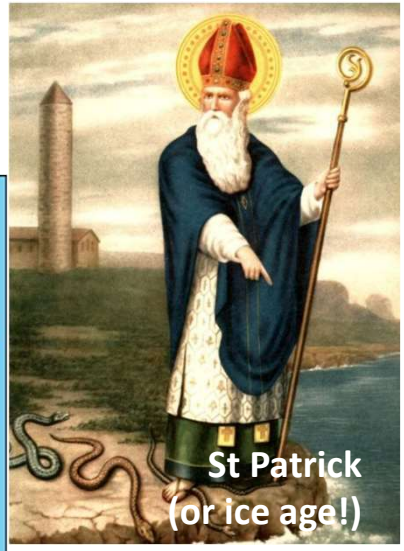
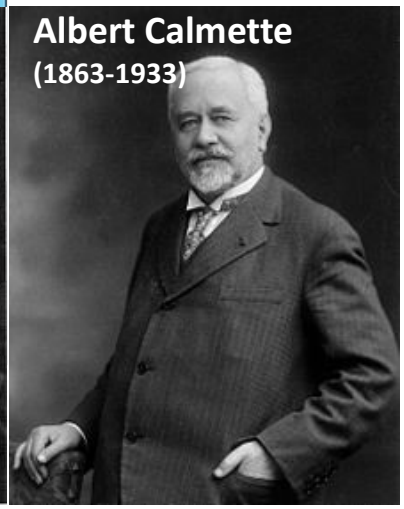
Can we “deconvolute” polyclonal anti-venoms to create defined recombinant antibody cocktails?

John McCafferty

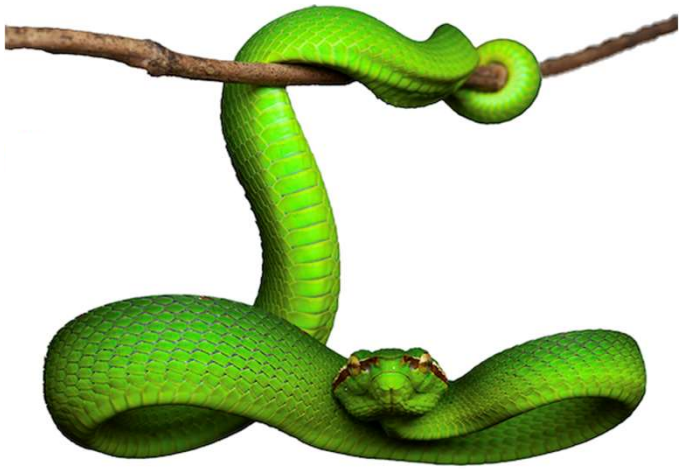
Pioneers addressing snakebites





Problem with animal-derived polyclonal antibodies

- Composition of immunogen will vary
- Variable immune response of animal -> variable composition of antibody cocktail
- Only a fraction of injected antibodies “relevant”
  - Directed to non-venom targets
  - Venom components unrelated to toxicity
- Low antibody titres against highly toxic but poorly immunogenic low MWt toxins
- Injection of up to 15g of foreign (equine) antibodies



The potential of venom therapeutics

IONTAS

maxion therapeutics

Aneesh Karatt-Vellatt, CSO
11.20 Wed 8th June

Venom-derived therapeutics

anti-venoms

Can we “deconvolute” polyclonal anti-venoms to create defined recombinant antibody cocktails?

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UNIVERSITY OF CAMBRIDGE

Department of Medicine

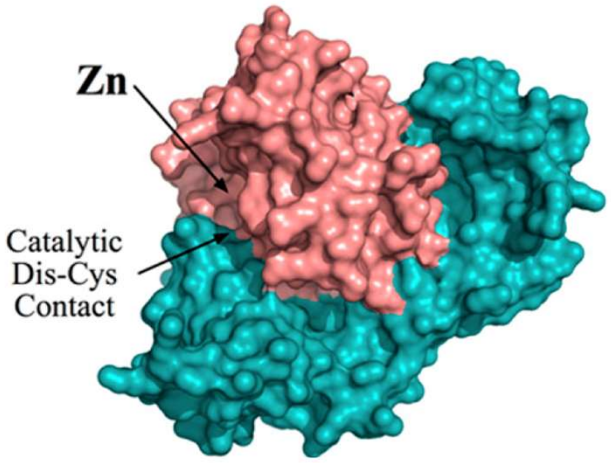
Venom-derived therapeutics

anti-venoms

INSTITUTO **CLODOMIRO PICADO**

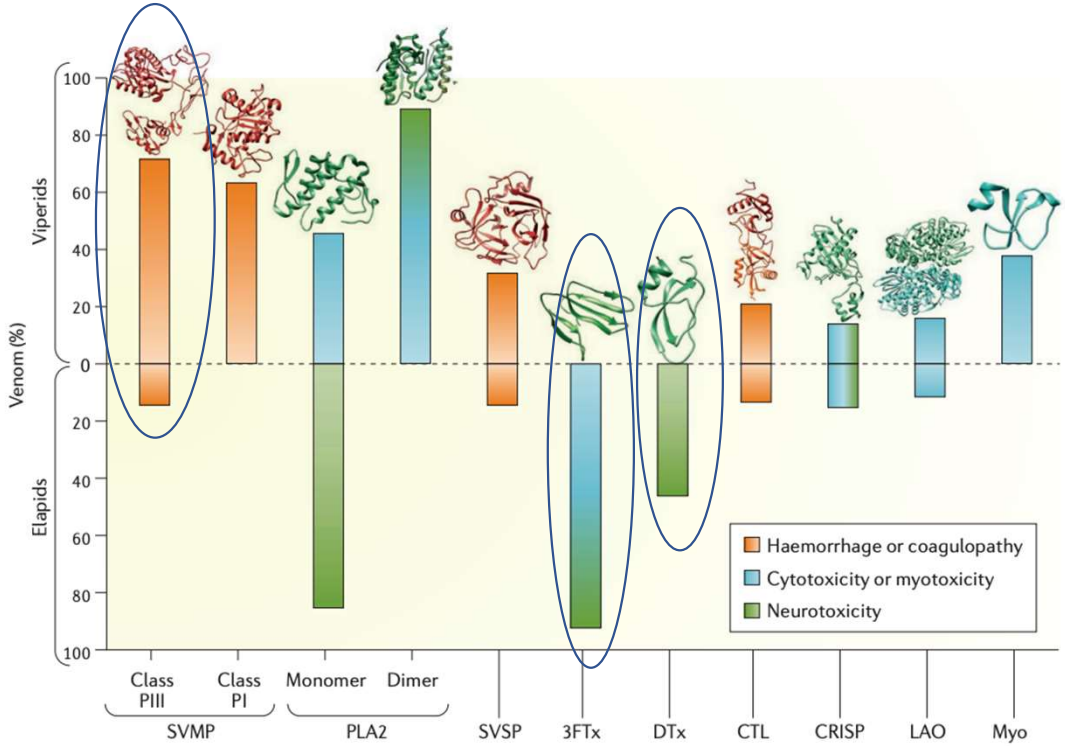
Andres Sanchez
Jose Maria Gutierrez (Chema)

