FAPESP France Week 2025

TOULOUSE 10-12 juin

From Deadly Animal Toxins to Life-Saving Drugs

Eliane Candiani Arantes, PhD
Laboratory of Animal Toxins
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Center for Translational Sciences & Biopharmaceuticals Development

FAPESP France Week 2025

FAPESP – Process 2021/11936-3

Center for Translational Science and Biopharmaceuticals Development

Grantee: Benedito Barraviera, *PhD, Full Professor*

Host Institution: CEVAP *(Center for the Study of Venoms and Venomous Animals)* – **UNESP** *(São Paulo State University "Júlio de Mesquita Filho")*

Board of Directors

Director: *Benedito Barraviera*
Associate-Director: *Eliane Candiani Arantes Braga*
Partnership Coordinator: *Rui Seabra Ferreira Junior*
Partnership Manager: *Carlos Roberto Prudêncio*
Communications Coordinator: *Saulo Phillipe Sebastião Guerra*
Communications Manager: *Ana Silvia Sartori Barraviera Seabra Ferreira*

Infrastructure

Lead Institution: unesp

Partner Institutions:

USP · Instituto Adolfo Lutz · Instituto Biológico · UNIFESP Universidade Federal de São Paulo 1933 · Instituto de Infectologia Emílio Ribas · Instituto SENAI de Inovação Biotecnologia

International partners: *KU Leuven (Belgium) & SRM University (India).*

BioPharma — **Pilot Biopharmaceutical Manufacturing Facility for Clinical Research** *(CDMO model - Contract Development and Manufacturing Organization) — unique in Latin America.*



Establishing a **CDMO specialized in monoclonal antibodies (mAbs) at one of major Brazilian public university** *(***UNESP***) is a strategic initiative that aligns with Brazil's* **biopharma ambitions, public health needs, and economic reality**

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BioPharma

The first Brazilian CDMO

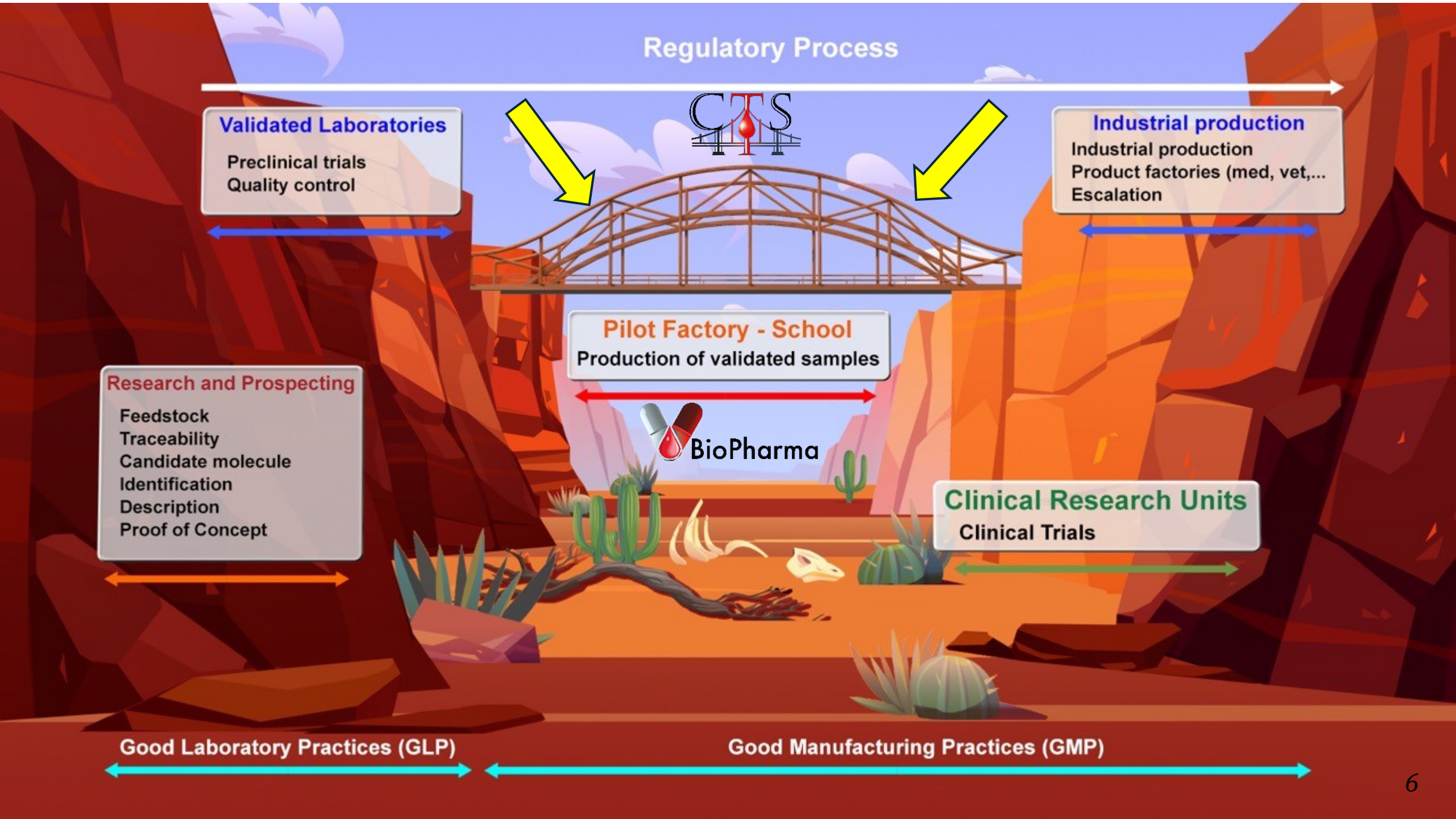
Supports GMP-compliant pilot-scale **manufacturing** *of validated* **drugs for clinical trials**.

Supported by:

FAPESP – *São Paulo Research Foundation*
MCTI – *Ministry of Science, Technology and Innovation;*
MS – *Ministry of Health*
MEC – *Ministry of Education*

V-BioPharma

1. *V-BioPharma delivers strategic solutions in biologics and* **CDMO** *services*
2. **Reduces dependence** *on imports of Active Pharmaceutical Ingredients (***APIs***) and medicines*
3. *Strengthens* **Brazil's self-sufficiency** *in pharmaceutical production*
4. *Positions itself as a leader in* **biologics**
5. *Paves the way for* **innovation** *in developing new treatments and advancing national biotechnology.*


Regulatory Process
Validated Laboratories
Preclinical trials
Quality control
CTS
Industrial production
Industrial production
Product factories (med, vet,...
Escalation
Pilot Factory - School
Production of validated samples
BioPharma
Research and Prospecting
Feedstock
Traceability
Candidate molecule
Identification
Description
Proof of Concept
Clinical Research Units
Clinical Trials
Good Laboratory Practices (GLP)
Good Manufacturing Practices (GMP)

CTS & V-BioPharma: Research-to-Clinical Translation

1. *V-BioPharma provides the* **technological infrastructure** *for CTS–CEVAP research*
2. *CTS-CEVAP-developed* **products will be scaled up** *at V-BioPharma's pilot facility*
3. *Enables production of* **clinical-grade compounds** *for future trials.*
4. *Integration between CTS–CEVAP and V-BioPharma ensures the* **translation** *from bench to the patient's bedside*

CTS – Objectives

*Improve **Fibrin Sealant** and **Apilic Antivenom**, research and produce recombinant molecules and mono and polyclonal antibodies.*

CTS - Research Lines

1. *Cloning and Expression of Bioactive Molecules*
2. *Construction of a Llama-Derived Phage Display Library*
3. *Monoclonal antibodies and biosimilars*
4. *Development and production of polyclonal antibodies - Apilic Antivenom*

Fibrin Sealant

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DA ECONOMIA
INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

CARTA PATENTE Nº BR 102014011432-7

O INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL concede a presente PATENTE DE INVENÇÃO, que outorga ao seu titular a propriedade da invenção caracterizada neste título, em todo o território nacional, garantindo os direitos dela decorrentes, previstos na legislação em vigor.

(21) Número do Depósito: BR 102014011432-7

Patente de trâmite prioritário

(22) Data do Depósito: 12/05/2014

(43) Data da Publicação Nacional: 08/12/2015

(51) Classificação Internacional: A61K 35/58; A61K 38/36; A61K 38/37; A61K 33/06; A61L 24/10; A61L 24/02; C12N 5/077.

(54) Título: ARCABOUÇO TRIDIMENSIONAL PARA CÉLULAS TRONCO, PROCESSO DE OBTENÇÃO DO MESMO E SEU USO

(73) Titular: UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO, Pessoa Jurídica. Endereço: Rua Quirino de Andrade, 215, Centro, São Paulo, SP, BRASIL(BR), 01049-010, Brasileira

(72) Inventor: RUI SEABRA FERREIRA JUNIOR; BENEDITO BARRAVIERA.

Código de Controle: C02D7474DCD7C886 BB7C565BC6CA57A3

Prazo de Validade: 20 (vinte) anos contados a partir de 12/05/2014, observadas as condições legais

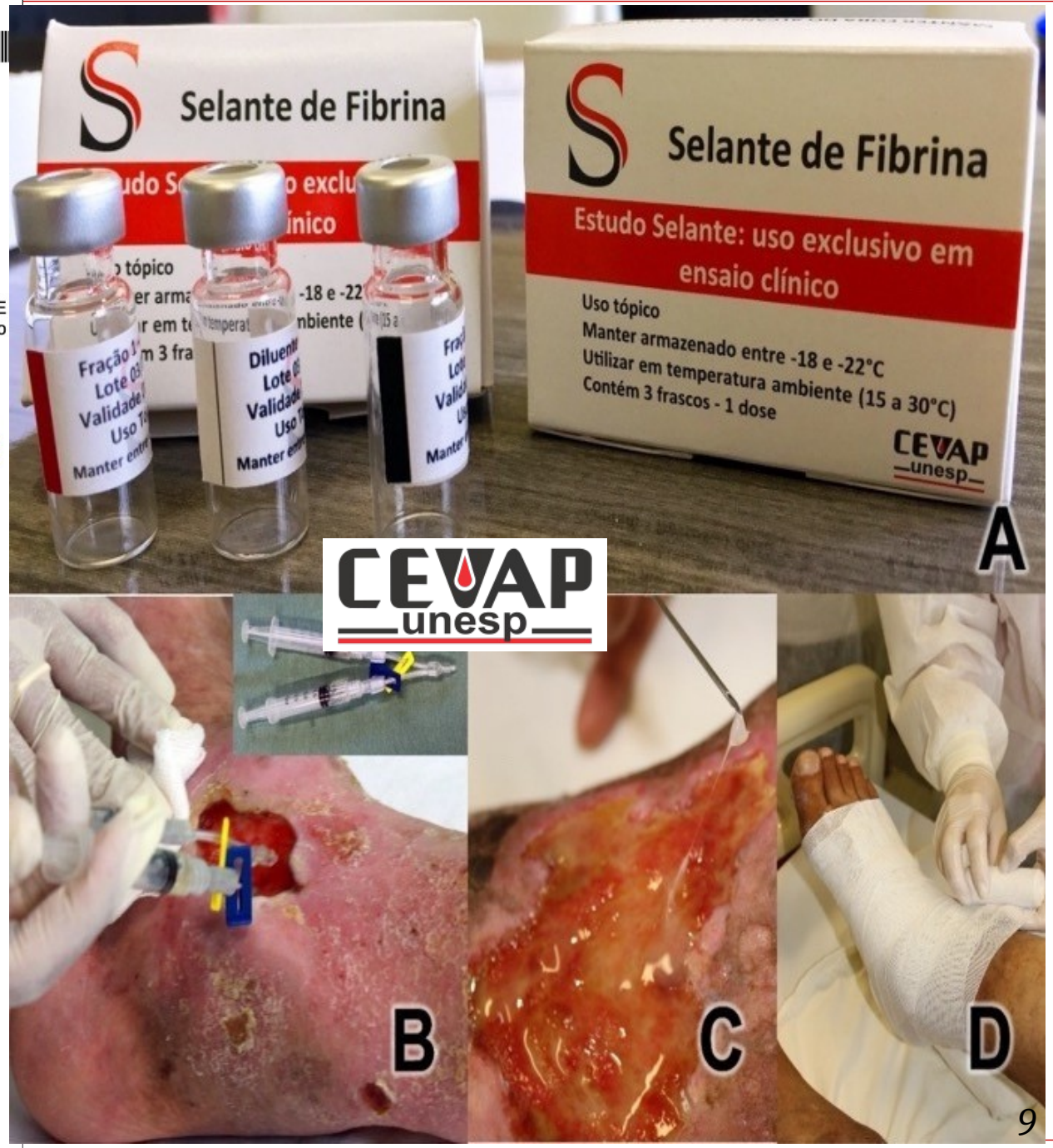
Expedida em: 07/06/2022

Assinado digitalmente por:
Alexandre Dantas Rodrigues
Diretor Substituto de Patentes, Programas de Computador e Topografias de Circuitos Integrados

https://pubmed.ncbi.nlm.nih.gov/33708219/

2022

Phase II clinical trial concluded!!!



