Recombinant antivenom based on oligoclonal broadly-neutralizing antibodies

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Parasitic diseases

Bacterial diseases

Snakebite antivenoms

Industrial biotech & BioAg

Scientific focus areas

Multi-protein targeting

Nanobody technology

Special properties (pH-sensitive)

Broadly-neutralizing antibodies

Affordable biotherapeutics for NTDs

Microbiome precision engineering
Recombinant oligoclonal broadly-neutralizing antivenom

Complex cocktails of toxic proteins

Complex antibody cocktails
Snakebite envenoming claims millions of lives

Global numbers (per year)
1.8-2.7 million envenomings
81,000-138,000 deaths

Asia
1.2-2.0 million envenomings
57,000-100,000 deaths

Africa & Middle East
453,000-580,000 envenomings
20,000-32,000 deaths

Latin America & Caribbean
137,000-150,000 envenomings
3,400-5,000 deaths

Europe
8,000-9,900 envenomings
30-128 deaths

The United States & Canada
3,800-6,500 envenomings
7-15 deaths

Oceania
3,000-59,000 envenomings
200-520 deaths

The United States & Canada
3,800-6,500 envenomings
7-15 deaths

Gutiérrez et al. Nature reviews 2017
Neurotoxic snakebites – Effect of alpha-neurotoxins

Laustsen. Recombinant antivenoms 2016
Neurotoxic snakebites – Effect of dendrotoxins
Myotoxic snakebites – Effect of myotoxins

Myotoxin → Muscle fiber

Diagram showing the effect of myotoxins on muscle fibers.
Are snakes venomous or poisonous? 

**VENOMS**
- Toxic when injected
- Not toxic when ingested

**POISONS**
- Toxic when injected
- Toxic when ingested
Antivenoms: a century old invention

Traditional antivenom production

- Animal immunization with snake venom
- Blood collection
- Purification of antibodies from blood
- Antivenom formulation

Recombinant antivenom production

- Genes of toxins specific antibodies
- Antibody expression in CHO cells
- Large scale antibody production
- Recombinant antivenom
Recombinant antivenoms based on monoclonal antibodies

Benefits

• Compatibility with human victims
• Enriched for toxin-neutralizing antibodies
• Consistent and reproducible production
• Tailor-made antibodies with optimal pharmacokinetics (PK) and pharmacodynamics (PD)
• Rapid administration of antivenoms
• Acceptance among clinicians
• No dependence on snakes and horses
• Potential prophylactic use

Phage display technology

Oligoclonal antibodies against black mamba venom

Oligoclonal antibodies against black mamba venom

Polyclonal phage ELISA

Monoclonal scFv ELISA

Monoclonal IgG ELISA

DNA sequencing

90 hits sequenced
35 unique $V_H^+V_L$ CDR3
29 unique $V_H$ CDR3

Laustsen et al. Nature Communications 2018
Oligoclonal antibodies against black mamba venom

*Dendroaspis polylepis*

Cocktail 1

Cocktail 2

Dendrotoxin

Laustsen et al. Nature Communications 2018
Oligoclonal antibodies against black mamba venom

Dendroaspis polylepis

Dendrotoxin

Cocktail 1

Cocktail 2

Survival %

Time [hours]

Cocktail 1 (4 IgGs)

Cocktail 2 (3 IgGs)

Laustsen et al. Nature Communications 2018
Wellcome: Recombinant antivenom for sub-Saharan Africa

26 snakes

18 elapids
8 viperids

Immune $V_{H}V_{H}$-libraries

Naïve scFv-library

Polyvalent recombinant antivenom
26 most medically relevant snakes of sub-Saharan Africa
Broadly-neutralizing antibodies against α-neurotoxins

A)

B)

C) Nk α-cobra toxin

D) Dp α-elapitoxin

E) Nm long neurotoxin 2

Ledsgaard et al. Submitted
Cross-panning to increase antibody cross-reactivity

Ahmadi et al. Scientific Reports 2020
Broadly-neutralizing antibodies against α-neurotoxins

A

- 368_01_C05 κ library
- 368_01_C05 λ library

**Round 1**
- α-cobratoxin 10 nM

**Round 2**
- α-elapitoxin 200 pM
- α-cobratoxin 1 nM

**Round 3**
- α-elapitoxin 4 pM
- α-cobratoxin 20 pM
- α-elapitoxin 20 pM
- α-cobratoxin 100 pM
Broadly-neutralizing antibodies against α-neurotoxins
Broadly-neutralizing antibodies against α-neurotoxins

A

B

C

D

Preincubation assay

Rescue assay

N. kaouthia

O. hannah

D. polylepis

Rescue N. kaouthia

Survival (%) vs Time (hours)

Survival (%) vs Time (hours)

Survival (%) vs Time (hours)

Survival (%) vs Time (hours)
Broadly-neutralizing antibodies against α-neurotoxins
Polyvalent recombinant antivenom

<table>
<thead>
<tr>
<th>Toxin subfamily-specific antibodies</th>
<th>Venom neutralized by combination (mix-and-match):</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Venom 1</td>
</tr>
<tr>
<td></td>
<td>Venom 2</td>
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<td></td>
<td>Venom 3</td>
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<td></td>
<td>Venom 4</td>
</tr>
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<td></td>
<td>Venom 5</td>
</tr>
</tbody>
</table>

Combining all four antibodies yields a polyvalent antivenom.
Discovery, development, and manufacturing feasibility

Cost

R&D cost

Manufacturing cost

Time

Laustsen & Dorrestijn. Toxins 2018; Knudsen et al. Toxicon 2019
Antibody formats and their sizes

- IgG (~150 kDa)
- F(ab')₂ (~110 kDa)
- Fab (~50 kDa)
- Diabody (~55 kDa)
- scFv (~25 kDa)
- V₉H (~15 kDa)

Laustsen et al. Toxicon 2018
Next-generation antivenoms can become affordable
Proof of Concept: Ultra-low-cost nanobody constructs

Divalent constructs

- Derived from llama hClgGs
- High affinity ($K_d$ pM to nM range)
- pH and heat stability
- Small size (30 kDa), single-chain
- Low COGS (demonstrated in >1000 L scale)
Quadrucept technology: Low-cost, high avidity binders

Potential
- Increased avidity with improved neutralization potency
- Lower dosing
- Generate multispecific molecules
- Low cost of manufacture due to self-assembly

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1. Fragment
2. Subunit selection
3. Self-assembly

$K_d$: 86.68 nM
Valence: 1
Size: 2.07 nm

$K_d$: 5.1 nM
Valence: 4
Size: 3.53 nm

$K_d$: 0.71 nM
Valence: 16
Size: 6.81 nm

Wade et al. In revision
Recombinant oligoclonal broadly-neutralizing antivenom

Complex cocktails of toxic proteins

Complex antibody cocktails
Our lead prototypes can diagnose envenomings the three medically relevant snake genera in Brazil:

- *Crotalus*
- *Lachesis*
- *Bothrops*

These snakes are responsible for combined 99% of snakebites in Brazil, as well as 60,000 snakebites annually in Latin America.

Future versions will allow for Point of Care use by packing the test-strips in a plastic casing.
Fangs for your attention
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