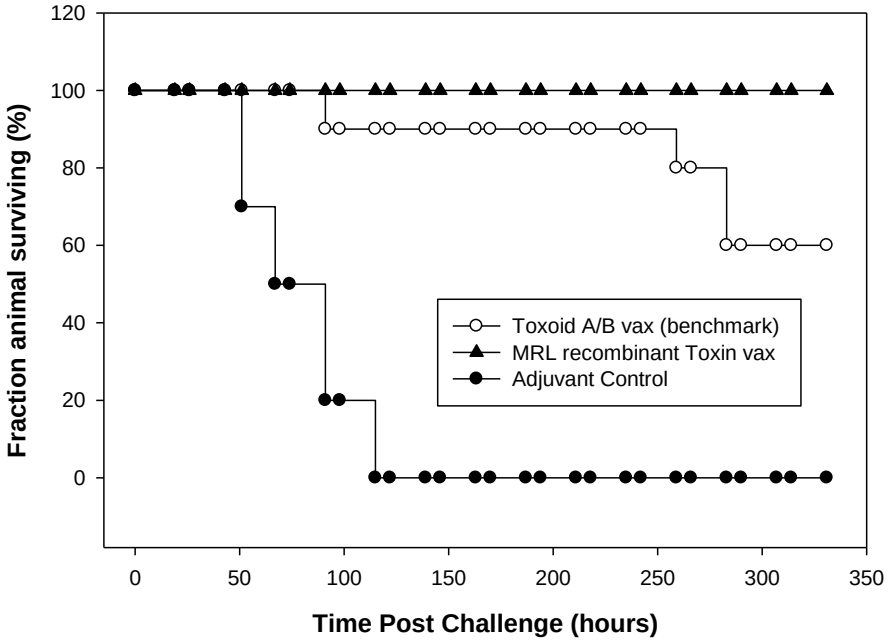
Correlation of neutralizing antibody titers and survival in hamster model



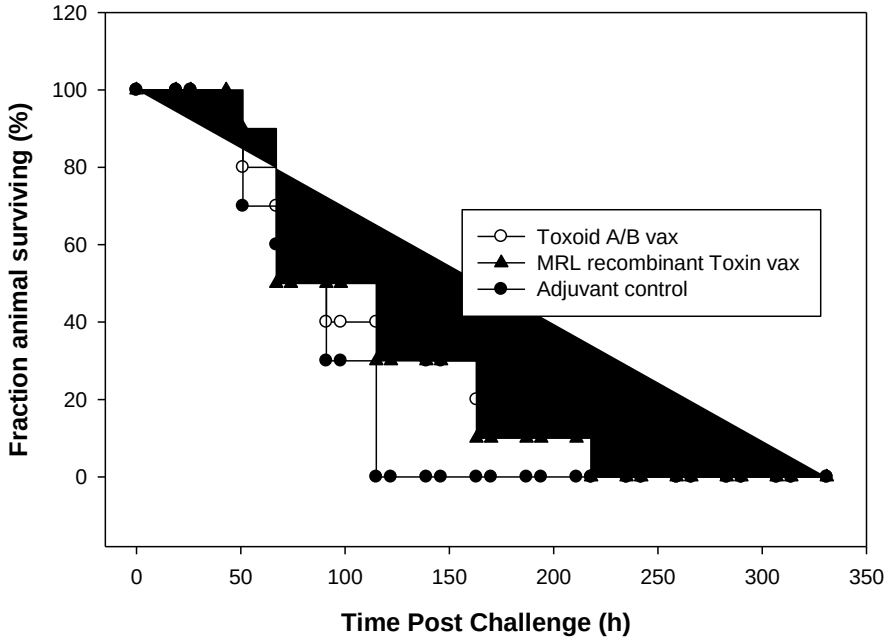
TcdA & B neutralizing antibodies correlate to protection following VPI 10463 challenge.

Efficacy of TcdA/TcdB vaccine in protection of hamsters from challenge with binary toxin expressing strains