Apilic Antivenom

REPÚBLICA FEDERATIVA DO BRASIL
MINISTÉRIO DO DESENVOLVIMENTO, INDÚSTRIA, COMÉRCIO E SERVIÇOS
INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL

CARTA PATENTE Nº BR 102015026308-2

O INSTITUTO NACIONAL DA PROPRIEDADE INDUSTRIAL concede a presente PATENTE DE INVENÇÃO, que outorga ao seu titular a propriedade da invenção caracterizada neste título, em todo o território nacional, garantindo os direitos dela decorrentes, previstos na legislação em vigor.

(21) Número do Depósito: BR 102015026308-2

(22) Data do Depósito: 16/10/2015

(43) Data da Publicação Nacional: 02/05/2017

(51) Classificação Internacional: A61K 39/395; A61K 35/16; C07K 16/18; A61P 39/02.

(54) Título: PROCESSO DE OBTENÇÃO DE SORO EQUINO ANTIAPÍLICO, SORO EQUINO ANTIAPÍLICO E SEUS USOS

(73) Titular: FUNDAÇÃO BUTANTAN. CGC/CPF: 61189445000156. Endereço: Av. Vital Brasil, nº 1500, Butantã, São Paulo, SP, BRASIL(BR), 05503900; INSTITUTO VITAL BRASIL S/A. CGC/CPF: 30064034000100. Endereço: Rua Maestro José Botelho, nº 64, Vital Brasil, Rio de Janeiro, RJ, BRASIL (BR), 24230410; UNIVERSIDADE ESTADUAL PAULISTA JULIO DE MESQUITA FILHO. CGC/CPF: 48031918000124. Endereço: Rua Quirino de Andrade, 215, Centro, São Paulo, SP, BRASIL(BR), 01049010

(72) Inventor: RUI SEABRA FERREIRA JUNIOR; BENEDITO BARRAVIERA; RICARDO DE OLIVEIRA ORSI; LUÍS EDUARDO RIBEIRO DA CUNHA; DANIEL PIMENTA.

Prazo de Validade: 20 (vinte) anos contados a partir de 16/10/2015, observadas as condições legais

Expedida em: 02/01/2024

Assinado digitalmente por:
Alexandre Gomes Ciancio
Diretor Substituto de Patentes, Programas de Computador e Topografias de Circuitos Integrados



2024

Phase II clinical trial concluded!!!

https://pubmed.ncbi.nlm.nih.gov/33841437/

Heterologous Fibrin Sealant Lyophilized (HFS-Lyo)

The **first CTS-Cevap product approved** *by the Brazilian* **Ministry of Health**, *with* **FAPESP support** *(Grants 2021/11936-3 and 2023/16514-5), through the Local Development and Innovation Program (PDIL),* **for a Phase I/II clinical trial** *(2025–2029)*





1. Cloning and Expression of Bioactive Molecules

- *Study and production of bioactive toxins (***collinein-1,** *giroxin,* **VEGF ...)**
- *Develop an advanced lyophilized Fibrin Sealant (version 2.0)*



Fibrinogen -rich cryoprecipitate - ***Bubalus bubalis*** **plasma**

Provides the structural matrix for fibrin clot formation



Giroxin: thrombin-like Enzyme

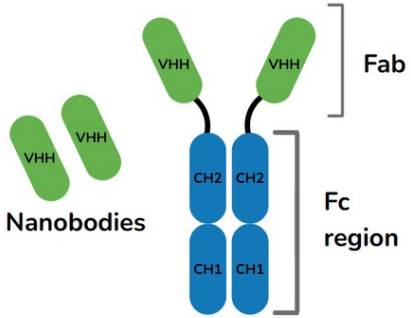
Catalyzes the conversion of fibrinogen into fibrin

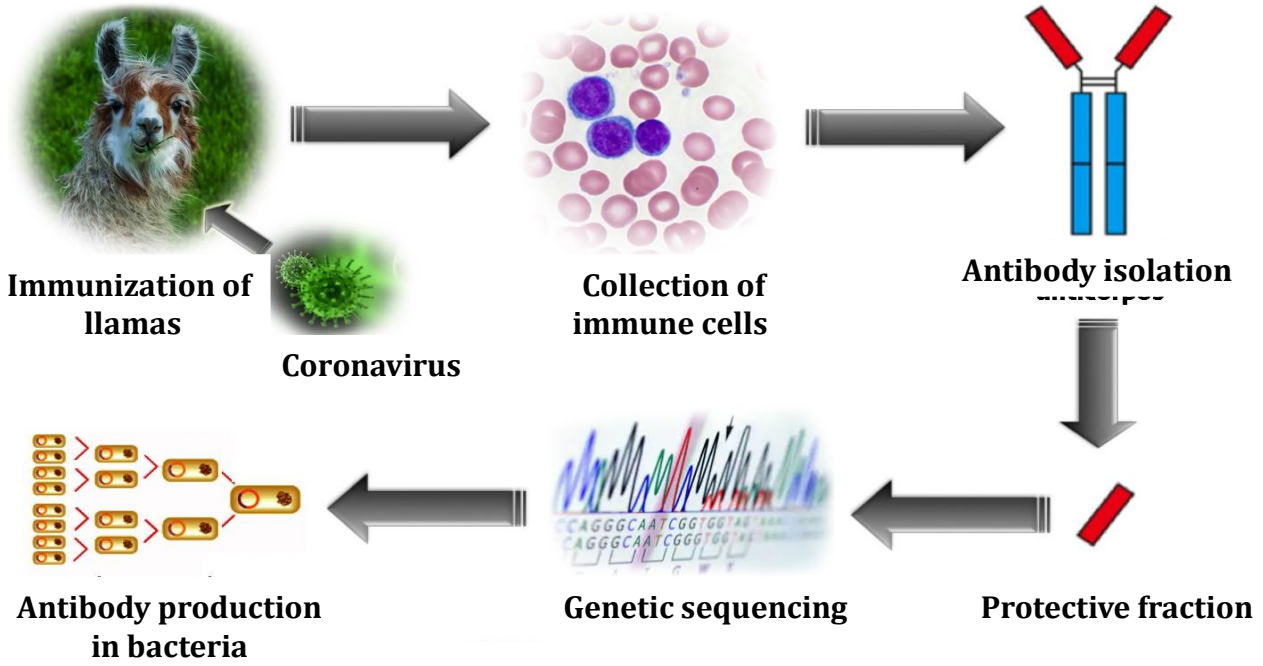


Fibrin Sealant

2. Llama-Derived Phage Display Library

- *Generation of recombinant* **nanobodies**
- *Applications: COVID-19 and HER2-positive breast cancer*

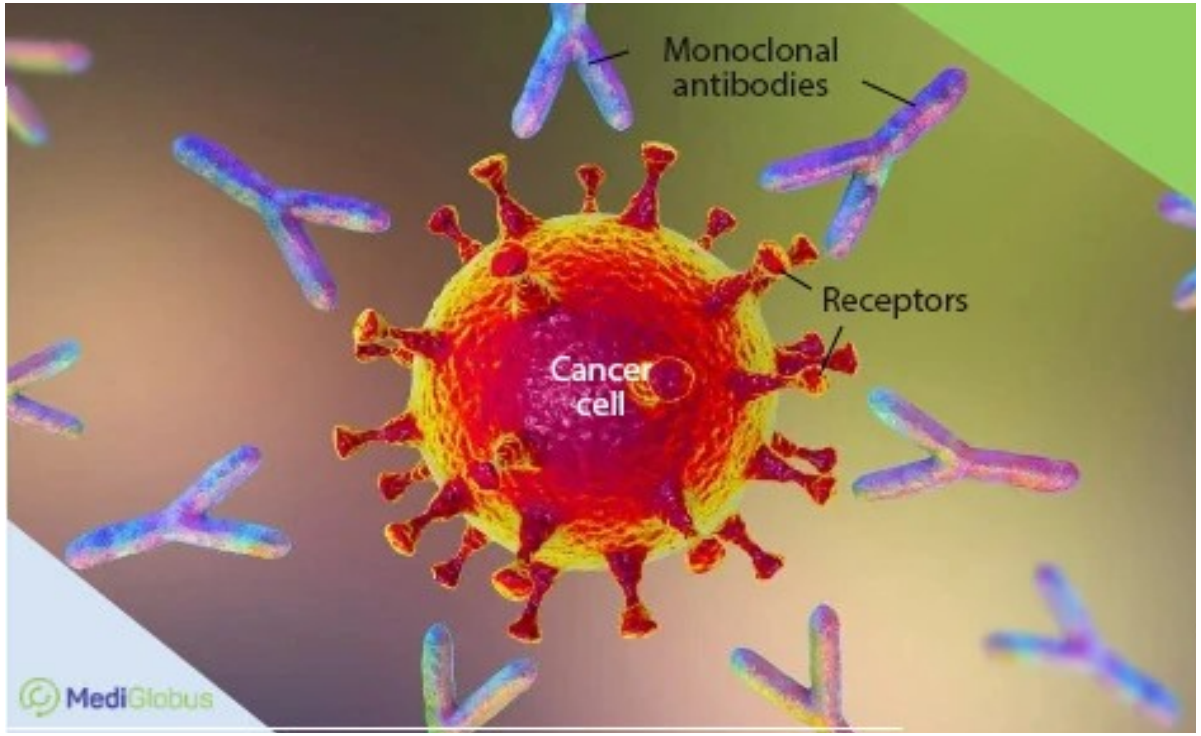






3. Monoclonal antibodies and biosimilars

- *Production of* **monoclonal antibodies (mAbs)** *targeting specific molecular markers.*
- *One of the key targets: HER2 receptor in* **breast cancer**.



Monoclonal antibodies *are a group of medicines that are laboratory-grown copies of immune cells called antibodies*

https://mediglobus.com/wp-content/uploads/2024/07/monoclonal-antibodies-in-action-eng.jpg.webp

4. Development and production of polyclonal antibodies

- *Production of equine polyclonal antibodies.*
- *Designed for treatment of multiple stings by honeybees (Apis mellifera).*



Apis mellifera **venom**



Equine polyclonal antibodies



Apilic Antivenom

Laboratory of Animal Toxins – CTS Research Lines

1. **Snake Venom VEGF and Serine Protease for Adhesive Biopolymer Development**
   - *Recombinant Production, PEGylation and Evaluation of Function, Stability, Solubility and Immunogenicity*
2. ***Tityus serrulatus* α-KTx Toxins for Immunosuppressive Therapies**
   - *Recombinant Expression, PEGylation, and Functional Evaluation of Ts-KTx-1 and Ts-KTx-2 in an Inflammatory Disease Model and potassium channels.*
3. **Human scFv Antibodies Against *Bothrops* Metalloproteases**
   - *Discovery of pH-Dependent and Cross-Reactive scFvs (single-chain fragment variable) via Phage Display for Next-Generation Antivenom Development*
4. **Scorpion Venom Peptide with Antitumor Potential**
   - *Discovery of a Novel Scorpine-Like Molecule from Brotheas amazonicus with Cytolytic Activity Against Breast Cancer Cells*

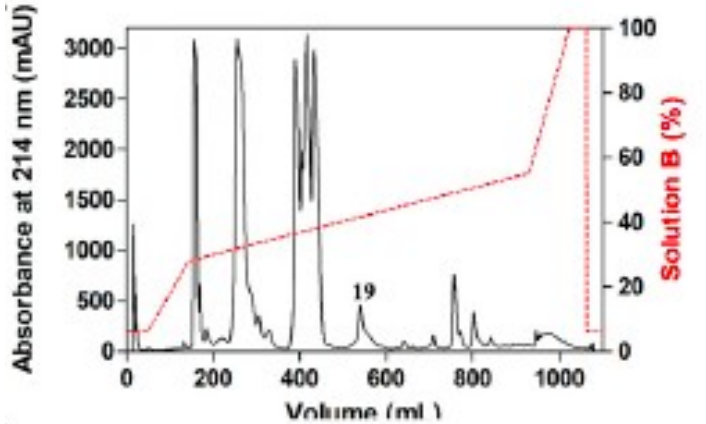
Why Animal Venom Toxins Are Promising Drug Candidates?