Profile of venom components in elapids and vipers



Russell viper venom metalloprotease (2E3X)

Gutierrez et al (2017) Nature Review Disease primers 3 No 17063



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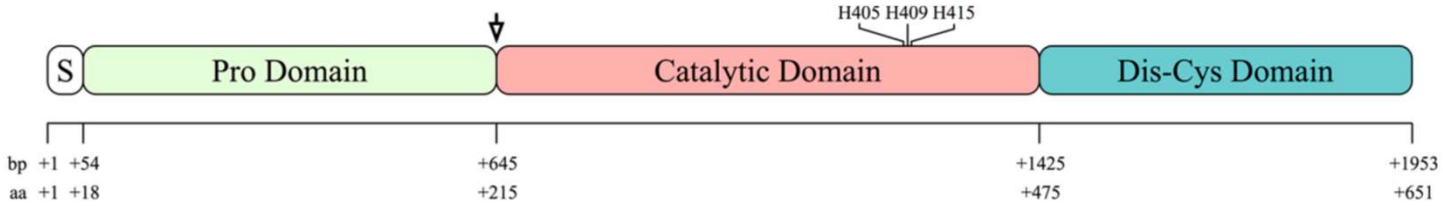
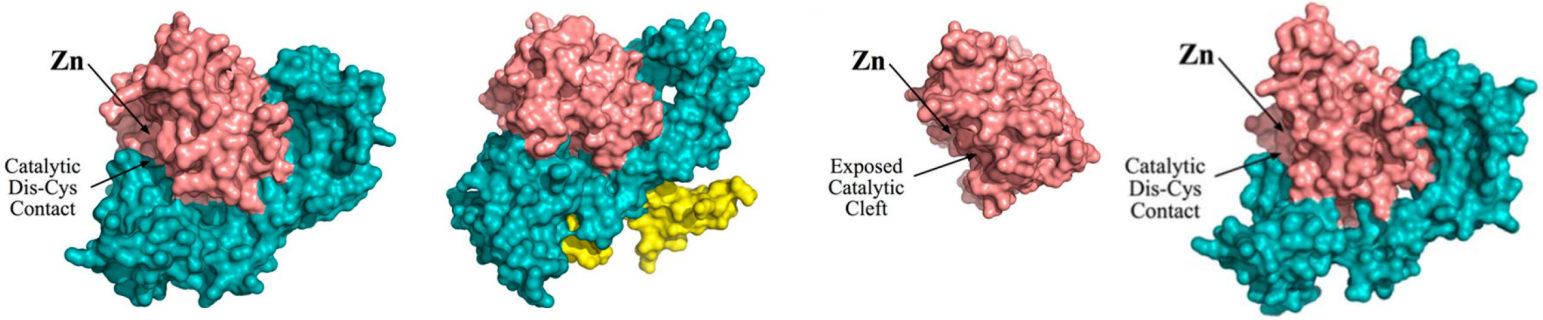
Example 1. Generation of metalloprotease blockers by phage display





Russell viper venom metalloprotease (2E3X)

human ADAM22 ectodomain (3G5C)

TACE catalytic domain (1BKC)

Model of TACE ectodomain

Catalytic domain conserved
Dis-Cys domain variable among MMPs

Tape et al (2011) Cross-Domain Inhibition of TACE Ectodomain PNAS 108 p5578–5583

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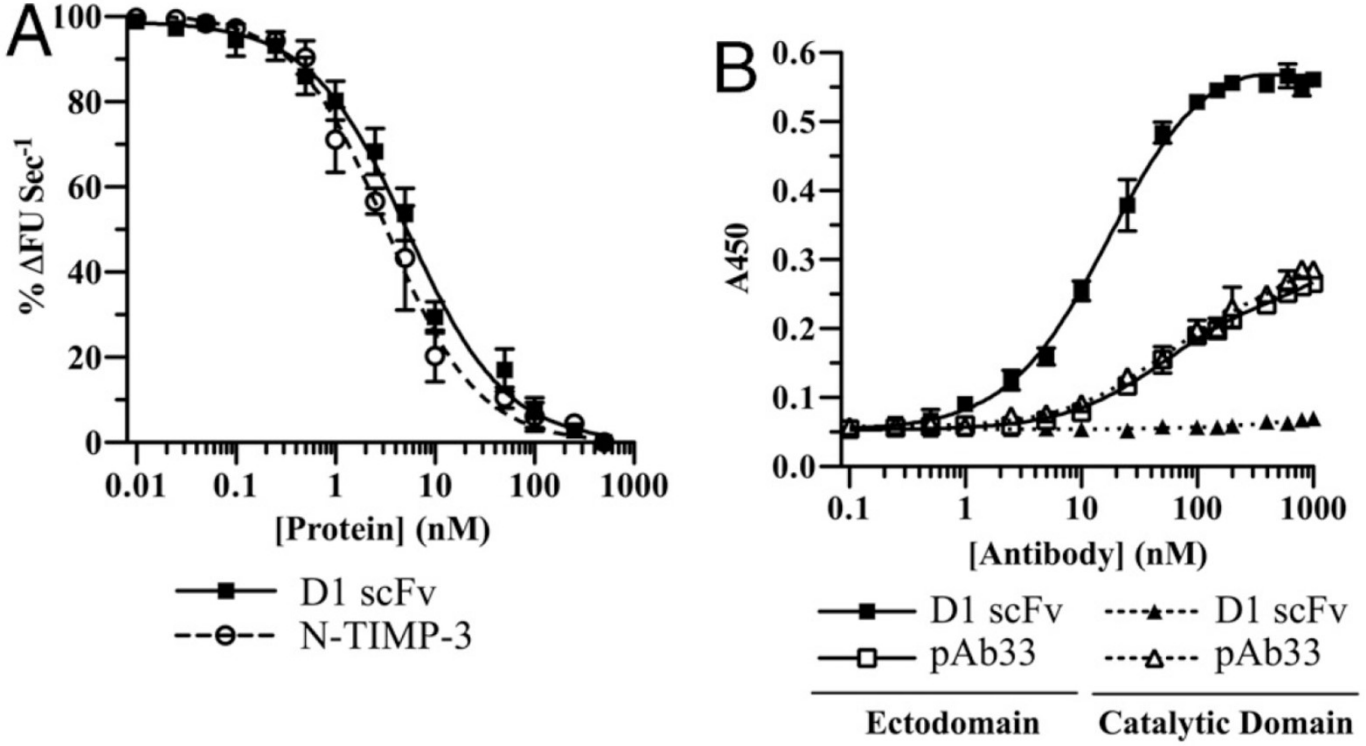
Anti-protease antibody D1 inhibits proteolytic activity of human TACE