VPI 10463 strain challenge
Binary toxin negative

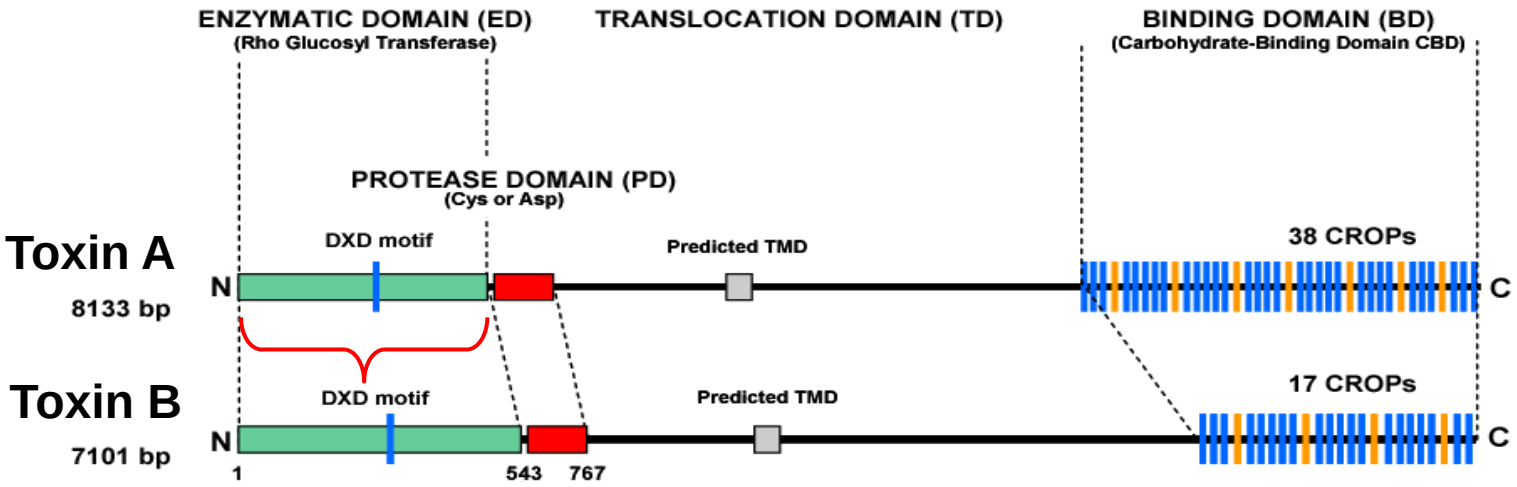


Epidemic (NAP1) strain challenge
Binary toxin positive



TcdA/TcdB vaccine fails to provide protection against NAP1 challenge in hamsters. BI17 strain obtained courtesy Dale Gerding.

Structure of *C. difficile* toxins



Binary toxin

CDTa

1389 bp

N — Interaction with CdtB — Enzymatic domain — C

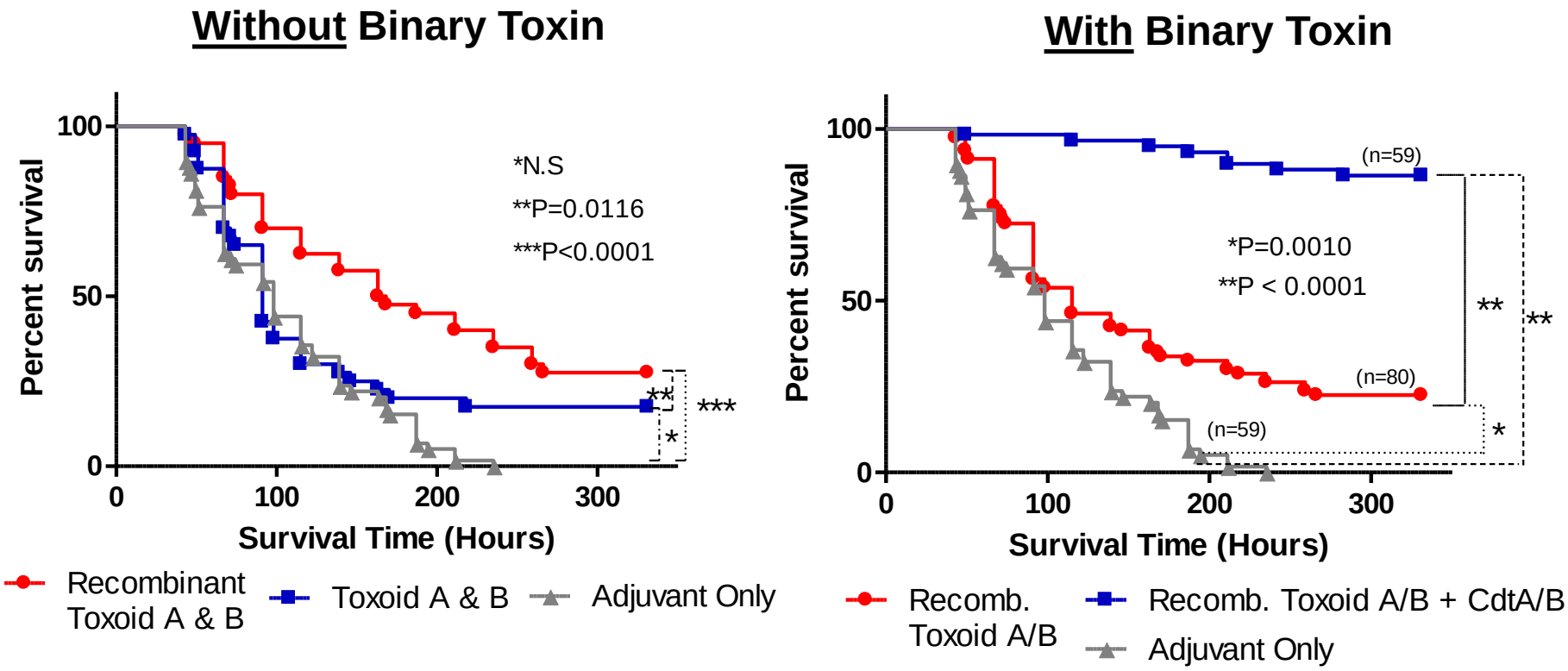
CDTb*

2505 bp

N — Activation Domain — Docking site for A — Membrane Insertion — Oligomerization — Receptor Binding — C

