OP7 - Epitopes and Mechanisms of Action of the Clostridium difficile Toxin-Neutralizing Antibodies Actoxumab and Bezlotoxumab

Alex Therien
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**Clostridium difficile as a Pathogen**

- Anaerobic spore-forming Gram positive bacillus
- Release of two toxins (A and B) causes *C. difficile*-associated infection (CDI)
- Damage to gut lining → diarrhea → pseudomembranous colitis → toxic megacolon → sepsis → death
- “Urgent threat” (CDC): 450,000 cases, 29,000 deaths in 2011 in the U.S. (Lessa et al, 2015)
- 20 to 30% recurrence rate with metronidazole or vancomycin
- Main mode of transmission is fecal-oral in hospital setting
MK-3415A: Monoclonal Antitoxin Antibody Combination

- Fully human mAbs that target and neutralize *C. difficile* toxins A and B
  - Actoxumab = MK-3415 / MDX-066 / CDA-1
  - Bezlotoxumab = MK-6072 / MDX-1388 / CDB-1
  - MK-3415A = actoxumab + bezlotoxumab
  - Co-developed by Medarex and MassBiologics Laboratories (MBL)
  - Licensed to Merck in 2009

- Administered IV in combination with standard antibiotic therapy (metronidazole, vancomycin, fidaxomicin) to patients with *C. difficile* infection (CDI)
  - Antibiotic to treat current infection, mAb to prevent recurrence

- Currently in Phase III development
  - Four-arm study: (i) anti-A alone, (ii) anti-B alone, (iii) anti-A and anti-B together, (iv) placebo
Mechanism of Action of MK-3415A: Unresolved Questions

Antibodies are predicted to cover all clinically important strains of *C. difficile*.

*Orth et al, JBC, 2014*

- MK-3415A acts on toxins to prevent the symptoms of CDI in critical window of susceptibility, until recolonization by gut microbiota.
- No evidence that MK-3415A can influence colonization of gut by *C. difficile* or normal biota.

*Hernandez et al, AAC 2015*

*Yang et al, IAI, 2015*

*Flattery et al, ICDS, Poster P69*

*Zhang et al, IAI 2015*

Toxin-dependent paracellular leakage, not Fc-dependent transcellular transport.
Toxin Structures and Peptides Used in this Study

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Formation of Antibody/Toxin Immune Complexes Assessed by SEC-MALLS

- Actoxumab binds to ≥2 sites in CROP domain and causes aggregation of TcdA
- Bezlotoxumab likely binds to 1-2 sites in CROP domain and does not cause aggregation
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Binding of Antibodies to Toxins Assessed by Western Blotting

- Actoxumab binds to at least two sites, one in N-terminal half, other in C-terminal half, of TcdA CROP
- Bezlotoxumab binds to at least one site in N-terminal half, not in C-terminal half, of TcdB CROP
**Binding of Antibodies to Toxins Assessed by Surface Plasmon Resonance**

- Actoxumab binds to TcdA at sites that have similar affinities and shows minimal binding cooperativity.
- Bezlotoxumab binds to TcdB at least at two sites in the N-terminal half, with different affinities for each site and/or cooperativity of binding.

### Binding of Bezlotoxumab to TcdA

<table>
<thead>
<tr>
<th>Toxin/fragment</th>
<th>$K_d$ (pM)*</th>
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</thead>
<tbody>
<tr>
<td>TcdA</td>
<td>610 ± 90</td>
</tr>
<tr>
<td>A1</td>
<td>220 ± 110</td>
</tr>
<tr>
<td>A4</td>
<td>800 ± 490</td>
</tr>
</tbody>
</table>

* Fits single binding site model best

### Binding of Actoxumab to TcdB

<table>
<thead>
<tr>
<th>Toxin/fragment</th>
<th>$K_{d1}$ (pM)**</th>
<th>$K_{d2}$ (pM)**</th>
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</thead>
<tbody>
<tr>
<td>TcdB</td>
<td>19 ± 5</td>
<td>370 ± 310</td>
</tr>
<tr>
<td>B1</td>
<td>41 ± 13</td>
<td>660 ± 35</td>
</tr>
<tr>
<td>B2</td>
<td>46 ± 21</td>
<td>810 ± 56</td>
</tr>
<tr>
<td>B3</td>
<td>NB</td>
<td>11,000 ± 6,000</td>
</tr>
<tr>
<td>B4</td>
<td>NB</td>
<td>NB</td>
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</tbody>
</table>

** Fits two binding site model best (Orth et al, JBC, 2014)
Binding of Antibodies to Toxins Assessed by HDX-MS

- Actoxumab binds to up to 5 homologous epitopes within TcdA CROP
- Bezlotoxumab binds to 2 homologous epitopes within TcdB CROP
- All epitopes appear centered around “long repeats” (LR) where putative carbohydrate receptor binding pockets are located (Greco et al, 2006)
Overlap between bezlotoxumab epitopes and putative carbohydrate receptor binding pockets modeled from TcdA (Greco et al, 2006)

Orth et al, JBC, 2014
Acto/bezlo Prevent Binding of Toxins to Mammalian Cells

**Western blot on isolated membranes**

**Flow cytometry**

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**Blockade not due to toxin aggregation**
Acto/bezlo Neutralize Functional Effects of Toxins

Cox-Hipkin et al, ICDS, *poster P91*
### Summary

<table>
<thead>
<tr>
<th>Acto/TcdA</th>
<th>Bezlo/TcdB</th>
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<tbody>
<tr>
<td>Actoxumab binds with high pM affinity to TcdA</td>
<td>Bezlotoxumab binds with low pM affinity to TcdB</td>
</tr>
<tr>
<td>Multiple Actoxumab bind to up to 5 homologous sites within CROP domain</td>
<td>A single bezlotoxumab binds to exactly 2 homologous sites within CROP domain</td>
</tr>
<tr>
<td>Causes formation of “large” immune complexes of varying stoichiometries</td>
<td>Causes small immune complexes of 1:1 or 1:2 stoichiometry</td>
</tr>
<tr>
<td>Epitopes overlap with putative receptor binding pockets</td>
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<td>Antibody neutralizes toxin by blocking toxin binding to target cells</td>
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**C. difficile**

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