……but doesn't bind the catalytic domain

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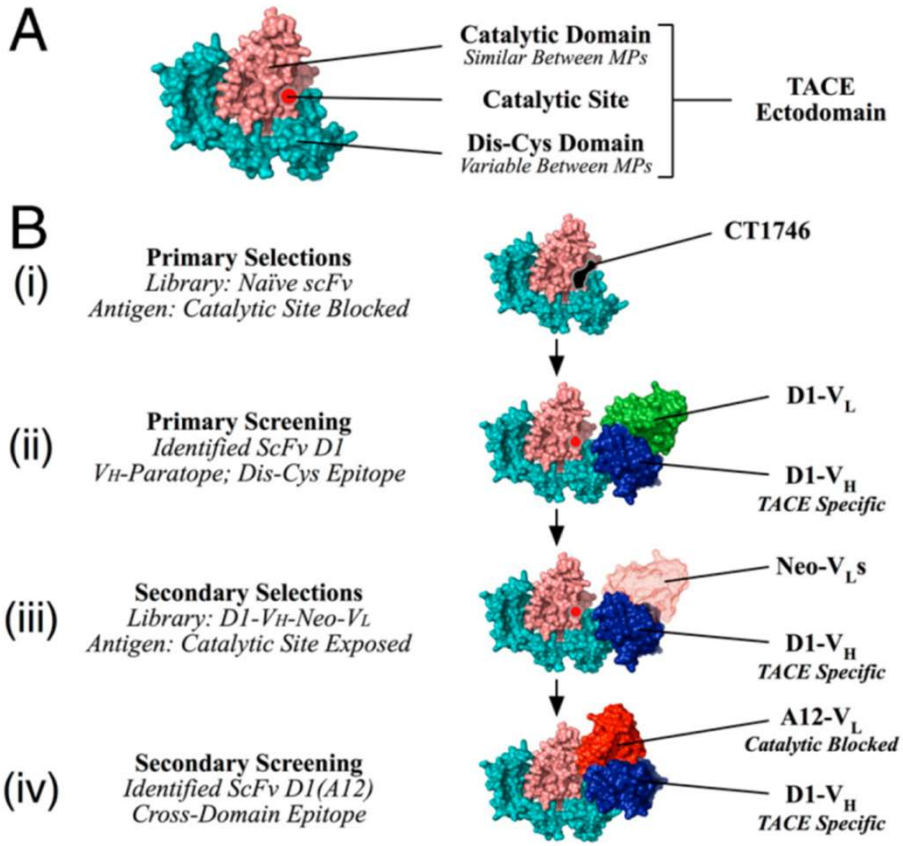
Affinity maturation generates a cross-domain inhibitor of TACE



K_D 26nM

K_D 0.4nM
(5.2nM on catalytic domain)

Tape et al (2011) Cross-Domain Inhibition of TACE Ectodomain PNAS 108 p5578–5583

+4 more anti-protease publications with Gill Murphy lab

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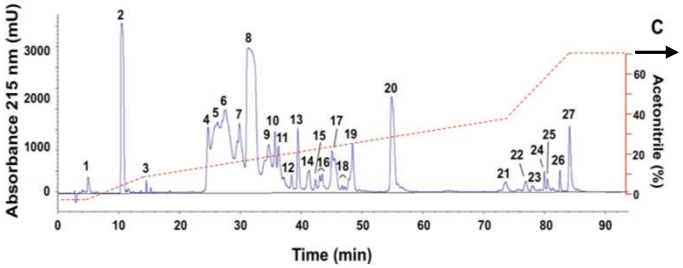
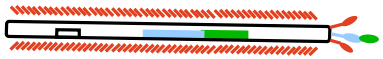
Example 2. Generation of blockers of dendrotoxin from black mamba (*Dendroaspis polylepis*)

Venom fractionation and biotinylation



Phage selection

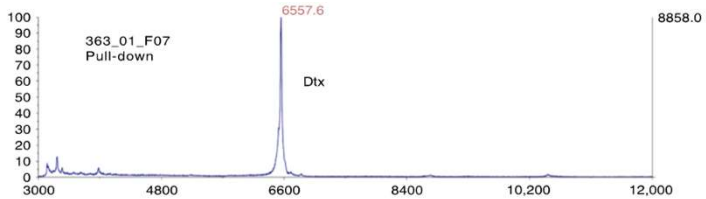


Antibody characterisation

Screen as scFv

Convert to IgG format

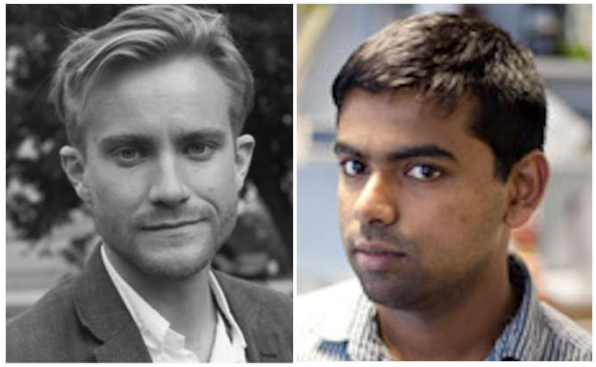
Identify target by affinity pull down and mass spec (dendrotoxin δ)



Andreas H. Laustsen
Technical University of Denmark

Aneesh Karatt Vellatt
John McCafferty
IONTAS Ltd

Prof. José María Gutierrez
Prof. Bruno Lomonte
Instituto Clodomiro Picado



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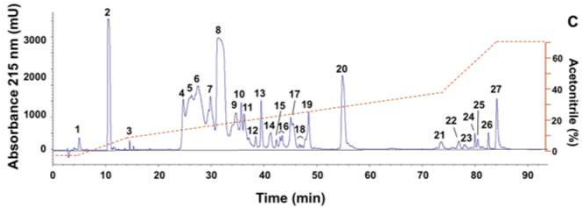
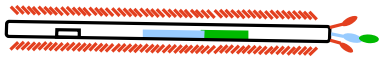
Venom fractionation and biotinylation



Phage selection



In vivo

Blockade of dendrotoxin in vivo using intracerebroventricular injection

In vivo neutralization of dendrotoxin-mediated neurotoxicity of black mamba venom by oligoclonal human IgG antibodies