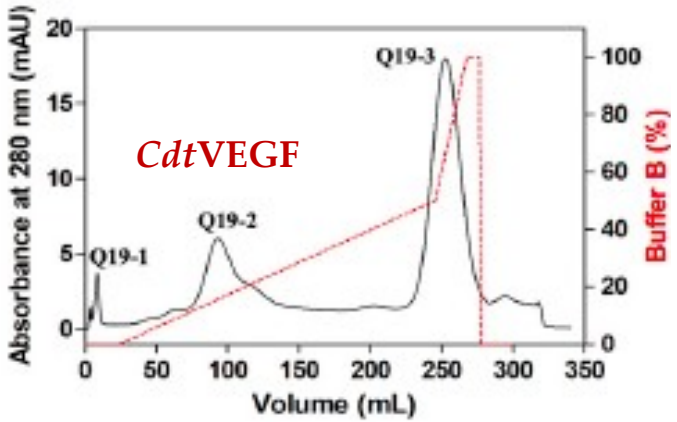
1. **High Specificity and Potency**
   Evolved to target molecular structures (ion channels, receptors) with precision and efficacy.
2. **Enhanced Stability**
   Structural features like disulfide bridges confer resistance to degradation, ensuring prolonged activity.
3. **Broad Therapeutic Targets**
   Capable of modulating complex biological pathways,
4. **Successful Clinical Inspiration**
   - **Captopril***: ACE inhibitor, from Bothrops jararaca venom*
   - **Ziconotide***: potent analgesic from Conus magus peptide*
   - **Ozempic (Semaglutide***): type 2 diabetes and obesity, from Gila monster (Heloderma suspectum).*

Native *Cdt*VEGF

Vascular Endothelial Growth Factor

Purification

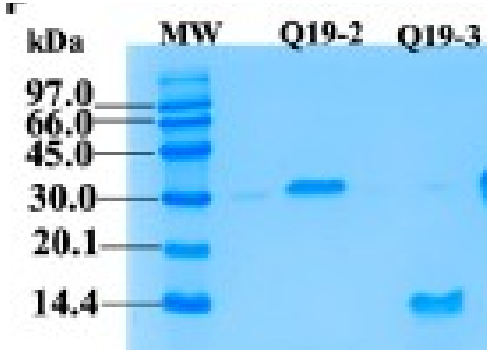




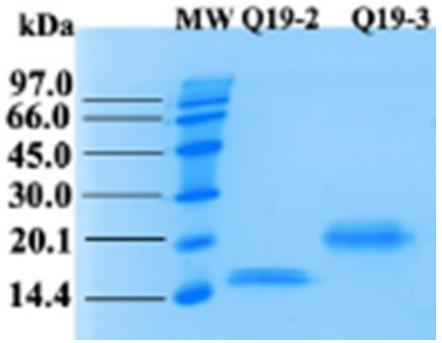
CdtVEGF: **2%** *of the C. d. terrificus venom*

SDS-PAGE

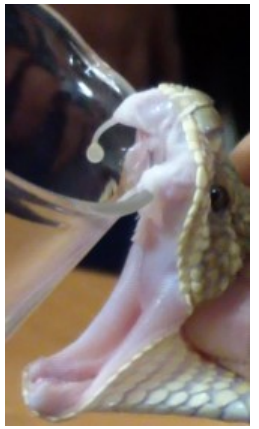
Non-reduced disulfide bridges



Reduced disulfide bridges



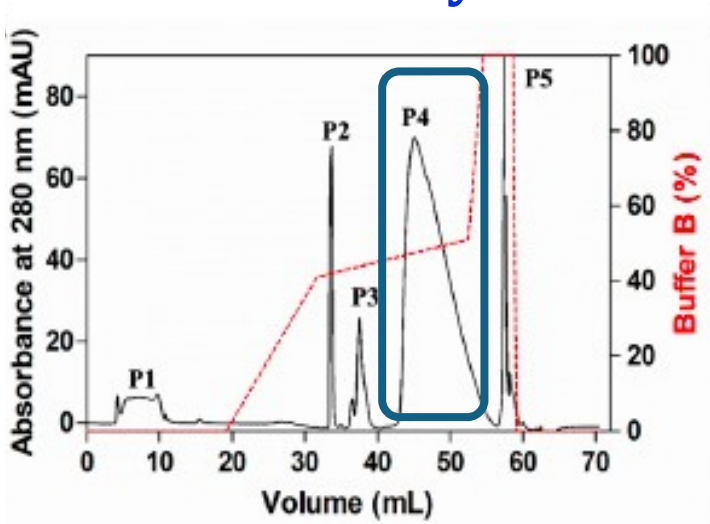
CdtVEGF is a **homodimeric molecule**

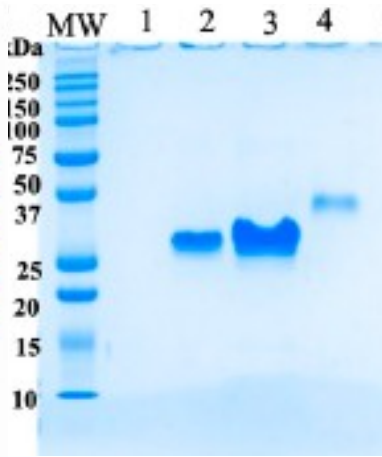


Crotalus durissus terrificus



PEGylation





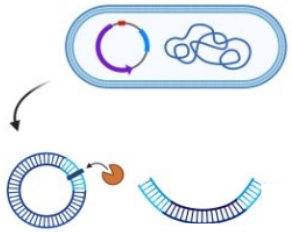
PEGylation – mPEG maleimide 5 kDa

Ferreira et al., **Toxins,** *v. 15, p. 483, 2023*

Expression of *Cdt*VEGF

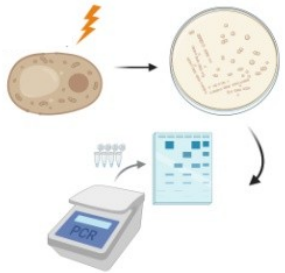
Expression in ***Pichia pastoris*** *and testing of snake venom* **VEGF** *and* **serine protease** *for developing a* **new adhesive biopolymer**

1 Plasmid



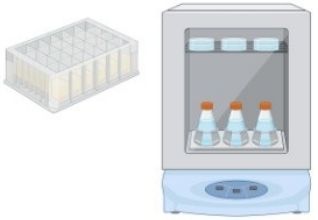
- *E. coli* propagation
- Linearization (PmeI)

2 Transformation and colony PCR



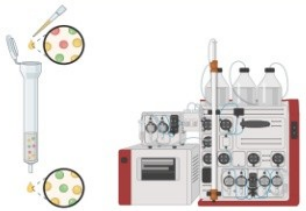
- Electroporation on *Pichia pastoris*
- 10 colonies
- PCR

3 Protein expression



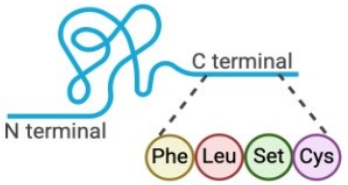
- Different pHs
- Large scale expression (144h, pH 7,0)

4 Protein purification



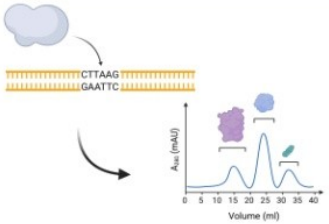
- IMAC
- Reversed-phase chromatography

5 Protein sequencing

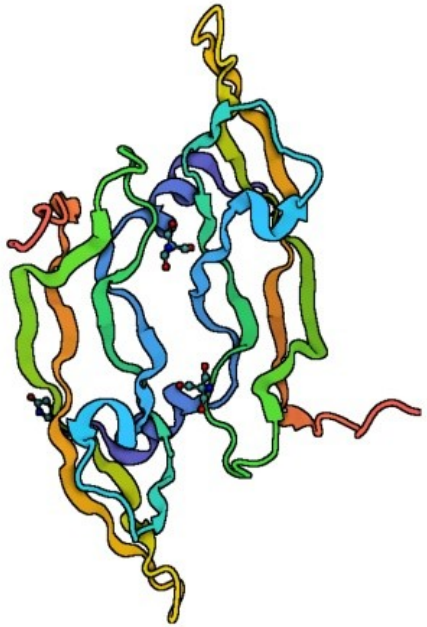


- Edman degradation

6 Poli-his cleavage



- TEV enzyme
- Reversed-phase chromatography



rCdtVEGF

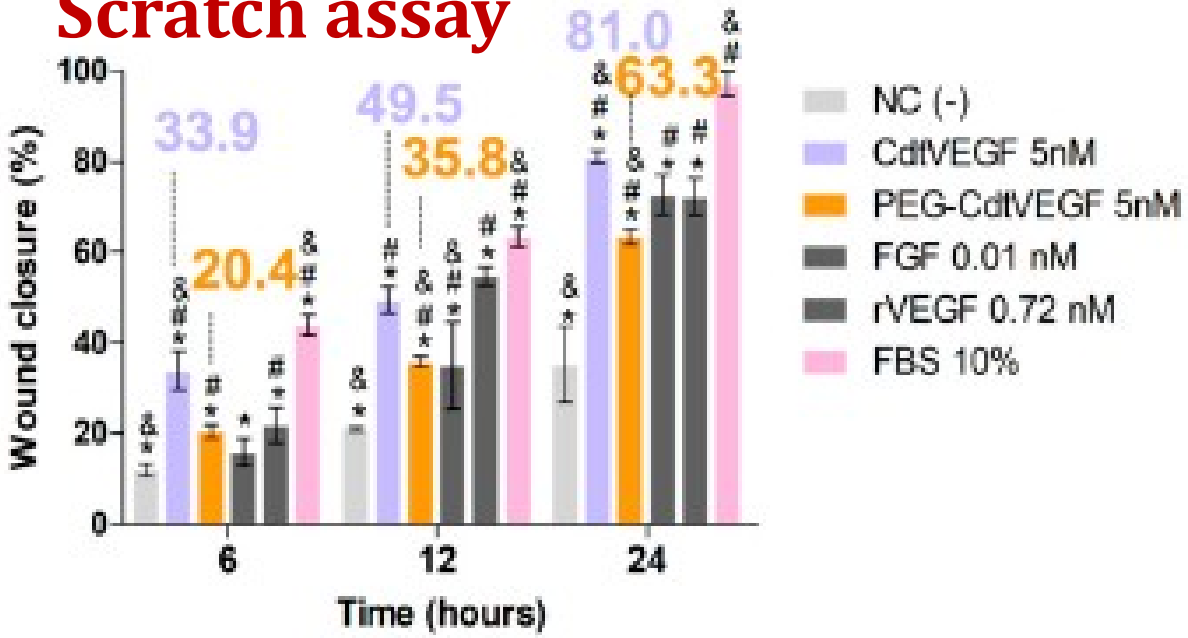
Recombinant Vascular endothelial growth factor