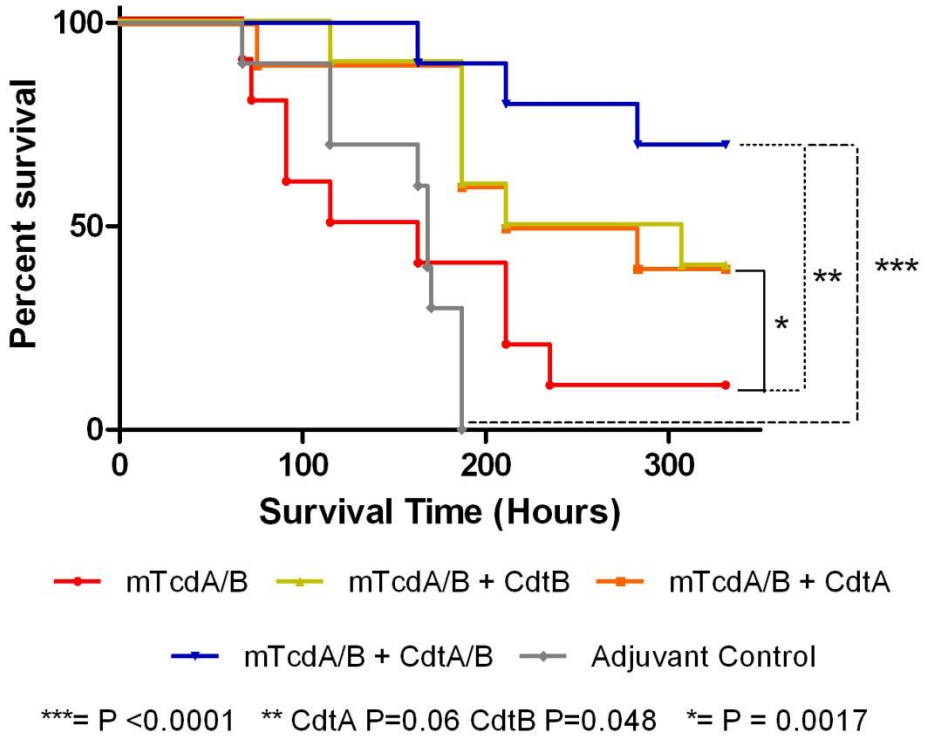
*Based on the structure of Iota toxin

Binary toxin provides enhanced survival following NAP1 challenge of hamsters



BI17 challenge

Inclusion of CDTa and CDTb enhances protection following NAP1 challenge of hamsters



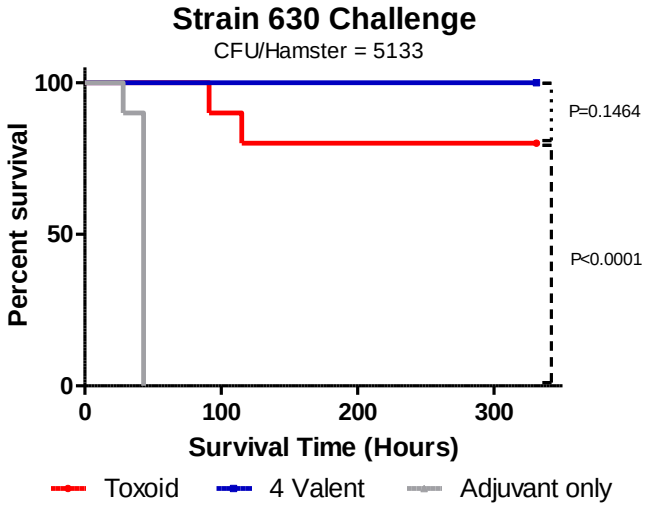
BI17 challenge

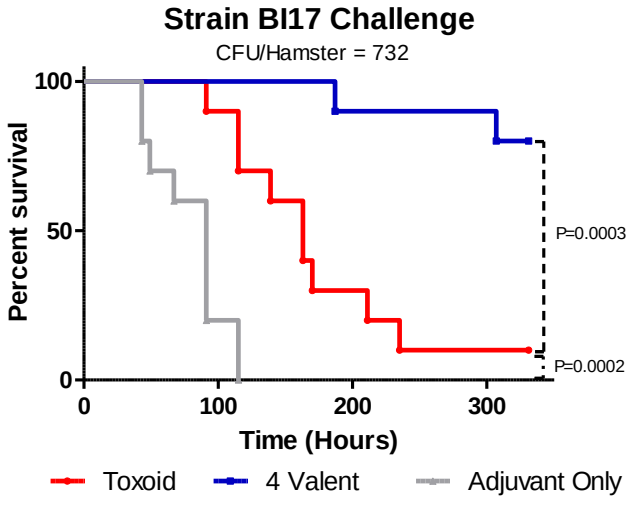
Binary Toxin components (CdtA/B) do not provide equivalent protection individually (with TcdA/B) compared to tetravalent vaccine.