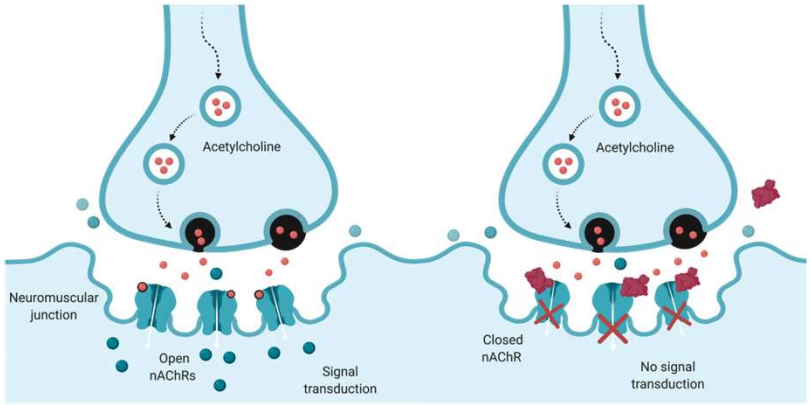
Andreas H. Laustsen[1], Aneesh Karatt-Vellatt[2], Edward W. Masters[2], Ana Silvia Arias[3], Urska Pus[1], Cecilie Knudsen[1], Saioa Oscoz[3], Peter Slavny[2], Daniel T. Griffiths[2], Alice M. Luther[2], Rachael A. Leah[2], Majken Lindholm[2], Bruno Lomonte[3], José María Gutiérrez[3] & John McCafferty[2]

(2018) nature COMMUNICATIONS

Urska Pus **Cecilie Knudsen**

Example 3. Generation of blockers of α-neurotoxin from monocled cobra (Naja kaouthia)

- Long chain a neurotoxin makes up 35% of venom of monocled cobra
- Antagonist of post-synaptic acetylcholine receptor
- Blocks channels causing neuromuscular paralysis



Ledsgaard et al (2022)
In vitro discovery of a human monoclonal antibody that neutralizes lethality of cobra snake venom **mAbs** (in press)

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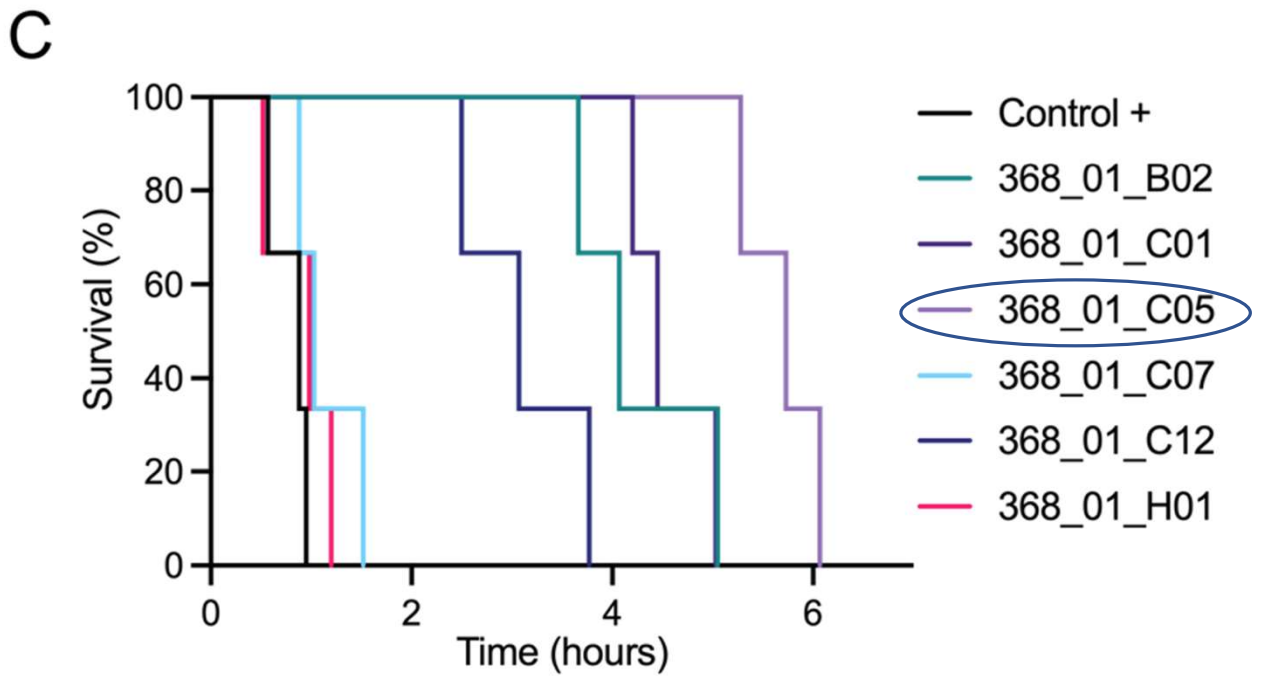
Neutralising human antibodies direct from non-immune IONTAS library



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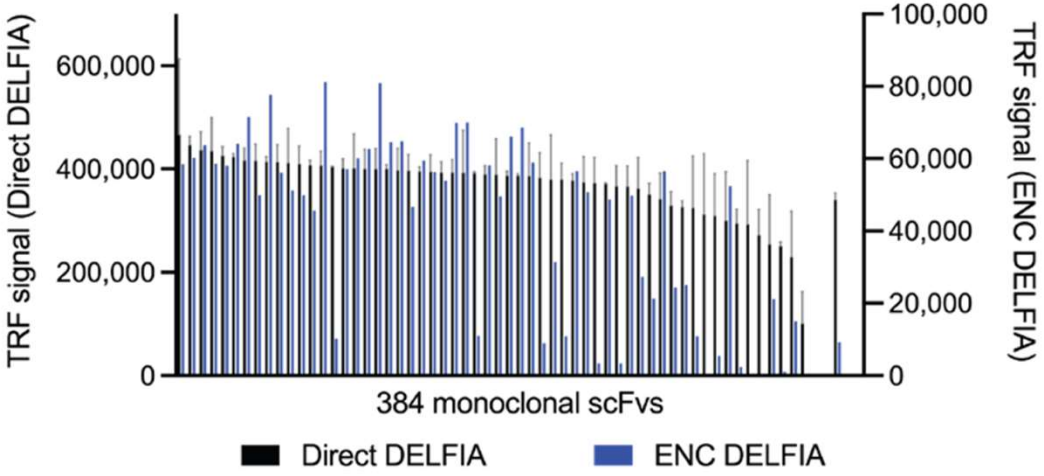
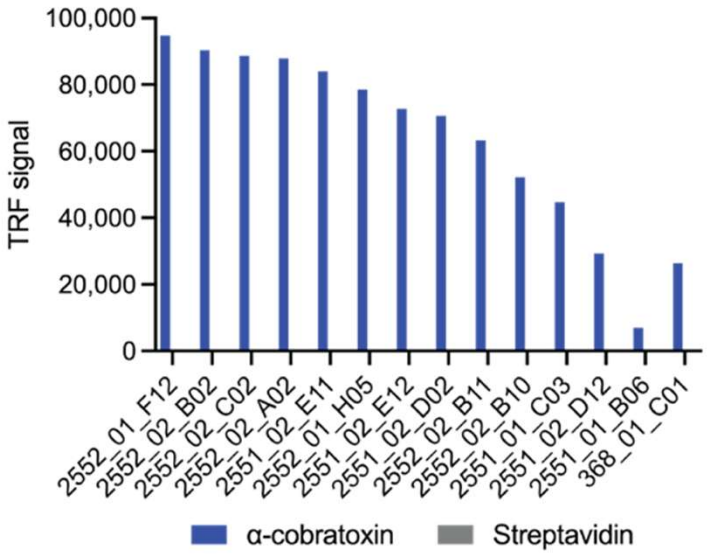
Improving α-neurotoxin binders by chain shuffling