Created in https://BioRender.com

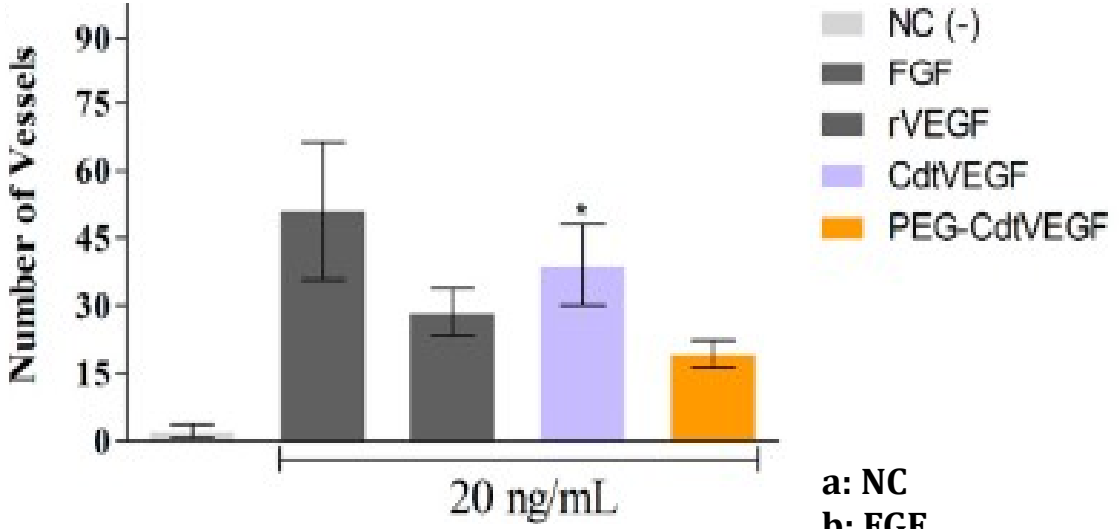
In vitro functional assays of *Cdt*VEGF

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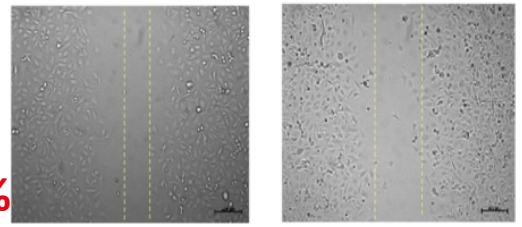
Scratch assay



Tube formation on Matrigel®



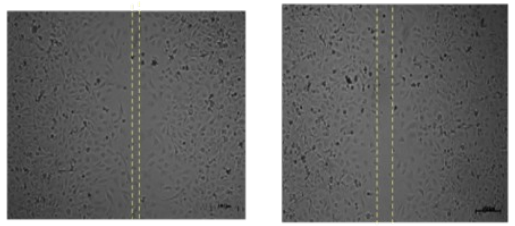
*Cdt*VEGF (5 nM) PEG-*Cdt*VEGF (5 nM)



12 h

49.5%

35.8%



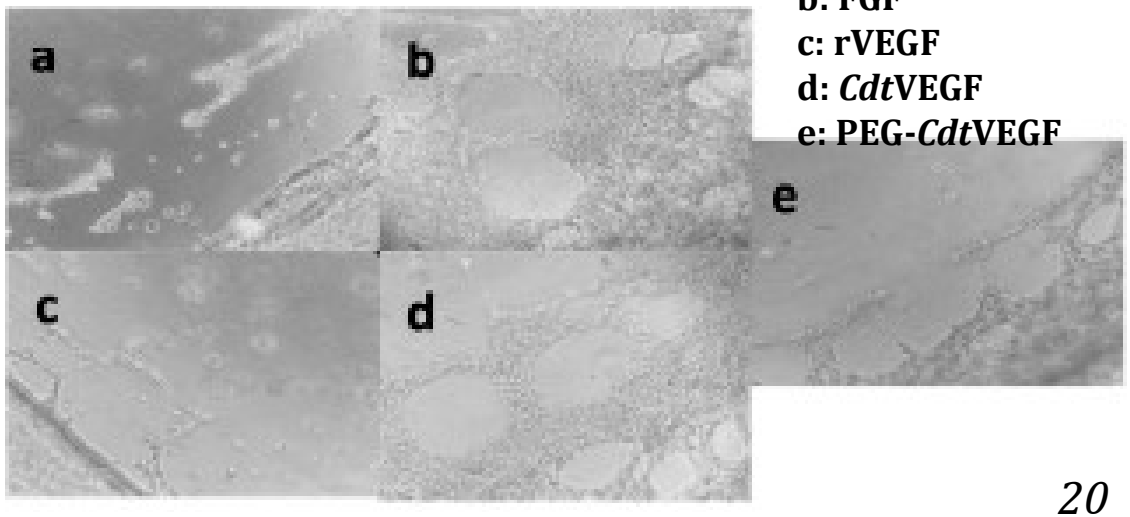
24h

81%

63.3%

Stimulates wound healing Proliferation

Promotes the formation of blood vessels Neovascularization

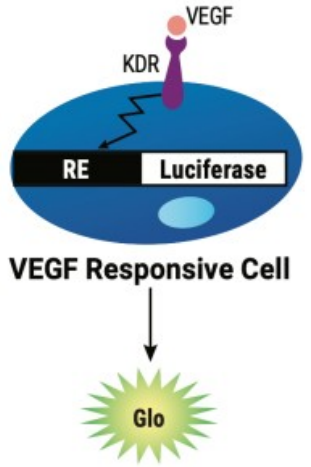


a: NC
b: FGF
c: rVEGF
d: *Cdt*VEGF
e: PEG-*Cdt*VEGF

Functional evaluation of *Cdt*VEGF

VEGF bioassay using KDR NFAT-RE HEK293 cells and luminescence readout.

Bio-Glo Luminescence assay



Created in https://BioRender.com

- KDR NFAT-RE HEK cells
- Binding of *Cdt*VEGF to VEGFR2 (KDR)

- ***cdt*VEGF binds to VEGFR-2**, *it activates intracellular signaling, leading to the expression of luciferase.*
- *Upon adding the* **Bio-Glo™ reagent**, **luminescence is produced**, *which is directly* **proportional to receptor activation**.

***Cdt*VEGF** *binds* **VEGFR-2,** *the receptor involved in endothelial cell migration, proliferation, increased vascular permeability and neovascularization, with an* **EC_{50} of 0.82 nM (21 ng/mL)**.

Biodiscovery Institute *from* **University of Nottingham**

rCollinein-1

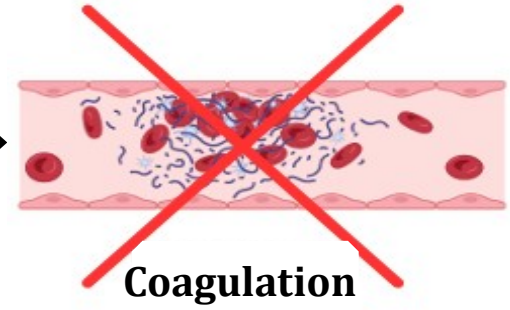
Snake Venom Thrombin-Like Enzyme (SVTLE)

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SVTLE → *Degrades fibrinogen* → *Fibrin* → *Loose clot* →

~~α~~ β γ

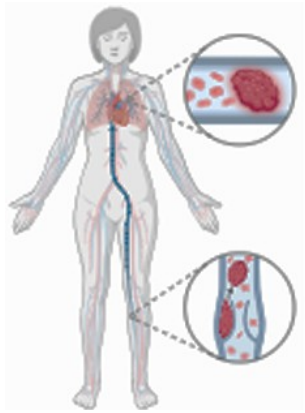
Factor XIII



Coagulation

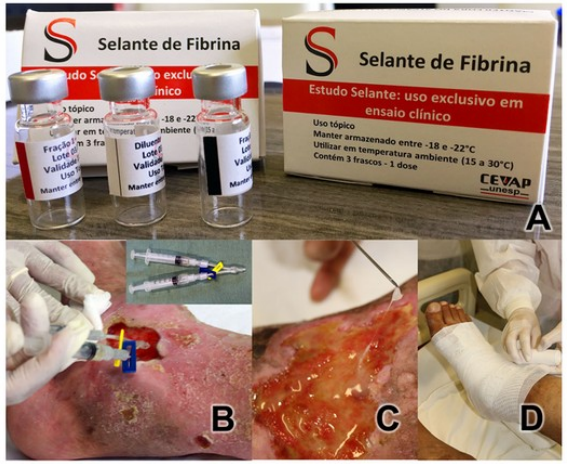
Possible applications

Vascular diseases



Embolism and thrombosis

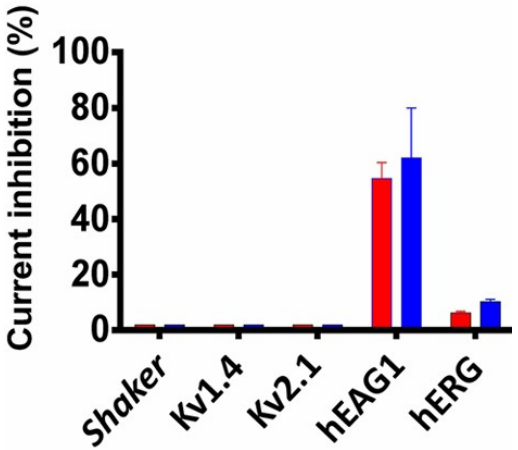
Heterologous fibrin sealant



Treatment of chronic venous ulcers (CVU)

Abbade, L. P. F *et al.*, 2021.

Potassium channel blockade



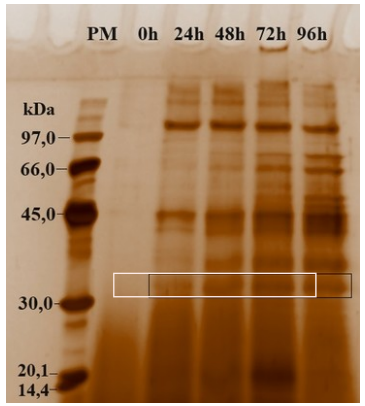
Potential antitumor activity

Boldrini-França *et al.*, 2020.

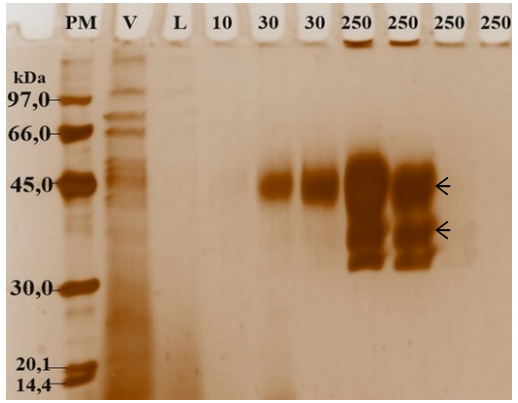
rCollinein-1

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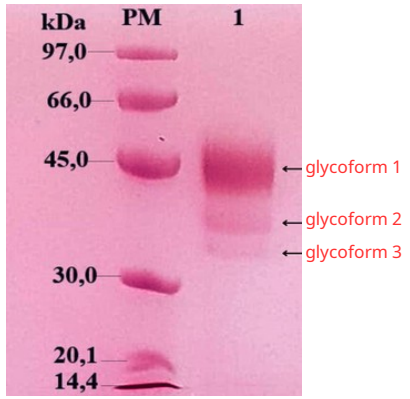
Heterologous expression