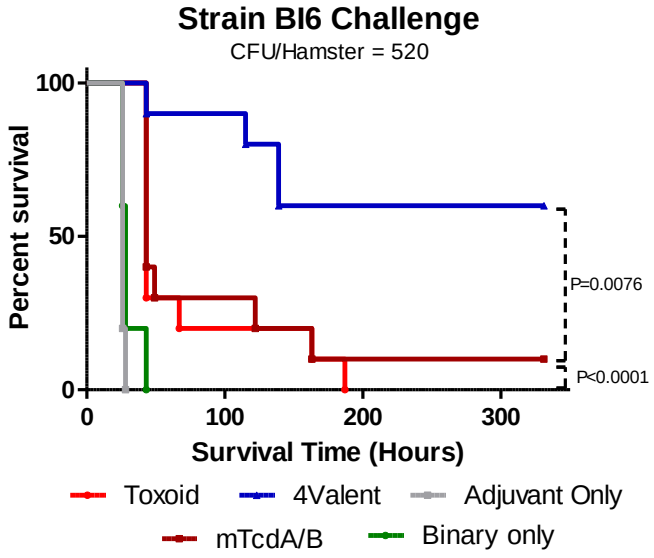
Merck's investigational *C. difficile* vaccine

- **Includes TcdA, TcdB and binary toxin (CDTa and CDTb)**
- **All molecules produced recombinantly**
- **TcdA, TcdB and CDTa contain mutations designed to eliminate enzymatic activity**

Tetravalent vaccine is protective against multiple strains

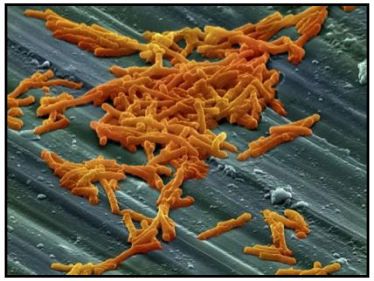
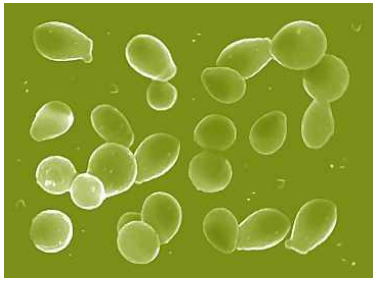
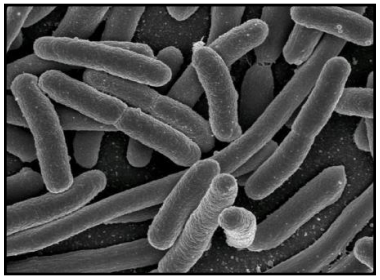
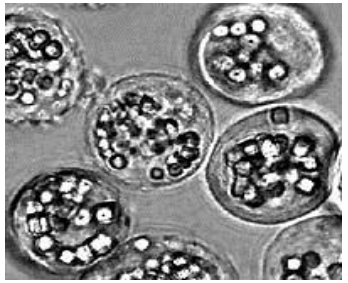






BI6 strain courtesy of Dale Gerding.

Heterologous host evaluation for expression of *C. difficile* toxins

Expression systems			
			
Clostridium difficile	***Saccharomyces cerevisiae***	***Escherichia coli***	***Baculovirus Infected Insect Cells***
Properties			
Gram-positive Low GC Content	Eukaryote, fungus	Gram-negative Med. GC Content	Med. GC Content
Sporulating	Non-Sporulating	Non-Sporulating	Non-Sporulating
Pathogenic	Non-Pathogenic	Non-Pathogenic	Non-Pathogenic
Low Productivity	Low productivity	Low productivity (TcdA)	High productivity
		Low quality (degradation)	High quality

Recombinant antigen production

Cell Substrate	*Microbial #1*	*Microbial #2*		Insect Cell		*Microbial #3*	
Toxin	TcdA/B	TcdA/B	CdtA/B	TcdA/B	CdtA/B	TcdA/B	CdtA/B
Productivity							
Product Quality							
Immunogenicity							

Use of the consolidated Insect cell (baculovirus) platform is optimal for tetravalent vaccine