scFv screen



IgG screen



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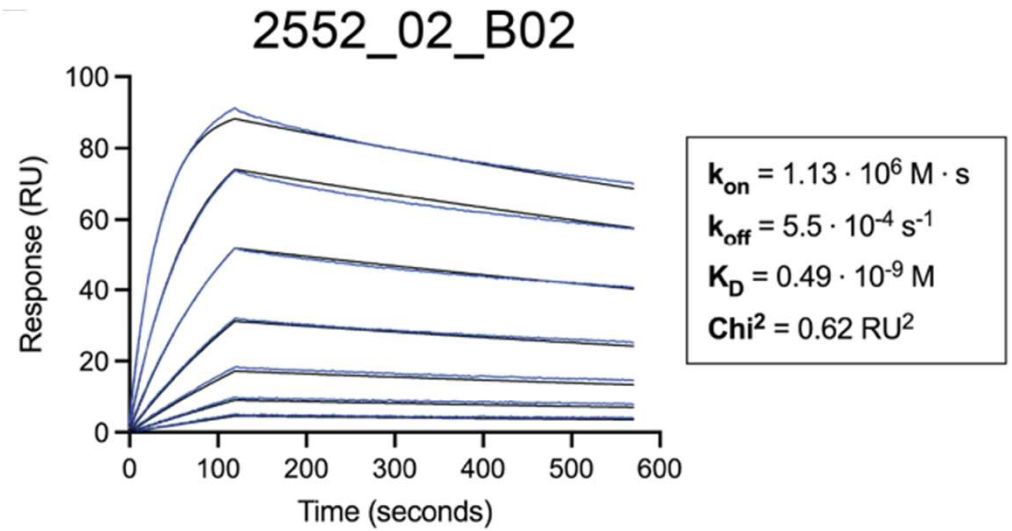
Improving α-neurotoxin binders by chain shuffling

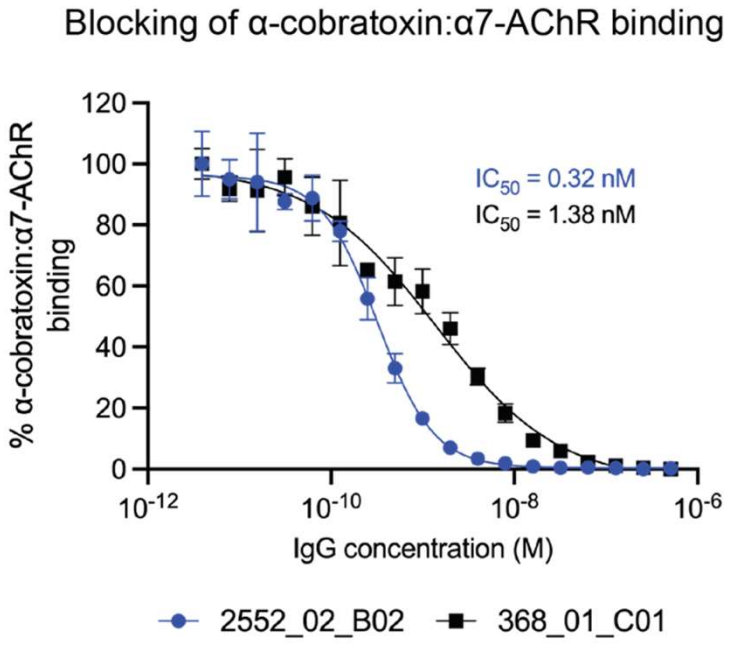
Affinity

3.8nM → 0.49nM



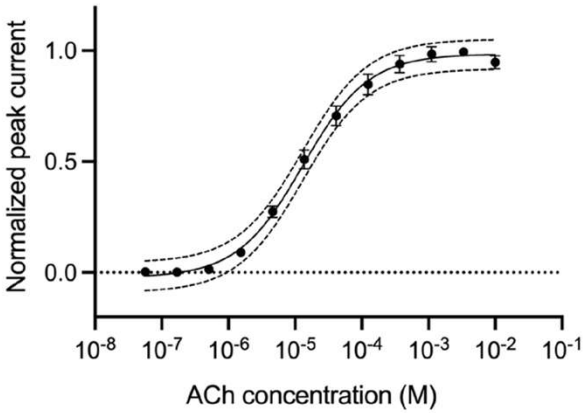
Receptor blocking

1.4nM → 0.32nM



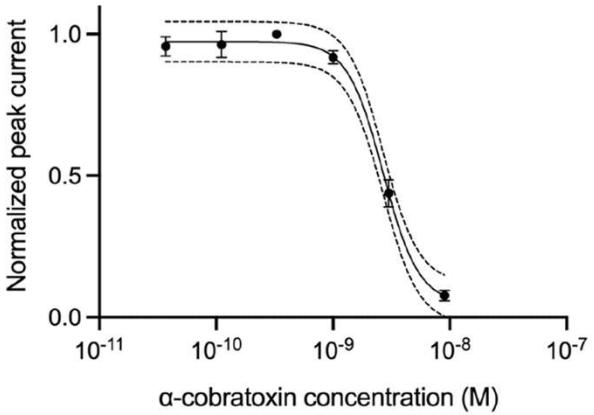
In vitro neutralisation of a-cobratoxin channel blockade Electrophysiology