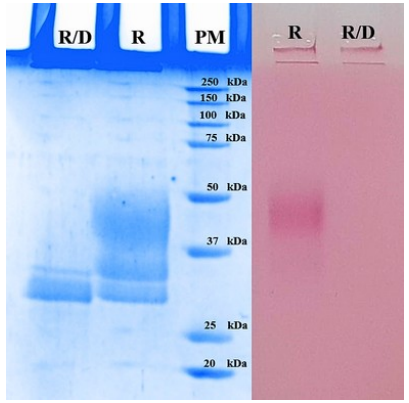
IMAC



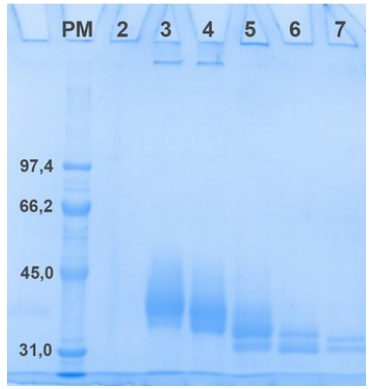
Glycosylation



N- Glycosylation

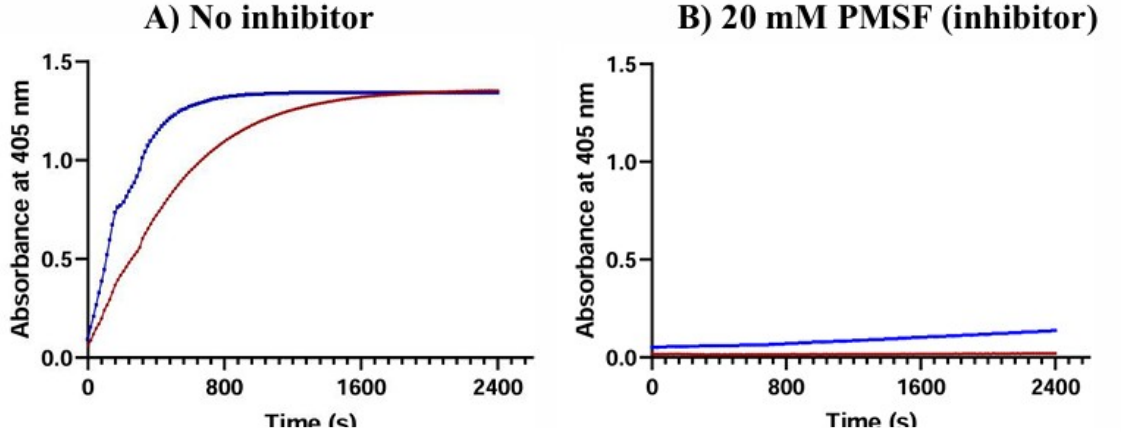


PEGylation



Pinheiro-Júnior *et al.*, 2021.

Fibrinogenolytic activity
Immunogenicity ↓



Glycosylated and Deglycosylated rColinein-1

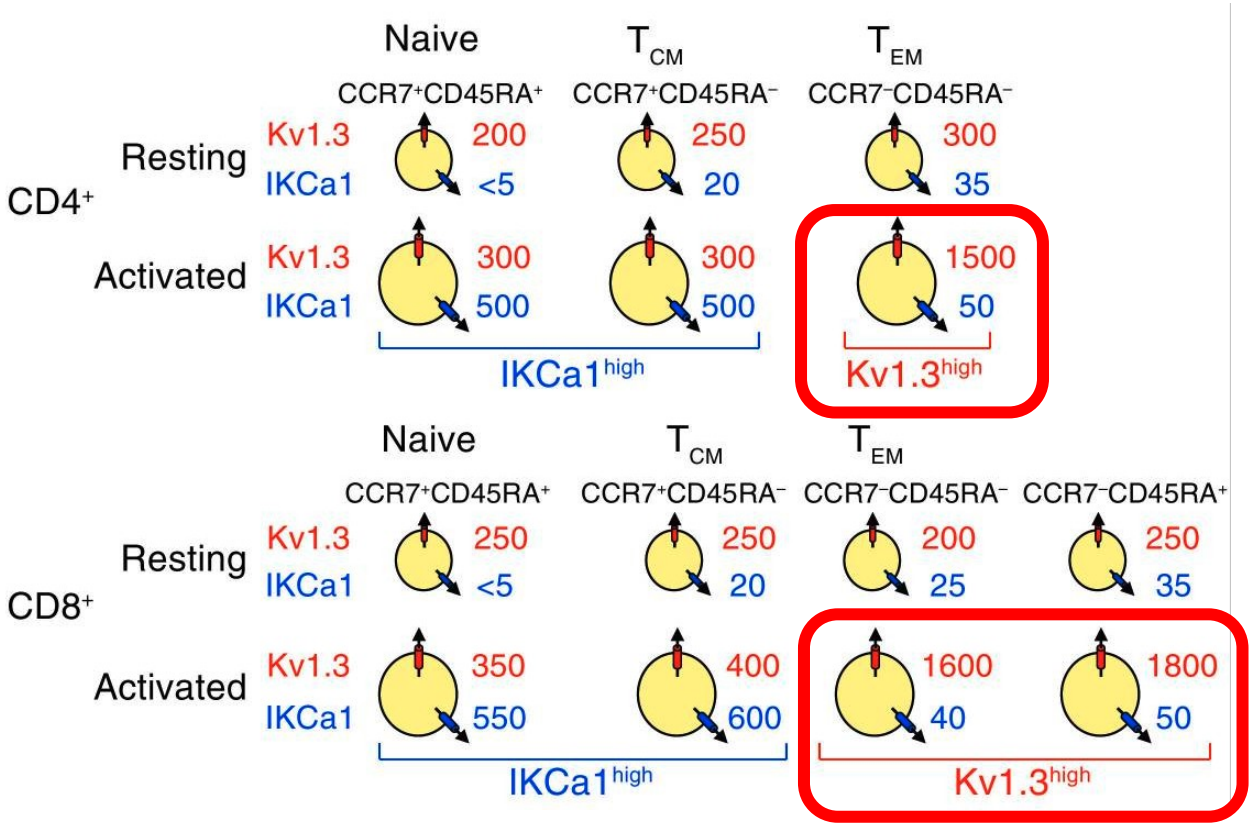
Glycosylation: important for activity and protection

Immunogenicity ? **Solubility ?**

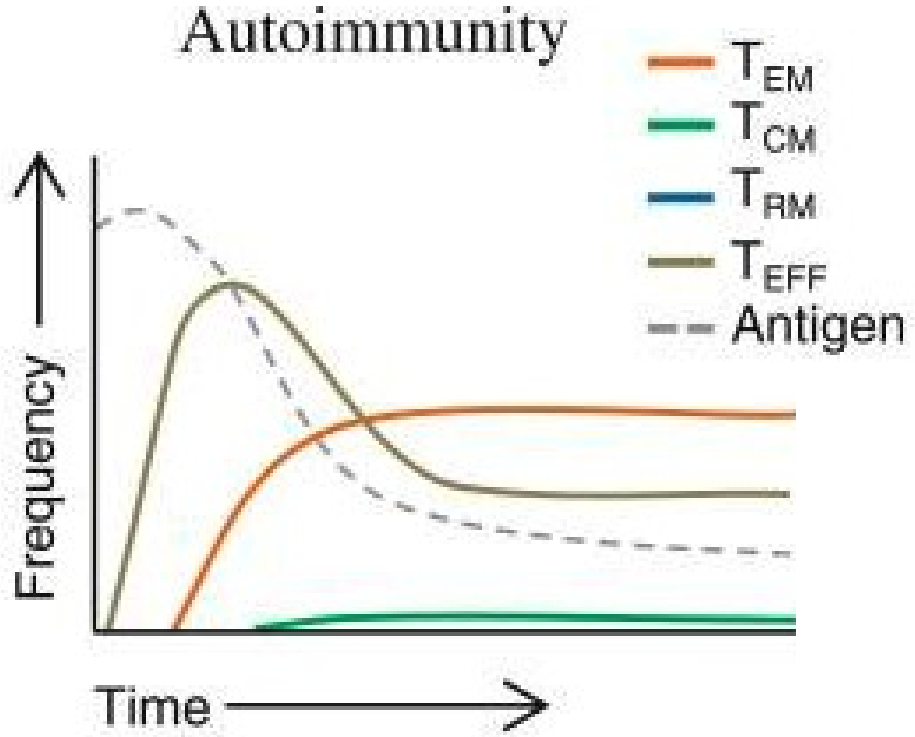
Stability ?

Fundamental properties for the formulation, transportation, storage and safety of a medicine

Kv1.3 channel – *Plays a key role in ion regulation and T cell function, particularly in* **Effector Memory T cells**



Wulff et al. The voltage-gated Kv1.3 K(+) channel in **effector memory T cells** *as new target for MS. J Clin Invest. 2003 Jun;111(11):1703-13. doi: 10.1172/JCI16921. Erratum in: J Clin Invest. 2003 Jul;112(2):298.*



Devarajan P, Chen Z. Autoimmune effector memory T cells: the bad and the good. Immunol Res. 2013 Dec;57(1-3):12-22.

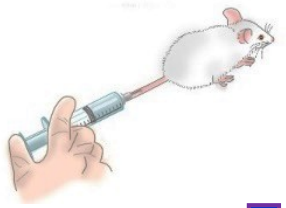
Kv1.3 channel blockade is a potential therapeutic alternative for autoimmune diseases

Ts-KTx-1 and Ts6: Potassium Channel Blocker with Immunosuppressive Action

(Cologna et al., 2011; Cerni et al., 2014)

In vivo
DTH, *delayed-type hypersensitivity*

Mediated predominantly by skin-homing T_{EM}



CFA
+ BSA (base of tail)

10 days after



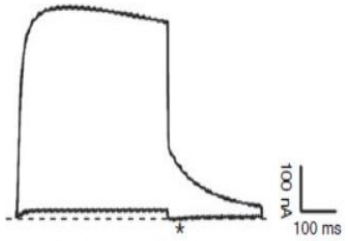
BSA (paw)
or BSA + Ts6
or BSA + Ts-KTx-1

24 h after

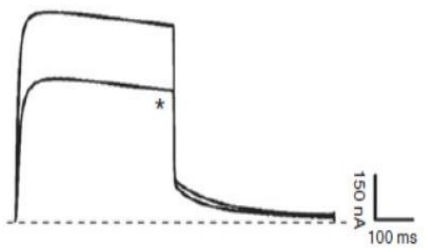


Paw edema (pachymeter)

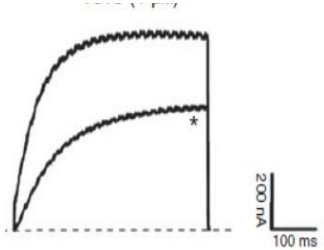
Kv 1.3
Ts6 (1 μM)