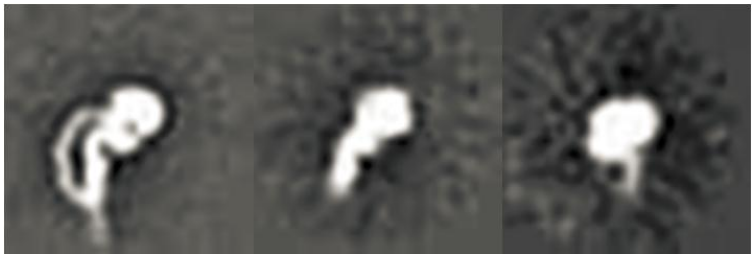
Red = Poor

Yellow = Average

Green = Good

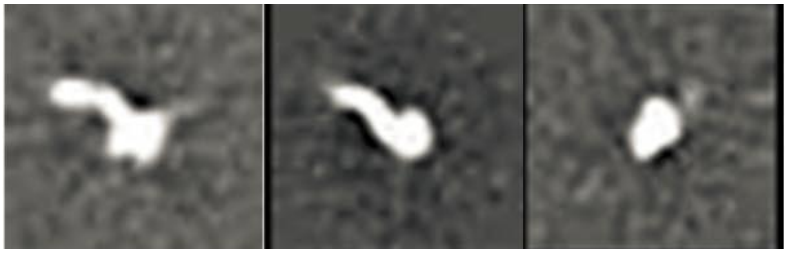
Cryo EM imaging of recombinant toxins

Insect Cell Produced TcdA



View 1 View 2 View 3

Insect Cell Produced TcdB



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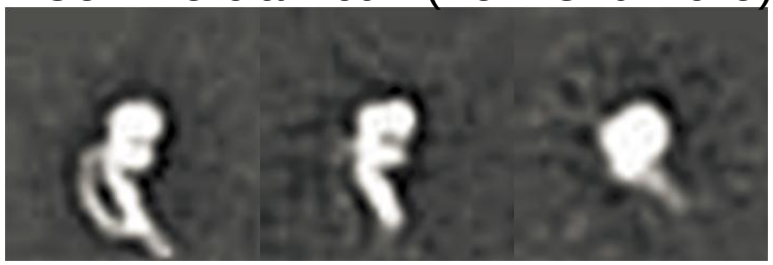
Commercial TcdA (from *C. difficile*)



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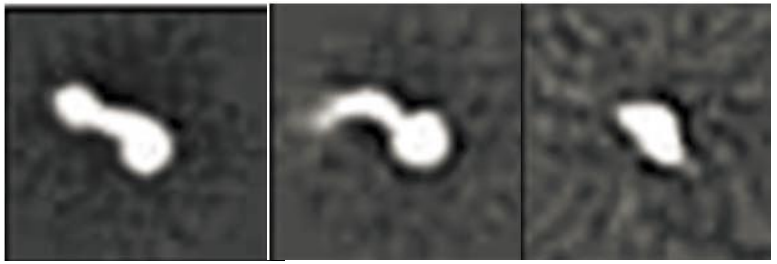
Microbial Produced TcdB



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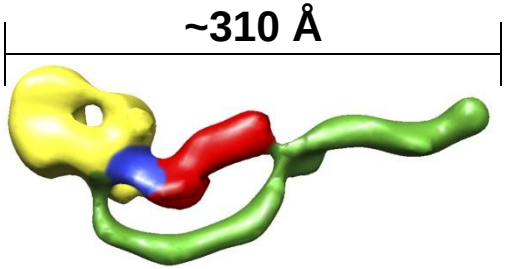