Acetylcholine titration



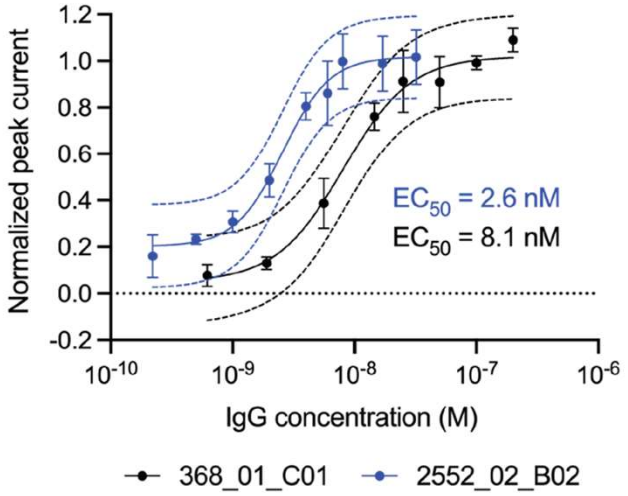
70uM Acetylcholine →

α-neurotoxin titration



4nM cobratoxin →

Antibody titration



SOPHION BIOSCIENCE

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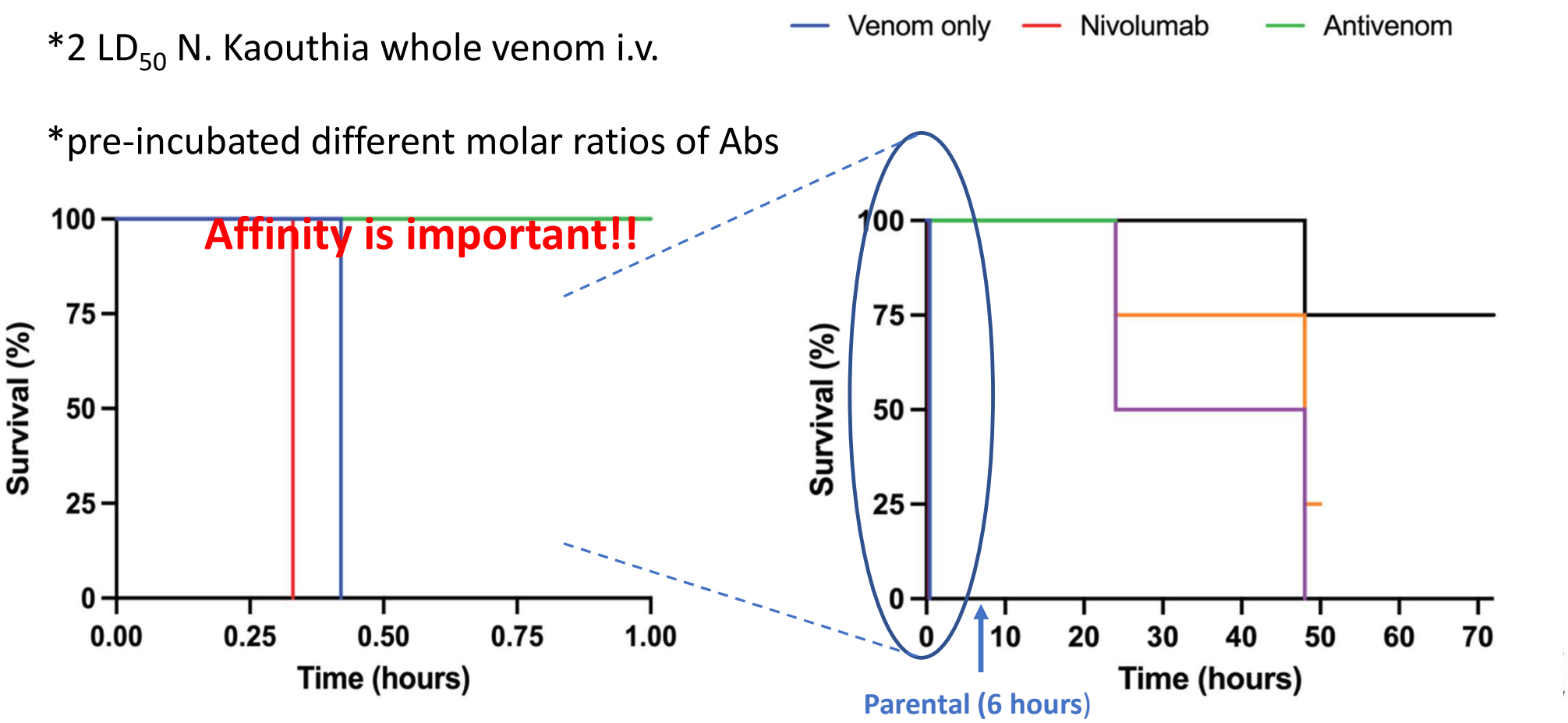
In vivo neutralisation of α-cobratoxin channel blockade

*2 LD_{50} N. Kaouthia whole venom i.v.

*pre-incubated different molar ratios of Abs



Despite problems polyclonal antibodies save lives!

- Can we harvest high affinity immune monoclonals to create defined recombinant antibody cocktails?

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Spitting cobra
Naja nigricollis

Carpet viper
Echis ocellatus

Puff Adder
Bitis arietans

EchiTAb-plus-ICP

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Polyclonal sera as a source of high affinity, neutralising antibodies

Table 2
Neutralization of the toxic activities of venoms by the antivenom.[a]

Venom	Lethality		Hemorrhagic		Coagulant		Necrotizing	
	ED_{50} µL/5 LD_{50s}	ED_{50} µL/mg venom	ED_{50} µL/5 MHDs	ED_{50} µL/mg venom	ED µL/2 MCDs	ED µL/mg venom	ED_{50} µL/1 MND	ED_{50} µL/mg venom
Echis ocellatus (Nigeria)	15 (8–22)	251 (135–352)	0.23 ± 0.01	377 ± 10	0.11 ± 0.005	188 ± 9	2.4 ± 0.2	61 ± 5
Echis leucogaster (Mali)	69 (50–98)	558 (398–787)	1.70 ± 0.22	259 ± 33	0.29 ± 0.02	56 ± 4	4.5 ± 1.4	95 ± 16
Echis pyramidum leakeyi (Kenya)	41 (31–55)	685 (513–917)	4.74 ± 0.32	816 ± 56	0.30 ± 0.06	133 ± 27	8.5 ± 1.6	237 ± 40
Bitis arietans (Nigeria)	26 (21–33)	393 (314–493)	0.15 ± 0.02	191 ± 22	ND[b]	ND[b]	7.0 ± 0.9	109 ± 19
Bitis gabonica (Nigeria)	102 (72–144)	680 (481–961)	0.81 ± 0.07	424 ± 38	ND	ND	5.1 ± 0.4	182 ± 6
Bitis gabonica gabonica	55 (42–73)	395 (300–521)	0.56 ± 0.01	570 ± 5	ND	ND	3.7 ± 0.4	129 ± 30
Bitis rhinoceros	80 (60–107)	645 (483–862)	0.21 ± 0.01	511 ± 21	ND	ND	4.2 ± 0.1	112 ± 4
Bitis nasicornis	139 (101–192)	1639 (1190–2273)	0.52 ± 0.01	1894 ± 37	ND	ND	8.8 ± 0.7	231 ± 19

[a] Neutralization is expressed as Effective Dose 50% (ED_{50}) (lethality, hemorrhagic and necrotizing effects) or Effective Dose (ED) (coagulant effect), in two different ways: (i) µL antivenom required to neutralize the 'challenge dose' of venom used (5 LD_{50s}, 5 MHDs, 2 MCDs, 1 MND); (ii) the ratio µL antivenom/mg venom. Results of lethality are expressed as ED_{50} and the 95% confidence limits are included in parentheses. For hemorrhagic, necrotizing and coagulant activities, results are presented as mean ± S.D. For details, see Materials and Methods.

[b] ND: these venoms did not induce coagulant activity and, therefore, neutralization was not studied.