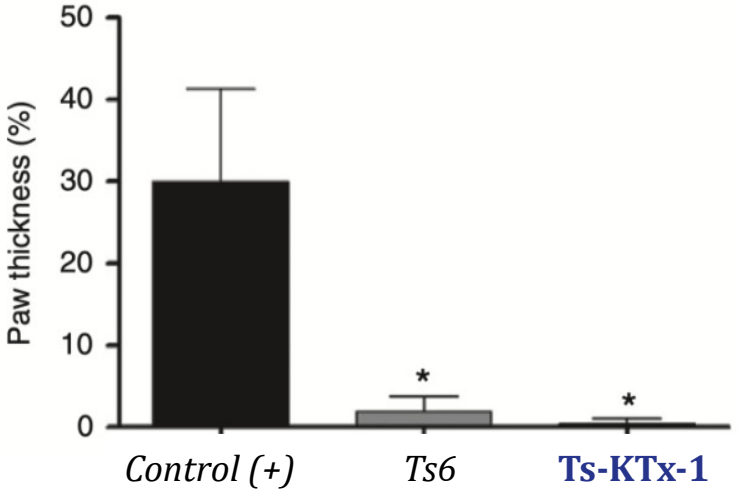
Kv 1.3
Ts-KTx-1 *(1 μM)*



Kv 2.1
Ts-KTx-1 *(1 μM)*

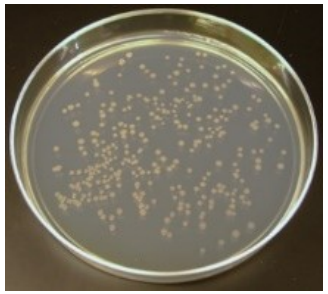


Both toxins block Kv1.3; Ts-KTx-1 also targets Kv2.1



The treatment with Ts6 and Ts-KTX-1 inhibited DTH

Recombinant Ts-KTx-1

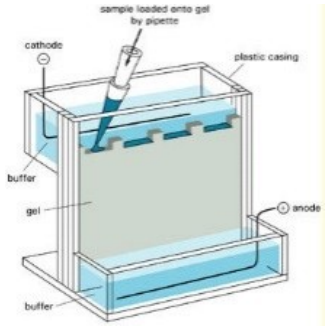




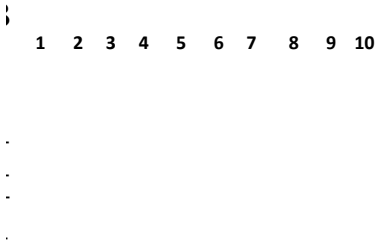
Expression in *P. pastoris* yeast cells



Immobilized-Metal Affinity Chromatography



SDS-PAGE at 16,5%



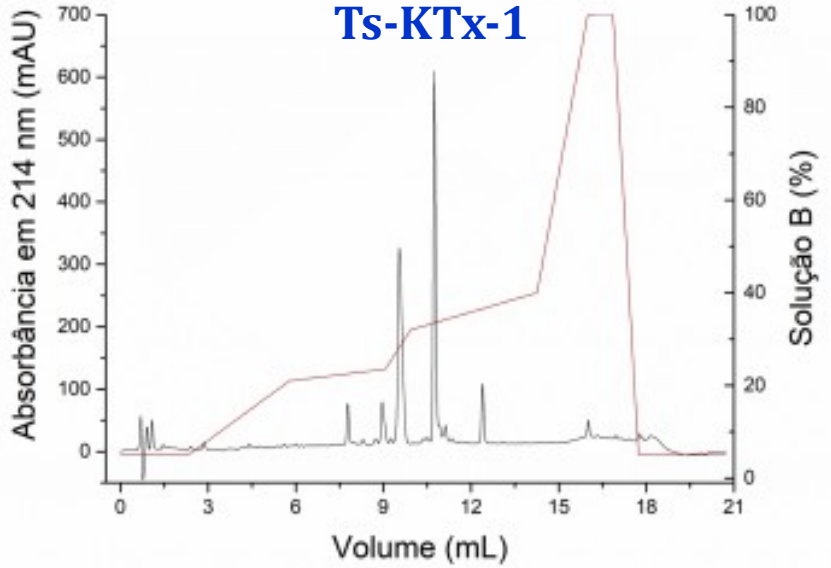
HHHHHH-ENLYFQ↓G-Ts-KTx-1

TEV cleavage to remove His-Tag

TEV: Tobacco Etch Virus protease



FPLC Akta UPC purification



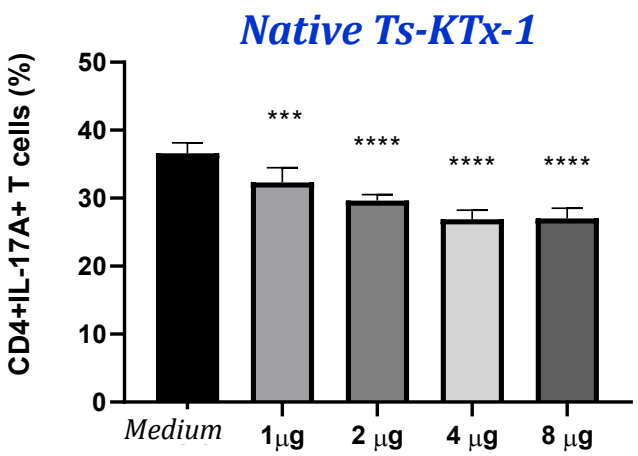
Immunomodulatory Effects of Ts-KTx-1

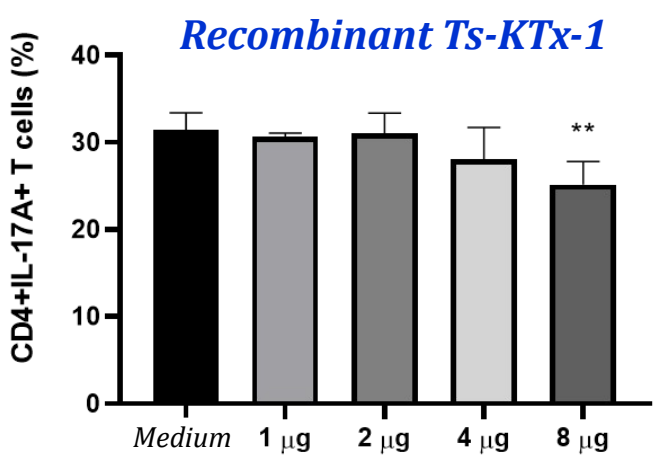
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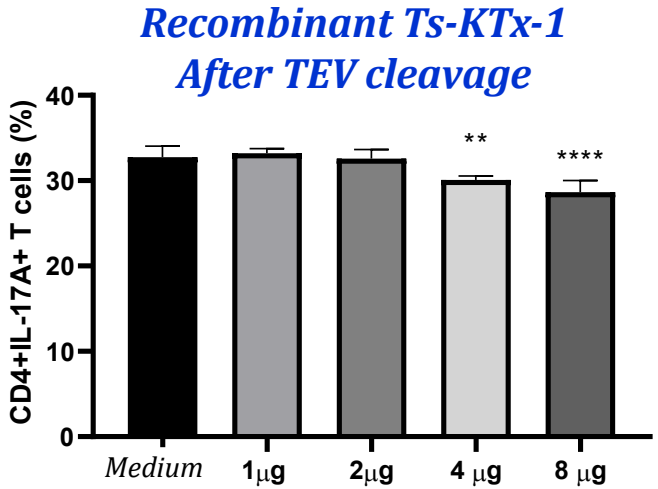
Th17

(T Helper 17 Cells)

Promotion *of inflammation*



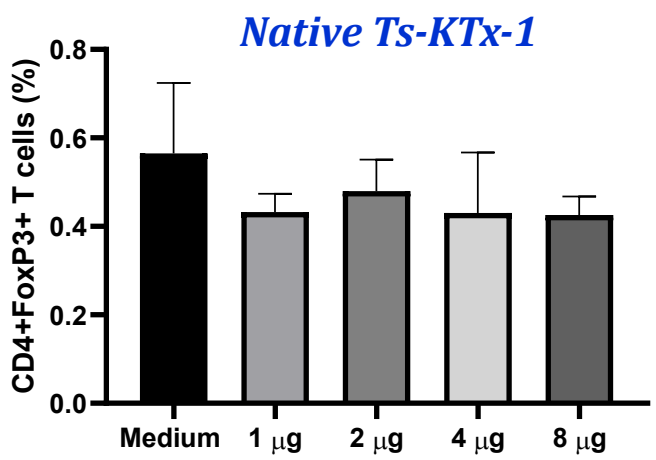


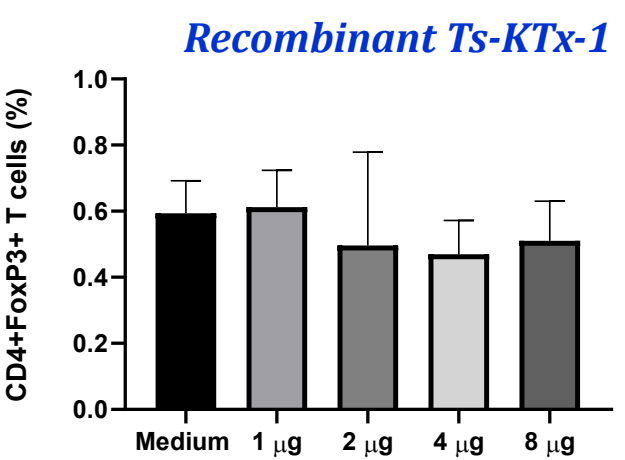


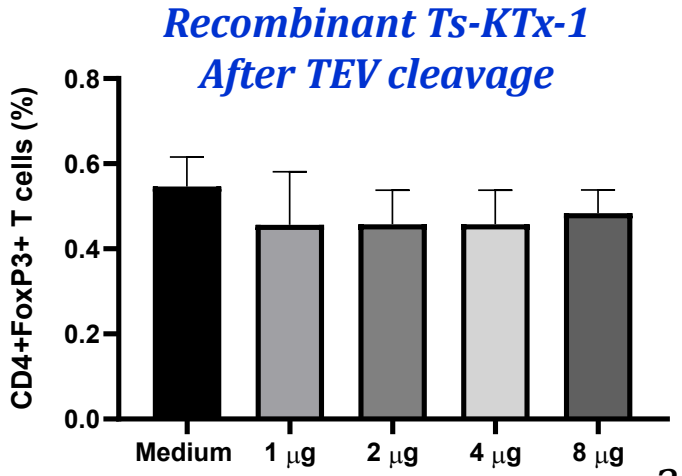
Treg

(Regulatory T Cells)

Suppression *of immune response*

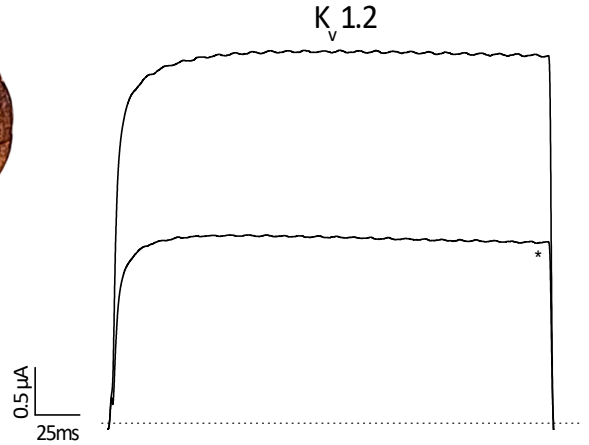






Ts-KTx-2





Kv 1.2
Ts-KTx-2 *(1 µM)*

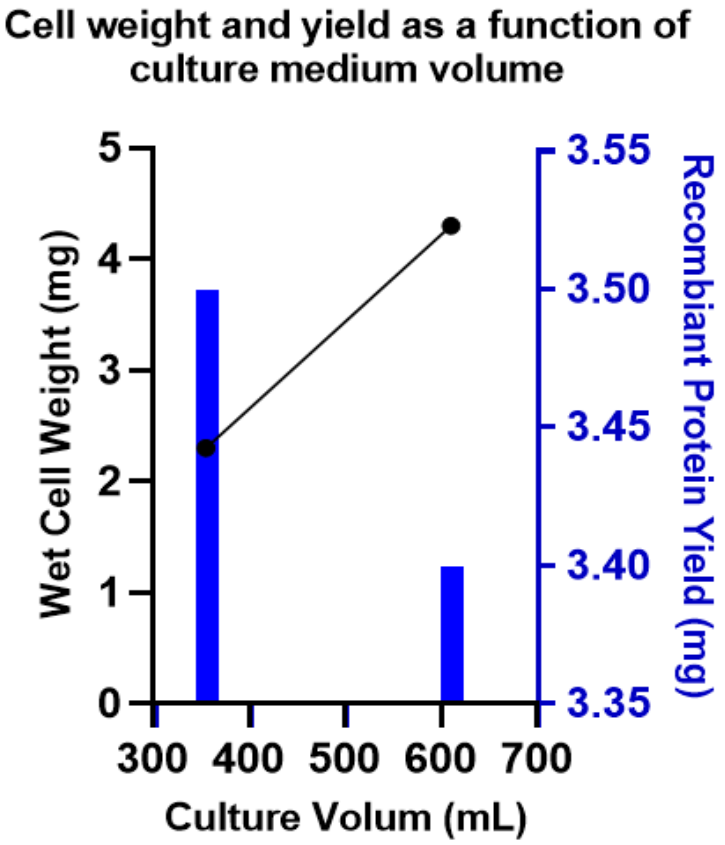
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Effect of **Ts-KTx-2** *(1µM) on Kv 1.2 expressed in Xenopus laevis. Ts-KTx-2* **specifically blocks Kv1.2 channels**

Recombinant Ts-KTx-2



Optimization of expression in *Komagataella phaffii*



- Decreased expression time
- Decreased volume of medium needed
- Maintained yield
- Using lower number of cells
- Significantly reduced production cost