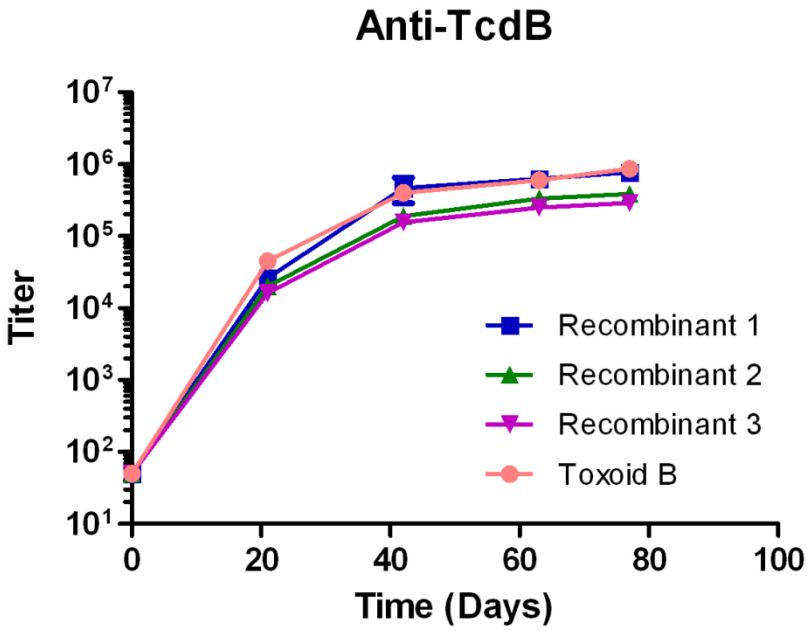
Model of TcdA

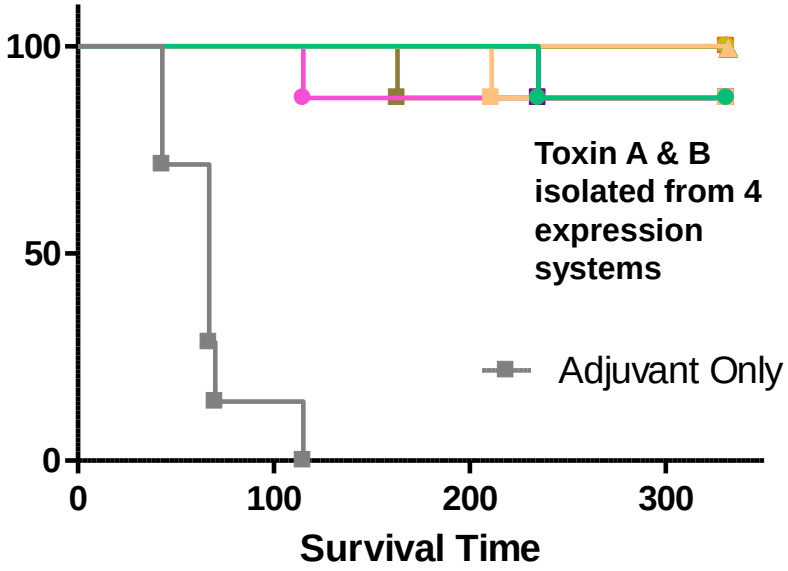
- Recombinant vaccine structure appears similar to *C. difficile* produced TcdA & TcdB

(Pruitt R N et al. PNAS 2010;107:13467-13472)

NanoImaging Services

Recombinant antigens elicit similar immune response and protection in hamsters compared to benchmark

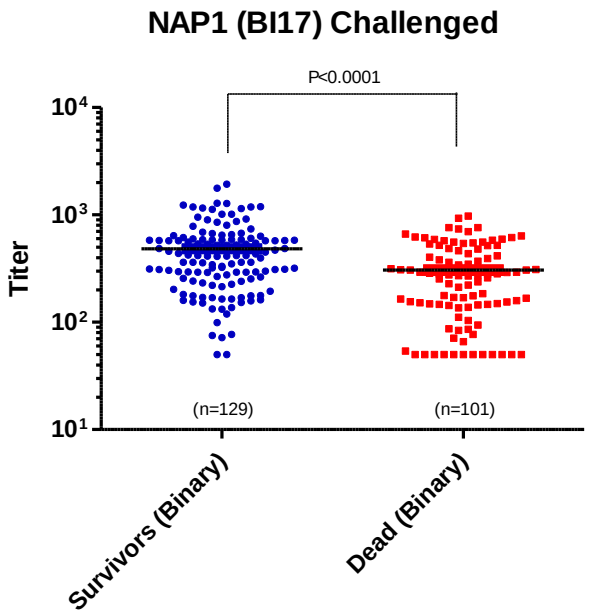




***Anti-Toxin A Titers Similar (not shown)**

Recombinant toxin showed equivalent efficacy when compared to formaldehyde-inactivated native toxin

Serum IgG responses to binary toxin correlate with hamster survival following BI17 challenge



Optimized conditions for anti-binary toxin NAb F-actin assay

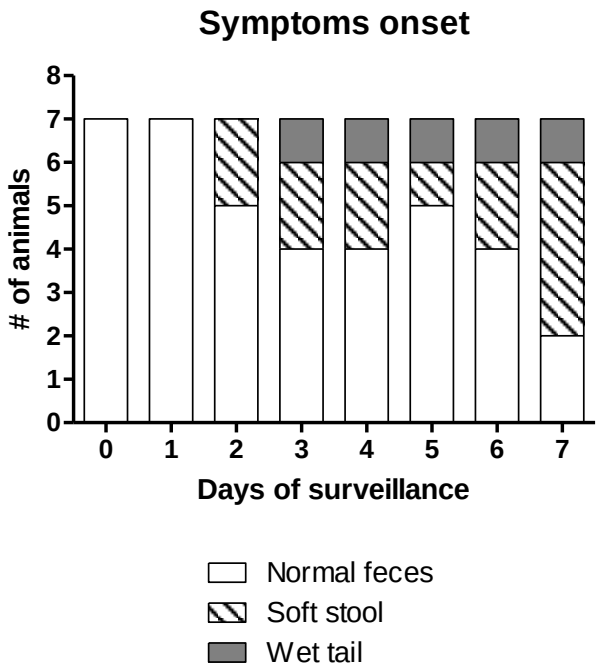
CDTa to a_CDTb molar ratio	1 to 7
CDTa concentration (ng/ml)	24
Cell Seeding Density (cell/well)	1500
Toxin-sera Pre-incubation time (mins)	75±15
Cell Intoxication Time (hours)	22-24

Binary toxin is lethal to mice

Group	Dose CDTa (ug)	Dose CDTb (ug)	Lethality (%)
rCDTa	0.8	-	0
rChymoCDTb	-	14	0
rCDTa/rProCDTb	0.8	14	0
rCDTa/rChymoCDTb	0.8	14	80
Control	-	-	0

CD1 mouse lethality following intraperitoneal challenge with recombinant (*E. coli* expressed) binary toxin components.

