Novel Approaches to the Treatment/Prevention of Clostridium difficile Disease

RE: Research Symposium - Festschrift for Professor Lyn Gilbert, Westmead Hospital, Sydney, Friday 18th March 2016

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Clostridium difficile Infection (CDI)

- Gram positive, toxin-producing,
- spore-forming anaerobic bacterium.
- One of three serious antibiotic resistant threats
- of nosocomial antibiotic-associated diarrhea
- sustained rise in the incidence of worldwide severe CDI
- Emergence of hypervirulent strains (NAP1/BI/027) with:
  - Increased toxin production and sporulation;
  - Altered antibiotic resistant pattern;
  - Liberation of an additional toxin
- Disease relapse are more common
Step 1 - Ingestion of spores

Step 2 - Germination into vegetative cells

Step 3 - Earlier altered intestinal microflora allows proliferation of *C. difficile* in colon

Step 4 - Toxin production leads to colonic damage +/- pseudomembrane, often systemic intoxication in severe cases

CDI: transmission through spores
Pathogenesis of *Clostridium difficile* infection (CDI)

Toxins A (TcdA) and B (TcdB)

↓

- disrupt intestinal barrier, activate inflammatory mediators

↓

- increase mucosal permeability, fluid secretion & cell migration

↓

- colonic inflammation

↓

- formation of pseudomembrane, fulminant colitis, or toxic megacolon

Systemic intoxication often leads to serious clinical consequences
Current options for treatment/control of CDI

**Antibiotics:** (target *C. difficile* but lead to recurrence)
- Vancomycin (only FDA-approved)
- Metronidazole (most commonly used)
- Fidaxomicin (lower recurrence rates)

**Normal flora restoration:** (variable results)
- probiotics and Lactic acid bacteria (*L. casei, L. bulgaricus, S. thermophilus*)
- Fecal trasnplantation therapy from normal donors

Non-toxingenic *C. difficile* under study

**Toxin binders:** e.g. tolevamer (poor efficacy)

**Immunological approaches:** (target toxins or prevent bacterial colonization)
- IgG infusion of HMab - protects against recurrence, costly, short-term.
- Active vaccination shows promise (experimental, some in Phase II)

**Proposed combining HMab IV and antibiotics** (Phase III) - reduces recurrence
Recurrent *C. difficile* infection

Common
- ~20% after first CDI episode
- ~40% after first recurrence
- ~60% after 2 or more recurrences
Current vaccine development

- Experimental vaccine candidates
  - Recombinant toxin fragments of TcdA and TcdB)
  - Surface adhesins and flagella
- Toxoid-based vaccine (Sanofi-Aventis)
  - Purified TcdA and TcdB from toxigenic and spore-forming C. diff
  - Prone to batch-to-batch variation
  - Safety: residual toxicity and formalin
  - Immunogenicity: formalin crosslinking masks neutralizing epitopes.
- Non-toxigenic C. difficile strains
Our Strategy for the prevention/control of CD Disease

- Develop an effective safe recombinant vaccine targeting individuals at risk (elderly, on prolonged AB therapy, immunosuppressed, about to undergo surgery, etc.)
- Develop an effective safe immune-based therapy
  - (Antitoxin, rather than antibacterial agents)
- **Combining vaccine with therapy** might be the answer to reduce disease burden and significantly prevents recurrence
- Develop non-invasive delivery strategies (S. mitis, B. subtilis, sublingual delivery, intradermal silk-microneedle, Adeno/41)
Two novel chimeric vaccines against *Clostridium difficile* infection
Generate chimeric, non-toxic vaccine 1: cTxAB

- **RBD of TcdA**: immunodominant domain, adjuvant activity
- **TcdB-RBD**: variable and less immunogenic
Two generations of recombinant mutant vaccine constructs, cTxAB and mTcd138, tested for efficacy in mice and hamsters

- cTxAB – had low yield and unstable construct
mTcd138 is atoxic
mTcd138 immunization via intraperitoneal (i.p.), intramuscular (i.m.) or intradermal (i.d.) routes induces similar levels of antibody response
mTcd138 immunization induced potent neutralizing antibodies against both toxins
mTcd138 immunization of mice provided full protection against infection with a hypervirulent *C. difficile* strain.
Protective efficacy of mTcd138 in hamster model of *C. difficile* infection
Protection against recurrence/relapse
Summary of Advantages of mTcd138 as Vaccine candidate

- **Safety:**
  - non-toxic and non-reversible
  - safe bacterium (Bacillus megaterium): environmental, non-pathogenic, endotoxin-free

- **A single recombinant protein: easy to produce and purify**

- **Immunogenicity:**
  - Immunodominant domains of TcdA and TcdB
  - Toxin-like conformation
  - Induces potent neutralizing antibodies against TcdA & TcdB