Monoclonal antibody fragments targeting metalloproteases from *Bothrops* snake venoms

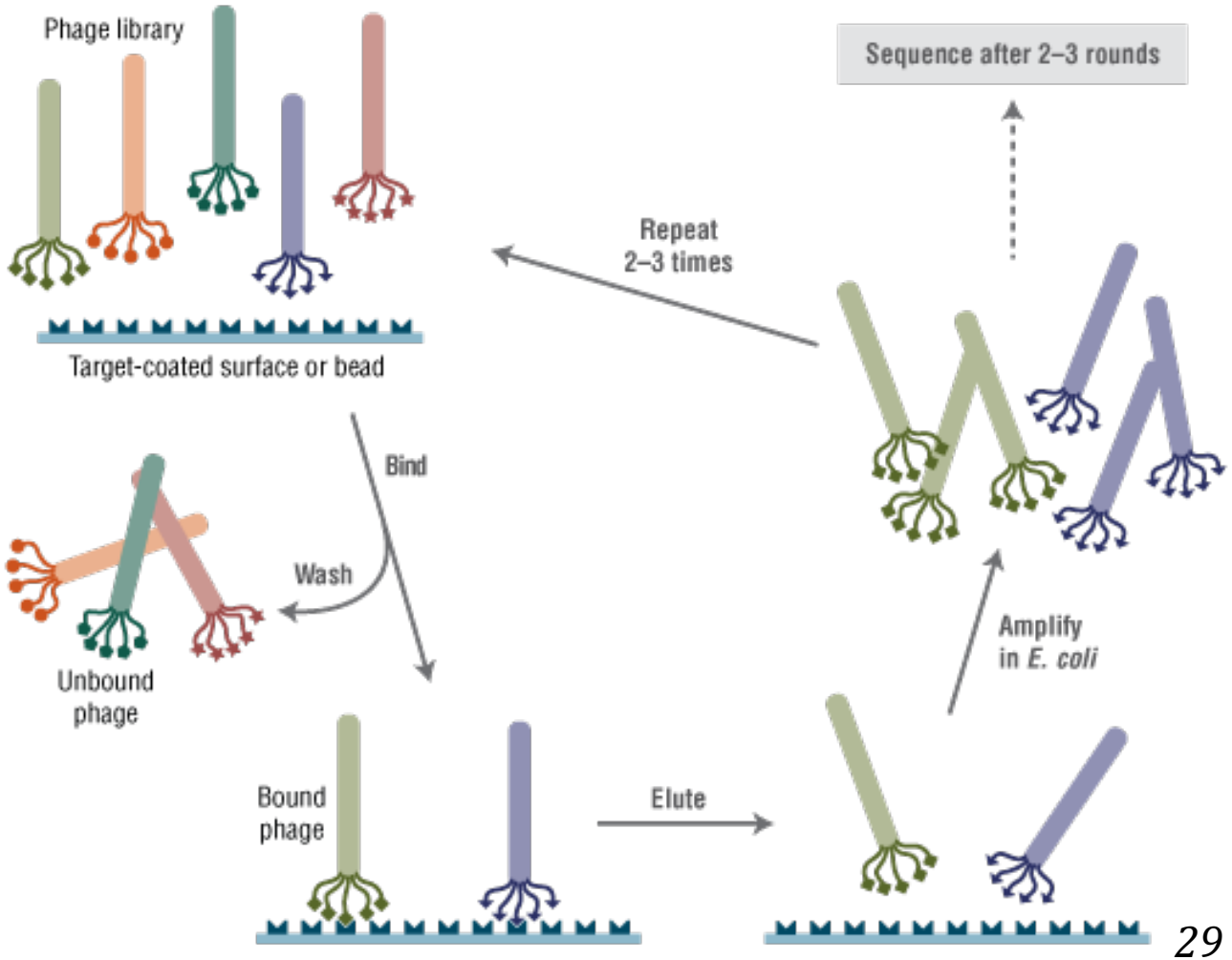
Selection and production of fragments of human monoclonal antibodies *(scFvs) targeting* **metalloproteases**, *through* **phage display technique**



scFv

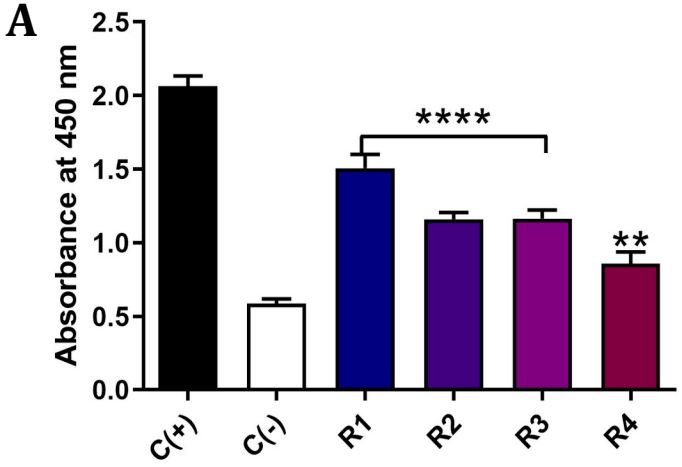
Phage display technique

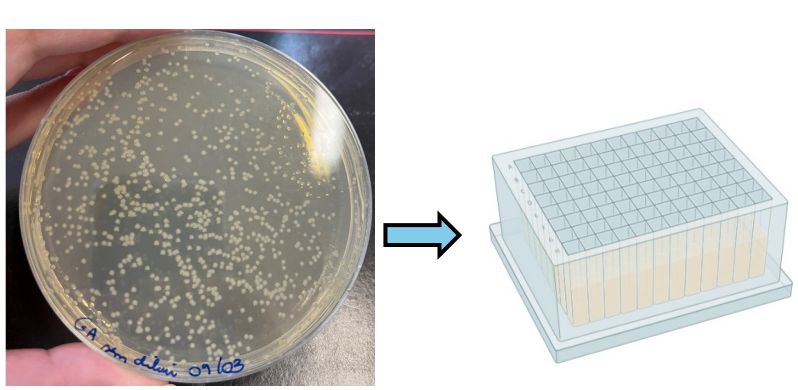
- **Griffin I library** *(human, non-immune)*
- **4 rounds of selection** *for increase the specificity of phage antibodies*
- **Jararhagin** *from B. jararaca venom (PIII class* - hemorrhagic metalloproteinase*)* **as the target** – *biotinylated for better antigen presentation*



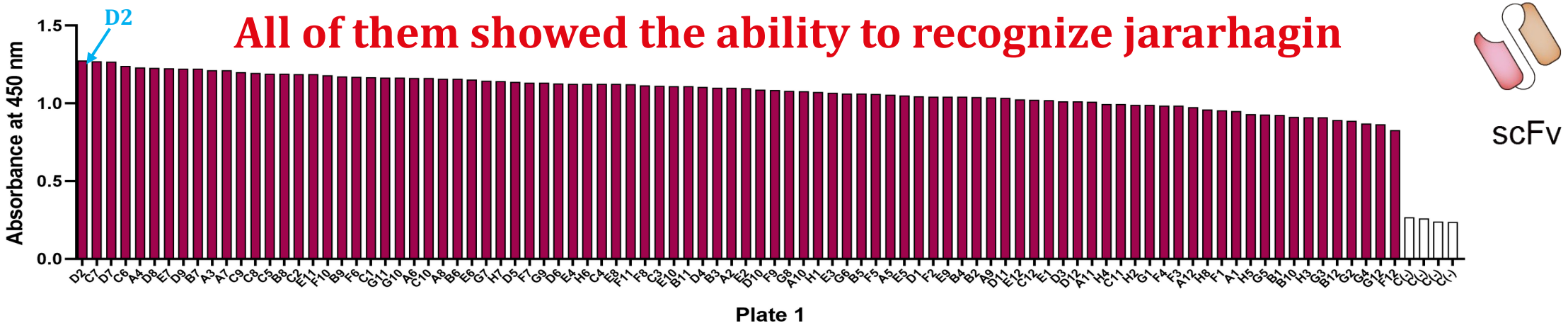
Ahmadi et al. (2020); Paine et al. (1992); Silva et al. (2018)

Clones from the 4th round were plated and picked individually.



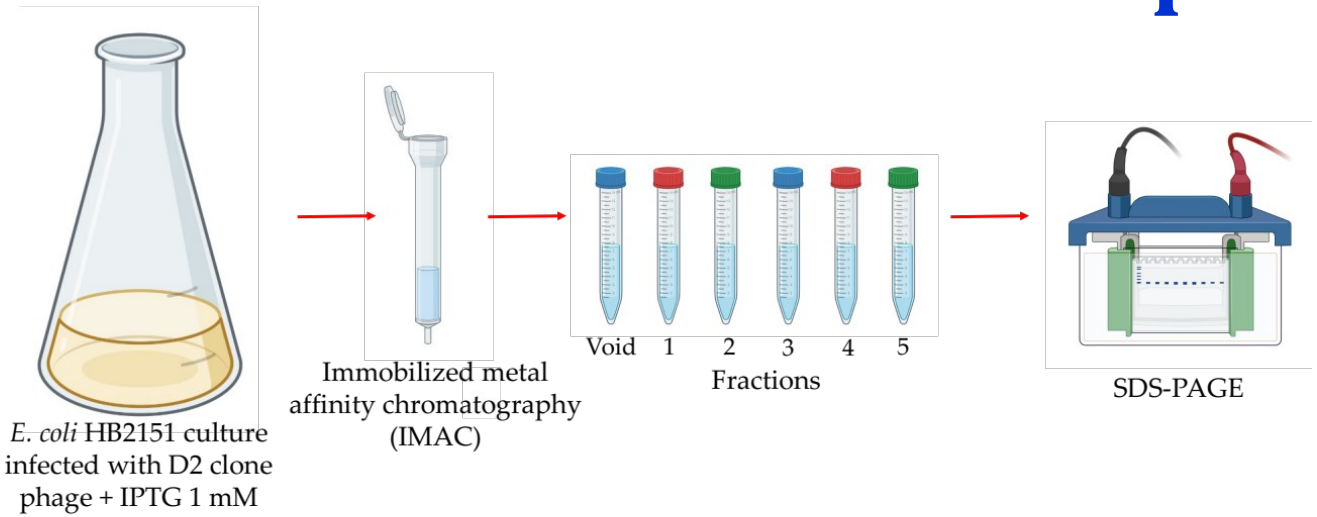


Selection of specific phage-antibodies against jararhagin. (A) *Phage-antibodies were analyzed by ELISA.* **(B)** *Culture plates demonstrate E. coli TG1 bacteria colonies growth, which were infected with phage-antibodies from the 4th round of panning.*

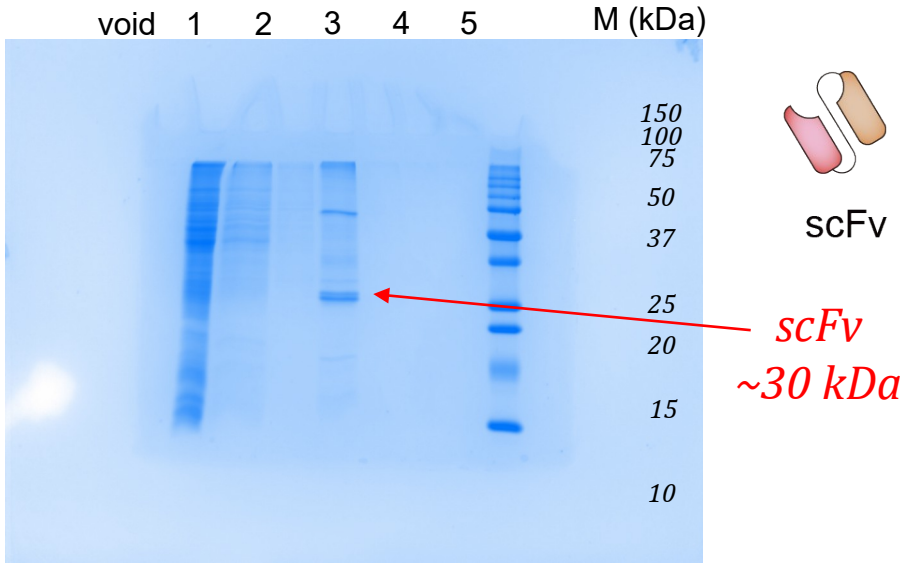


Monoclonal ELISA with phage-antibodies from 4th round.