Binary toxin expressing strains induce disease but are not lethal for hamsters

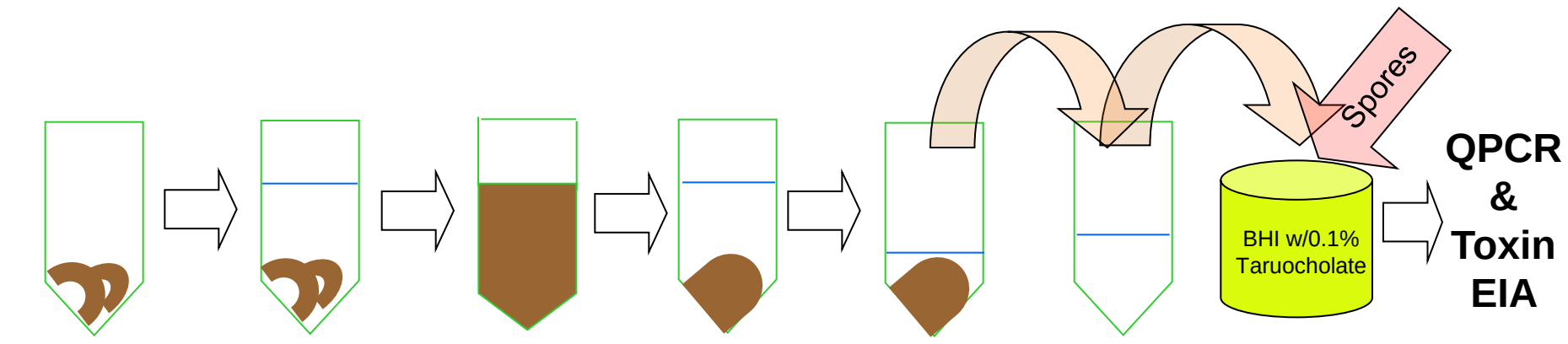


Animal	Toxin EIA	qPCR (copies/g)
1	Neg.	$2.2x10^{10}$
2	Neg.	$6.5x10^{8}$
3	Neg.	$2.7x10^{10}$
4	Neg.	$1.5x10^{9}$
5	Neg.	$7.8x10^{8}$
6	Neg.	$9.8x10^{9}$
7	Neg.	$4.2x10^{9}$

Hamster spore challenge with strain IS58 (TcdA-, TcdB-, Binary toxin +).
Strain courtesy of Maja Rupnik

A Tool to Study Colonization *in-vitro*: Fecal Filtrate Culture

An *In-vitro* method of determining the amount and timing of Cleocin disruption of gut microbiota of the hamster and how it relates to *C. difficile* ability to establish infection.



Collect Fecal Pellets

Dissolve Fecal Pellets in PBS 1:10 W/V

Mix

Pellet

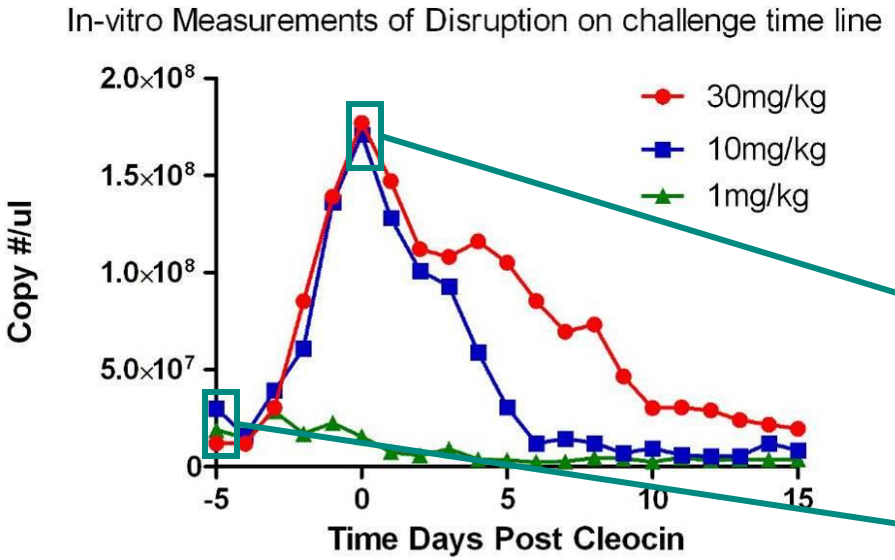
Collect Supernatant

Filter Sterilize (.22um)