- **Protection:**
  - Rapid and long-lasting
  - Against classic and epidemic strains
  - Against both primary and recurrent CDI
Novel immune-based therapeutic agents against *Clostridium difficile* infection
VHHs are single-domain Abs from Camelid heavy-chain-only Abs

- **Immunize alpaca with pathogen or antigens**
- **Obtain RNA encoding the VHH repertoire (heavy-chain-only antibody RNA)**
- **Express the VHH repertoire on phage; select clones that display pathogen-binding VHHs**
Conventional antibody

Camel Heavy-Chain antibody

VHH or Nanobody
VH domains from heavy chain only Abs (VHHs)

Favorable features of VHHs

- Small proteins (14 KDa)
- Easy to clone coding DNA (single domain)
- Functionally express at high levels
- More stable to extreme pHs and temperatures
- Commonly bind conformational epitopes
- Amenable to crystallization when bound to target
- Multimerization for improved properties
Bispecific VHH heterodimers acquire dramatically improved \textit{in vivo} efficacy demonstrated in 10 different toxin challenge animal models.
Favorable properties of VNAs

- Multi-specific proteins with 14 kDa VHH components
- Two different neutralizing VHHs to a toxin reproducibly acquire excellent in vivo antitoxin potencies
- Serum half-life enhanced by including an albumin binding peptide
- Target multiple toxins with one VNA
- Promote Ab effector functions by addition of anti-tag mAb
- Excellent for gene therapy delivery
- High level expression in cultures and in gene therapy
Options to target multiple toxins with one VNA

1. Multiple toxins successfully targeted in three animal models: *C. difficile* infection (TcdA + TcdB); enterohemorrhagic *E. coli* (Stx1 + Stx2); anthrax (EF + LF)

2. A hexaspecific VNA to BoNT/A, /B, /E has been successfully tested *in vitro*
Adenovirus/VHH, given to mice or piglets provide protection against toxin challenges (BoNT/A, Ricin, Stx1 & Stx2, TcdA & TcdB). Synthetic RNA was tested only in mice with BoNT/A.
Tcd-specific VHH protects mice with CDI
## Disease severity in piglets treated with Tcd-specific peptides or adenovirus expressing Tcd-specific VHH

<table>
<thead>
<tr>
<th>Treatment (number of animals)</th>
<th>Gastrointestinal Disease (%)</th>
<th>Systemic Disease (%)</th>
<th>Fatal Disease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UK6 + VHH-Tcd peptide (6)</td>
<td>mild-moderate (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>UK6 (6)</td>
<td>moderate-severe (100)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>UK6 + VHH-Tcd - Adeno (9)</td>
<td>mild-severe (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>UK6 + control-Adeno (6)</td>
<td>moderate-severe (100)</td>
<td>33</td>
<td>50</td>
</tr>
<tr>
<td>Uninfected control (3)</td>
<td>Mild (100)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Investigating the efficacy of anti-TcdA and anti-TcdB HMab in piglets, and in humans with CDI in Phase III clinical trials

- Anti-TcdA and anti-TcdB HMab (Merck Pharmaceuticals), were shown to be effective against the two toxins in mice and hamsters and safe in human phase I & II clinical trials (Lowy et al. 2010, N Engl J Med. 2010: 21;362)

- Piglets with CDI show clinical symptoms and colonic mucosal lesions that mimic those observed in humans (Steele et al. 2010. JID;201(3):428)

- The Merck anti-TcdA and anti-TcdB Hmab were evaluated in the CDI piglet diarrhea model (Steele et al. 2013. JID 207:323)

- Outcome of the Phase III studies show remarkable parallel to the piglet study (A. Therien, Merck; personal communication; 2015)
Clinical outcome in piglets with CDI treated IV with 10mg/kg anti-TcdA and 10mg/kg anti-TcdB

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Severity of GI Disease&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Systemic Disease&lt;sup&gt;b&lt;/sup&gt;</th>
<th>p&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Fatal Disease&lt;sup&gt;d&lt;/sup&gt;</th>
<th>p&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti-TcdA only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpaca polyclonal (n=6)</td>
<td>moderate to severe</td>
<td>100%</td>
<td>0.454</td>
<td>83%</td>
<td>0.067</td>
</tr>
<tr>
<td>HMab (n=6)</td>
<td>moderate to severe</td>
<td>100%</td>
<td>0.400</td>
<td>67%</td>
<td>0.545</td>
</tr>
<tr>
<td>anti-TcdB only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpaca polyclonal (n=6)</td>
<td>mild</td>
<td>0%</td>
<td>0.061</td>
<td>0%</td>
<td>0.400</td>
</tr>
<tr>
<td>HMab (n=5)</td>
<td>mild</td>
<td>0%</td>
<td>0.047</td>
<td>0%</td>
<td>0.167</td>
</tr>
<tr>
<td>anti-TcdA + anti-TcdB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpaca polyclonal (n=6)</td>
<td>mild</td>
<td>0%</td>
<td>0.061</td>
<td>0%</td>
<td>0.454</td>
</tr>
<tr>
<td>HMab (n=6)</td>
<td>mild to moderate</td>
<td>0%</td>
<td>0.033</td>
<td>0%</td>
<td>0.133</td>
</tr>
<tr>
<td>Controls (HMab x Stx)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpaca polyclonal (n=5)</td>
<td>moderate to severe</td>
<td>60%</td>
<td>20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMab (n=4)</td>
<td>moderate to severe</td>
<td>75%</td>
<td>50%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> severity of disease: diarrhea and mucosal lesions.