Segura et at (2010) Preclinical assessment of the efficacy of a new antivenom (EchiTAb-Plus-ICP) for the treatment of viper envenoming in sub-Saharan Africa. *Toxicon* **55** 369-374

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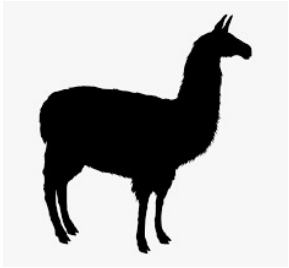
Harvesting polyclonal anti-venoms to create defined recombinant antibody cocktails



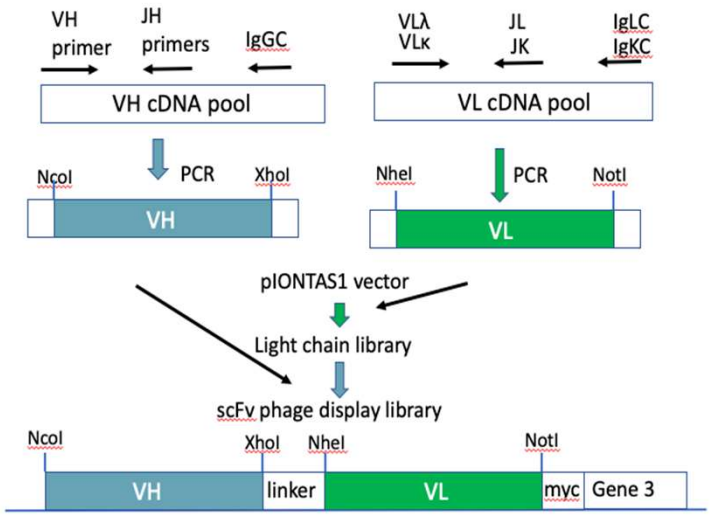
Horse and llama immunization

↓

Clone anti-venom immune repertoire

↓

Immune phage display libraries



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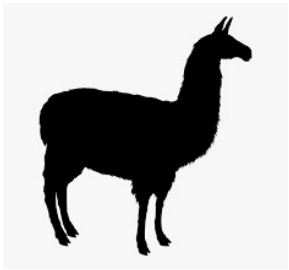
Harvesting polyclonal anti-venoms to create defined recombinant antibody cocktails



Diverse population of 1,000s (chimeric) binders

Horse and llama immunization

Clone anti-venom immune repertoire

Immune phage display libraries

Purified protein

Fractionated venom

Target specific assays

Mass spectrometry target identification

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OPEN ACCESS Freely available online

PLoS NEGLECTED TROPICAL DISEASES

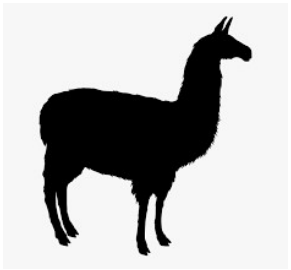
Randomised Controlled Double-Blind Non-Inferiority Trial of Two Antivenoms for Saw-Scaled or Carpet Viper (*Echis ocellatus*) Envenoming in Nigeria

Isa S. Abubakar[1], Saidu B. Abubakar[2], Abdulrazaq G. Habib[3]*, Abdulsalam Nasidi[4], Nandul Durfa[4], Peter O. Yusuf[5], Solomon Larnyang[†], John Garnvwa[6], Elijah Sokomba[7], Lateef Salako[8], R. David G. Theakston[6], Ed Juszczak[9], Nicola Alder[9], David A. Warrell[10], for the Nigeria-UK EchiTab Study Group

Table 2. Summary of primary and secondary (safety) outcomes plus comparative statistics (intention-to-treat population).

Outcome	EchiTAb Plus-ICP	EchiTAb G	Relative Risk[1]	P-value[2]
	(n = 194)	(n = 206)	(one-sided 95% CI)	
PRIMARY OUTCOME				
Permanent restoration of blood coagulability (20WBCT) 6 hr after 1st dose of antivenom	161 (83.0%)	156 (75.7%)	1.10 (Lower limit 1.01)	0.05
SECONDARY (SAFETY) OUTCOMES				
Early anaphylactic-type reactions				
Patients experiencing ≥1 reaction	50 (25.8%)	39 (18.9%)	1.36 (Upper limit 1.86)	0.06
Early pyrogenic reactions	0	0	inestimable	N/A
Late serum sickness type reactions	5/49 (10.2%)	3/58 (5.2%)	1.97 (Upper limit 6.28)	0.27

Potential of chimeric antibodies



Horse and llama immunization

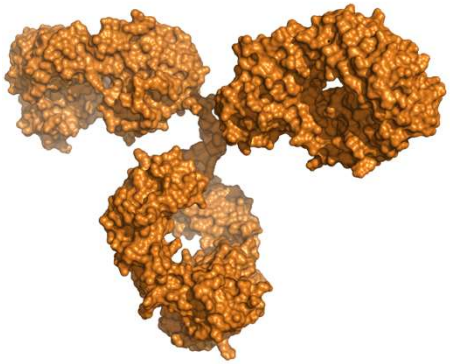
↓

Clone anti-venom immune repertoire

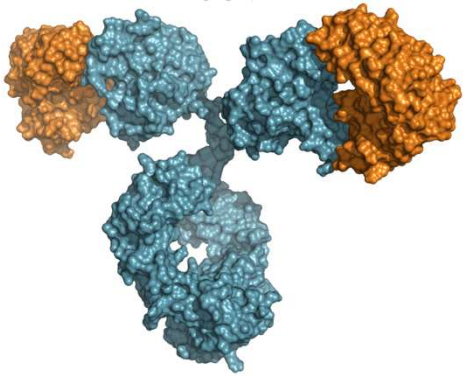
↓

Immune phage display libraries

Murine
1975

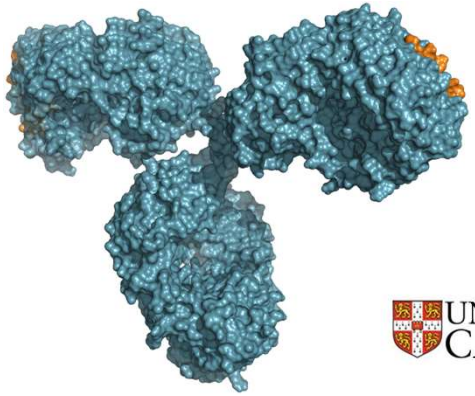


Chimeric
1984



Humanised
1988

Human
1990



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Acknowledgements

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CLODOMIRO PICADO

IONTas

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Andreas Lausten
Line Ledsgaard
Urska Pus
Cecilie Knudsen