Add Fecal filtrates 1:100 to BHI w/ 0.1% Taurocholate

Cultured for 72hr under anaerobic conditions then collect samples for qPCR and EIA

Analysis of the hamster microbiome



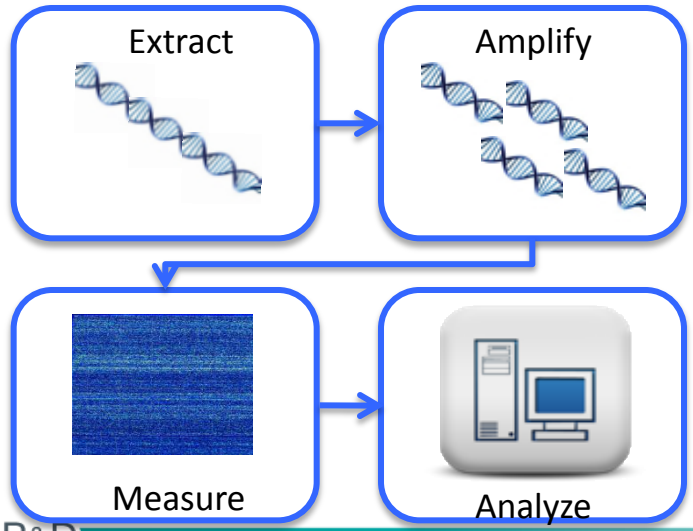


Figure 48

(./Circular_tree/C30_Extremes/itol_tree.eps) Circular tree comparing Day 0 samples (inner rings) with Day 5 and Day 9 samples (outer rings) after 1 mg/kg Clindamycin. From the 32,916 OTUs present in the study, 473 OTUs (within 86 families) produced p values < 10^{-9}. In 2 families, some of those OTUs had a higher mean abundance scores in the treated samples, while other OTUs had lower mean abundance scores. In these 2 families two OTUs were selected (one exhibiting each abundance pattern), for a total of 88 OTUs displayed in the tree. The color saturation indicates the degree of difference from the mean Day 0 value, where dark blue indicates a log ratio of 0.34, white = 1.0, dark red = 2.45.

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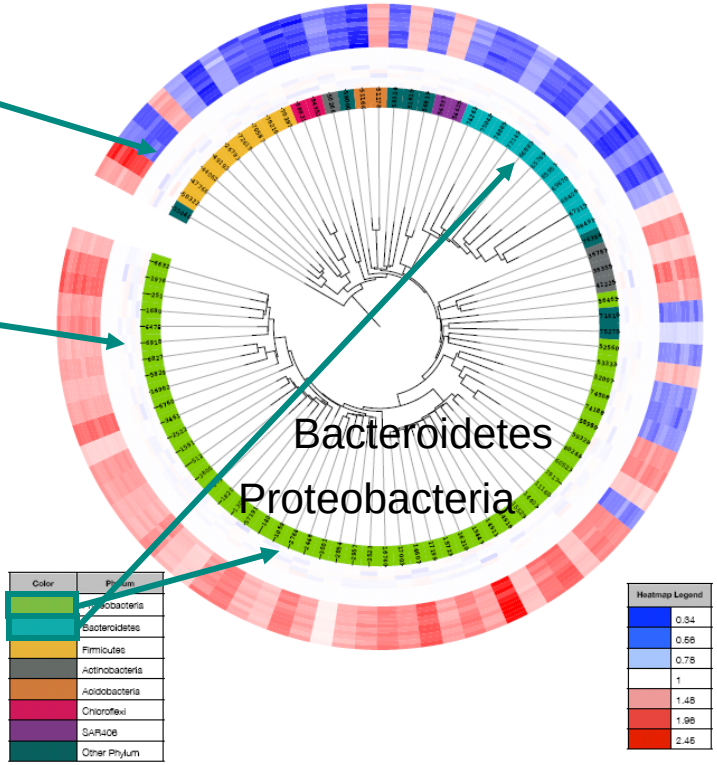

Microbiome analysis performed by Second Genome, Inc., San Bruno, CA.

For additional details see poster by Miezeiewski, et al.

C. difficile vaccine program conclusions

- **Immunization of hamsters with TcdA and TcdB is sufficient to confer protection against strains expressing only the large clostridial toxins**
  - Addition of binary toxin is required for protection of hamsters against NAP1 strain challenge
  - Binary toxin is lethal to mice when injected i.p. and induces disease in hamsters following spore challenge
- **Recombinant vaccine components perform equivalently to formaldehyde-inactivated toxoid from *C. difficile***
- **TcdA, TcdB, CDTa and CDTb can be expressed and purified from insect cells following baculovirus infection**
- **Incorporation of mutations in TcdA and TcdB results in a reduction in toxicity in cell culture**
- **Susceptibility of hamsters to *C. difficile* infection correlates with changes in their gut microbiomes following antibiotic treatment**