<sup>b</sup> systemic disease: severe, systemic (anorexia or dyspnea):

<sup>c</sup> p value systemic disease treated vs. controls: pE Value for Survival
Necropsy Findings in a piglets infected with C. difficile

- Hemorrhage and ulceration
- Pseudomembrane
- Mesenteric edema
- Dilatation, inflammation, edema
Severe injury of the colon’s mucosa of piglet treated with Human mab against TcdA

Pseudomembrane on colonic mucosa

Neutrophilic aggregate in colonic mucosa
Piglets treated with Human Mab against TcdB

Intact mucosa, no neutrophilic aggregates, mild submucosal edema

Mucosal closeup: few neutrophils, intact mucosa
# Adverse Event Summary

(All Patients as Treated)

<table>
<thead>
<tr>
<th></th>
<th>Acto+Bezlo (N=387)</th>
<th>Acto (N=235)</th>
<th>Bezlo (N=390)</th>
<th>Placebo (N=400)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>4 weeks following infusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One or more adverse events (AE)</td>
<td>59.7</td>
<td>67.2</td>
<td>65.4</td>
<td>62.0</td>
</tr>
<tr>
<td>Drug-related AE</td>
<td>6.2</td>
<td>7.2</td>
<td>8.2</td>
<td>5.0</td>
</tr>
<tr>
<td>Serious AE</td>
<td>14.7</td>
<td>27.7 (^1)</td>
<td>21.5</td>
<td>20.0</td>
</tr>
<tr>
<td>Serious &amp; drug-related AE</td>
<td>0.5</td>
<td>1.3</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Deaths</td>
<td>3.1</td>
<td>6.0</td>
<td>4.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Study medication discontinued due to AE</td>
<td>0.0</td>
<td>0.4</td>
<td>0.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Infusion-specific reactions, in first 24 hrs</td>
<td>8.8</td>
<td>11.1</td>
<td>11.8</td>
<td>7.5</td>
</tr>
<tr>
<td><strong>12 weeks following infusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One or more serious AE</td>
<td>24.3</td>
<td>44.3 (^2)</td>
<td>30.8</td>
<td>31.5</td>
</tr>
<tr>
<td>Deaths</td>
<td>5.2</td>
<td>11.5 (^3)</td>
<td>7.9</td>
<td>6.5</td>
</tr>
</tbody>
</table>

\(^1\) p=0.027 vs placebo; \(^2\) p=0.001 vs placebo; \(^3\) p=0.028 vs placebo.
# Most Common Adverse Events, ≥4%

*(All Patients as Treated)*

<table>
<thead>
<tr>
<th>Gastrointestinal disorders</th>
<th>Acto+Bezlo (N=387)</th>
<th>Acto (N=235)</th>
<th>Bezlo (N=390)</th>
<th>Placebo (N=400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>4.9</td>
<td>6.4</td>
<td>5.4</td>
<td>4.8</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>7.0</td>
<td>5.5</td>
<td>6.7</td>
<td>5.0</td>
</tr>
<tr>
<td>Nausea</td>
<td>7.8</td>
<td>11.9</td>
<td>7.4</td>
<td>6.5</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3.6</td>
<td>4.3</td>
<td>5.4</td>
<td>3.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>General disorders</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>3.1</td>
<td>4.7</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>3.1</td>
<td>4.7</td>
<td>5.6</td>
<td>2.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infections and infestations</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. difficile</em> infection</td>
<td>3.9</td>
<td>8.5</td>
<td>1.8</td>
<td>6.3</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>3.6</td>
<td>5.5</td>
<td>3.6</td>
<td>4.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nervous system disorders</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>5.2</td>
<td>6.0</td>
<td>5.1</td>
<td>3.3</td>
</tr>
</tbody>
</table>
Acknowledgements and Thanks

- Merck, NIAID/NIH, Pfizer,
- The organizers of this Symposium
- Charles Shoemaker’s lab generated the data on the development of VHH
- Hanping Feng and Xingmin Sun developed the vaccine candidates
- The technical contribution of the staff of the Infectious Disease group