Andres Sanchez
Jose Maria Gutierrez (Chema)
Bruno Lomonte

Aneesh Karatt-Vellatt

Gill Murphy
Chris Tape

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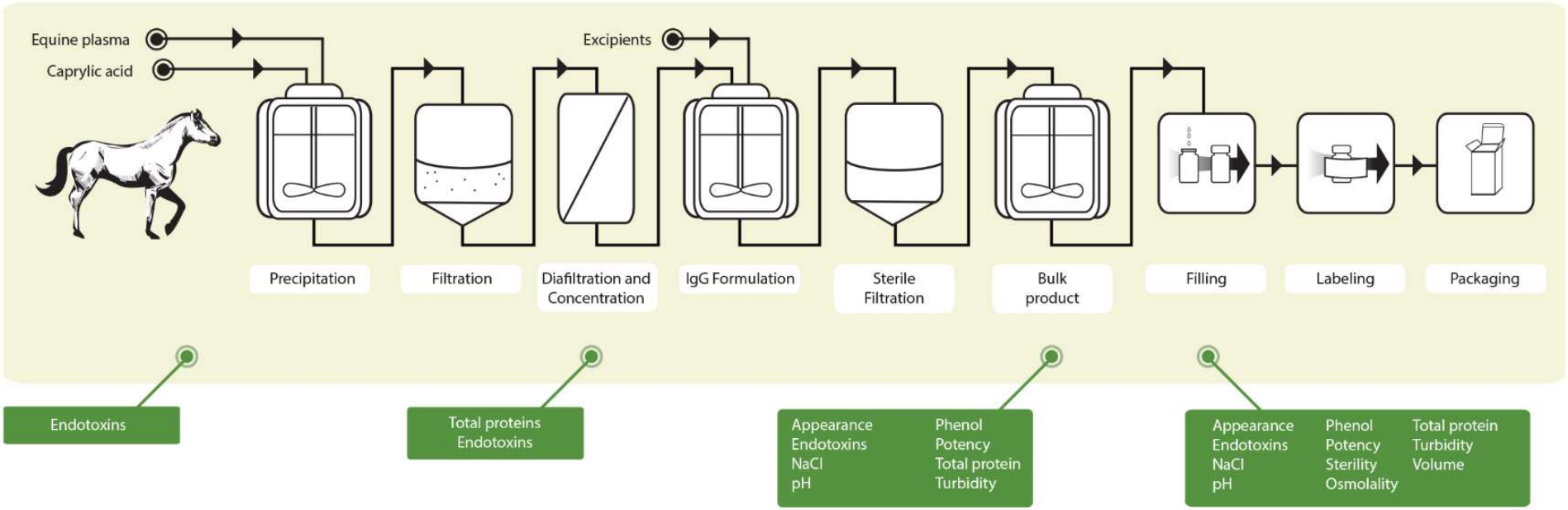
Antivenom production process



Gutierrez et al (2005) transactions of Royal Society of Tropical Medicine and hygeine 99 468-475

Alape-Girón et al., 2021. Frontiers in Medicine

Preclinical assessment of the efficacy of a new antivenom (EchiTAb-Plus-ICP®) for the treatment of viper envenoming in sub-Saharan Africa

Álvaro Segura [a], Mauren Villalta [a], María Herrera [a], Guillermo León [a], Robert Harrison [b], Nandul Durfa [c], Abdusalami Nasidi [c], Juan J. Calvete [d], R. David G. Theakston [b], David A. Warrell [b,e], José María Gutiérrez [a,*]

5x Median Lethal dose (LD_{50})
5x Minimum haemorrhagic dose (MHD)
1.5x Minimum myotoxic dose (MMD)
1x Minimum necrotizing dose (MND)
2x Minimum coagulant doe (MCD)
2x Minimum defibrinogenating dose (MDD)

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Echis pyramidum leakeyi (Kenya)	41 (31–55)	685 (513–917)	4.74 ± 0.32	816 ± 56	0.30 ± 0.06	133 ± 27	8.5 ± 1.6	237 ± 40
Bitis arietans (Nigeria)	26 (21–33)	393 (314–493)	0.15 ± 0.02	191 ± 22	ND[b]	ND[b]	7.0 ± 0.9	109 ± 19
Bitis gabonica (Nigeria)	102 (72–144)	680 (481–961)	0.81 ± 0.07	424 ± 38	ND	ND	5.1 ± 0.4	182 ± 6
Bitis gabonica gabonica	55 (42–73)	395 (300–521)	0.56 ± 0.01	570 ± 5	ND	ND	3.7 ± 0.4	129 ± 30
Bitis rhinoceros	80 (60–107)	645 (483–862)	0.21 ± 0.01	511 ± 21	ND	ND	4.2 ± 0.1	112 ± 4
Bitis nasicornis	139 (101–192)	1639 (1190–2273)	0.52 ± 0.01	1894 ± 37	ND	ND	8.8 ± 0.7	231 ± 19

[a] Neutralization is expressed as Effective Dose 50% (ED_{50}) (lethality, hemorrhagic and necrotizing effects) or Effective Dose (ED) (coagulant effect), in two different ways: (i) µL antivenom required to neutralize the 'challenge dose' of venom used (5 LD_{50s}, 5 MHDs, 2 MCDs, 1 MND); (ii) the ratio µL antivenom/mg venom. Results of lethality are expressed as ED_{50} and the 95% confidence limits are included in parentheses. For hemorrhagic, necrotizing and coagulant activities, results are presented as mean ± S.D. For details, see Materials and Methods.

[b] ND: these venoms did not induce coagulant activity and, therefore, neutralization was not studied.

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Pan-African polyspecific antivenom produced by caprylic acid purification of horse IgG: an alternative to the antivenom crisis in Africa

J.M. Gutiérrez[a,*], E. Rojas[a], L. Quesada[a], G. León[a], J. Núñez[a], G.D. Laing[b], M. Sasa[a], J.M. Renjifo[c], A. Nasidi[d], D.A. Warrell[e], R.D.G. Theakston[b], G. Rojas[a]

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Table 4 Neutralization of toxic activities of three African snake venoms by Pan-African antivenom[a]

Activity	*Echis ocellatus*		*Bitis arietans*		*Naja nigricollis*	
	μl/challenge dose	μl/mg venom	μl/challenge dose	μl/mg venom	μl/challenge dose	μl/mg venom
Lethality	26 (20–32)	454 (357–555)	20 (15–26)	238 (181–312)	735 (594–909)	7042 (5682–8696)
Haemorrhagic	0.27 ± 0.02	168 ± 15	0.19 ± 0.01	148 ± 8	ND	ND
Myotoxic	30 ± 5	353 ± 63	78 ± 10	830 ± 106	4.7 ± 0.5	1353 ± 149
Dermonecrotic	4.8	109	10.0	178	9.8	233
Coagulant	3.7 ± 0.03	1078 ± 8	ND	ND	ND	ND
Defibrinogenating	5	1000	ND	ND	ND	ND

Results are presented as mean ± SD, or for lethality as mean (95% confidence limits); ND: neutralization was not studied because the venom lacks the activity.

[a] Neutralization was expressed in two different ways: (i) μl antivenom required to neutralize the challenge dose of venom utilized for each effect, and (ii) μl antivenom per mg of venom.