The best-performing clone was used to infect *E. coli* for scFv production



Selection *and* **production** *of* **human antibody fragments** *against Bothrops* **metalloproteases for future potential inclusion in next-generation antibotropic antivenom.**





The resulting scFv will be evaluated in vitro and in vivo to assess its capacity to **inhibit metalloprotease activity**

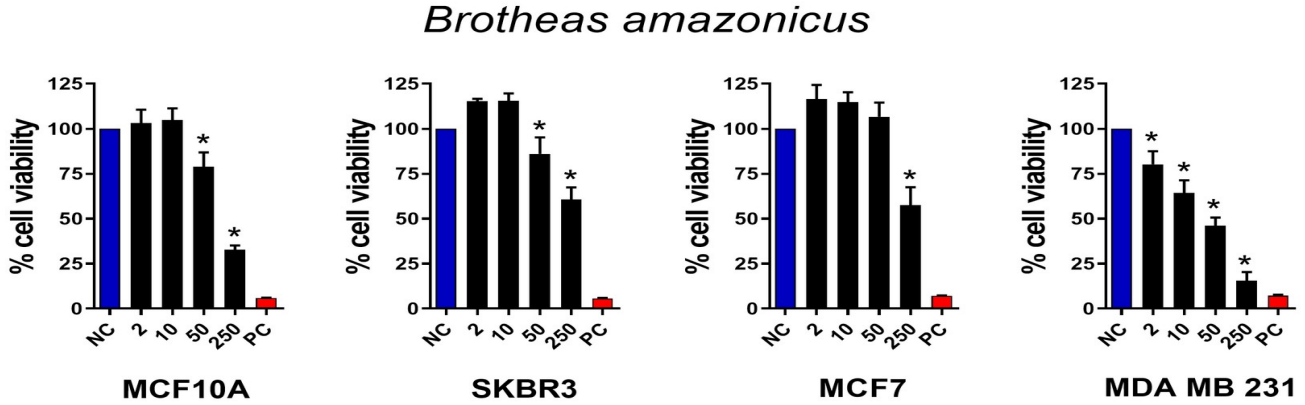
Scorpine-like peptide from the Amazonian Scorpion venom with cytolytic activity



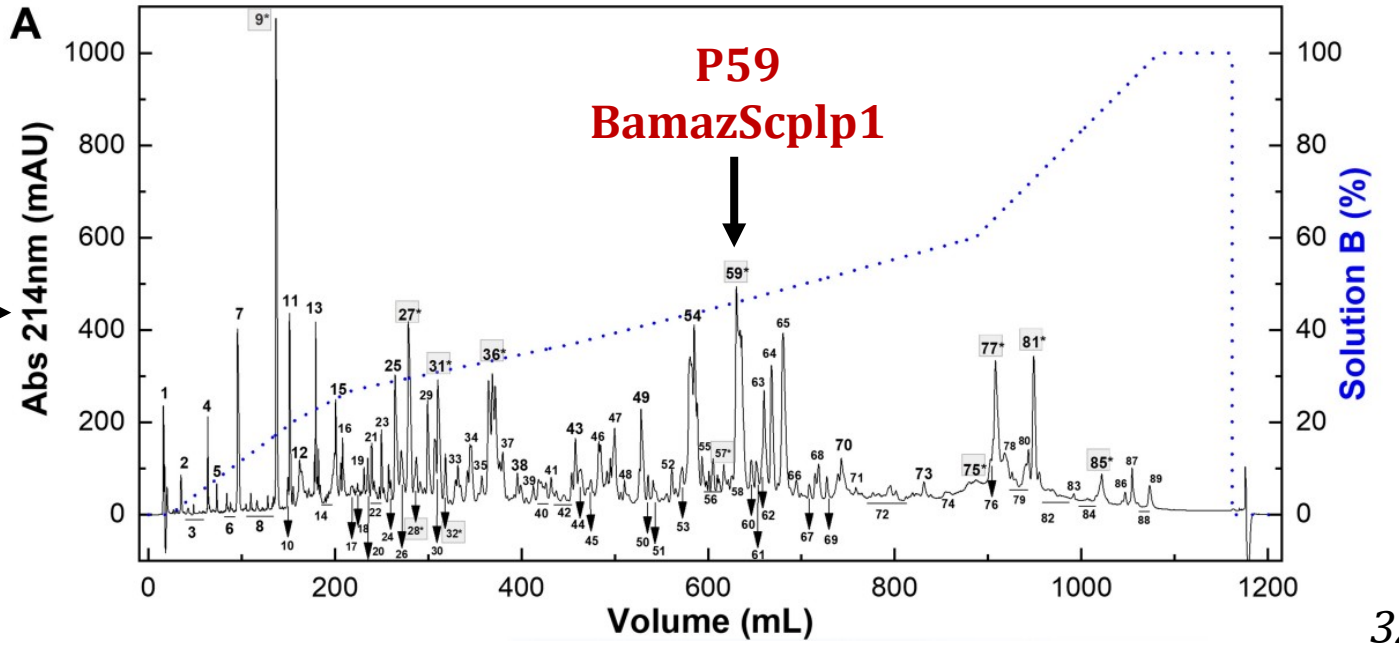
Jonas Gama Martins

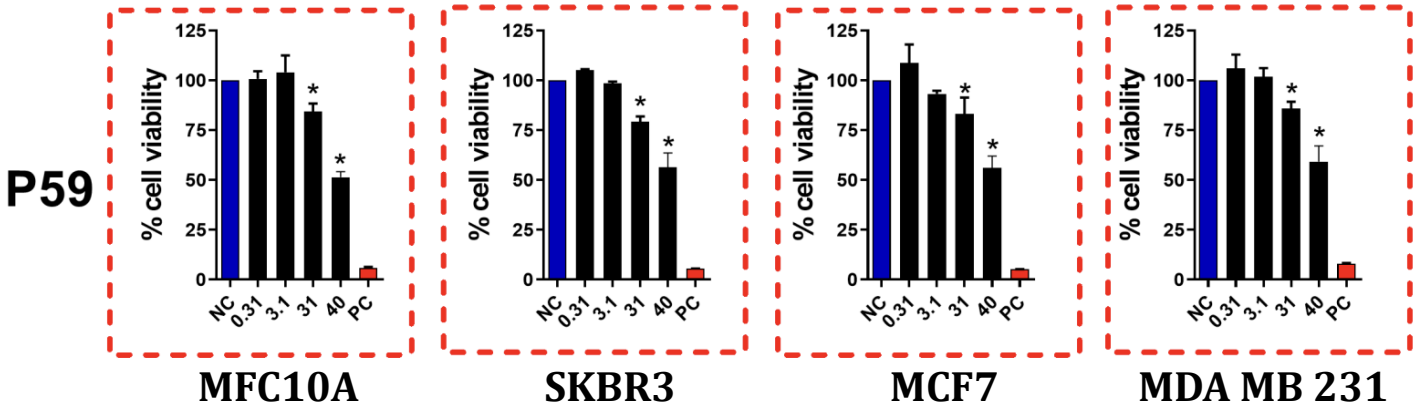
Brotheas amazonicus

Venom on Human **Breast Cancer Cell Lines**



Chromatographic profile of fraction >10 kDa on RP-FPLC system.



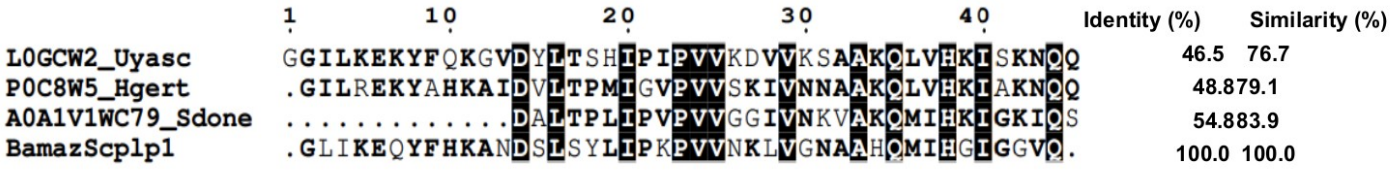


Cytolytic activity of P59 from *Brotheas amazonicus* venom

BamazScplp1 (P59)

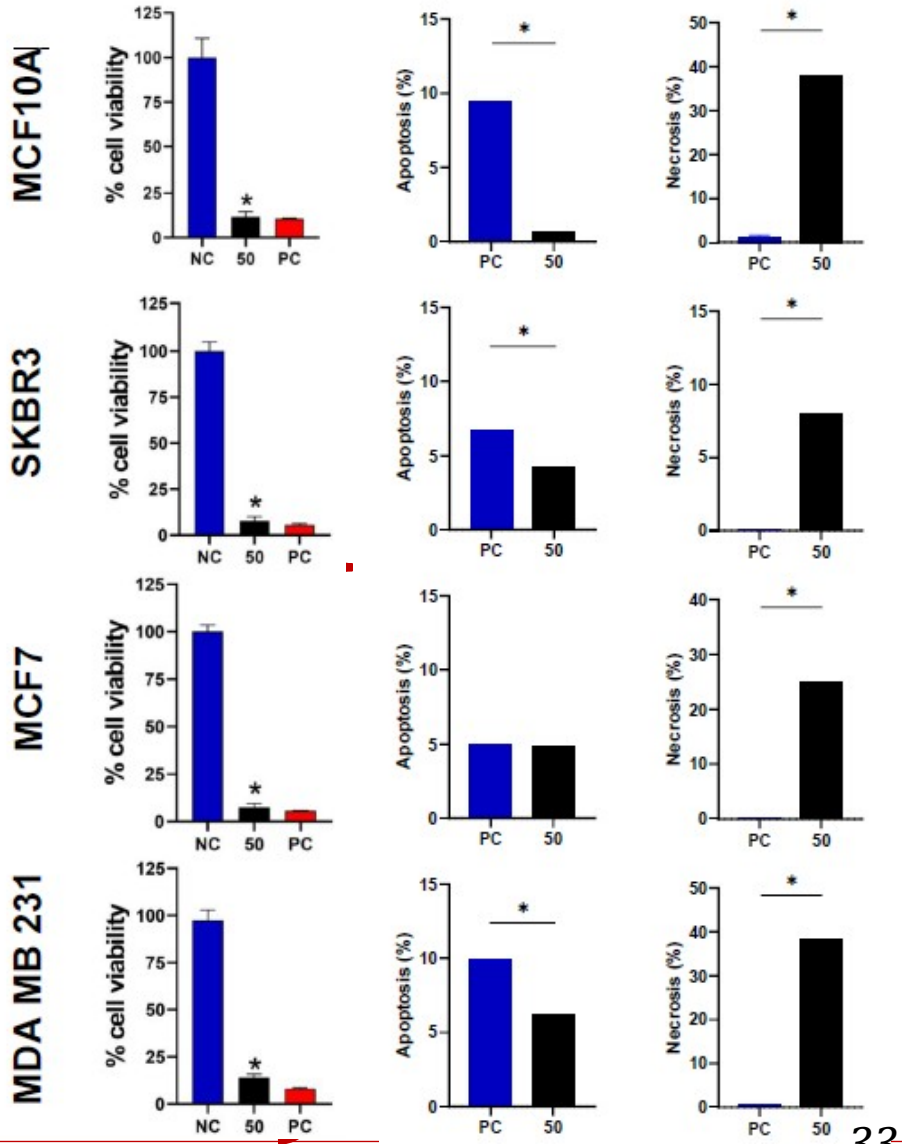
Scorpine-like peptide

Mechanism of death: **NECROSIS**



N-terminal alignment of BamazScplp1 with scorpine-like sequences

Scorpine-like-2 (Urodacus yaschenkoi, L0GCW2), Hg-scorpine-like-2 (Hoffmannihadrurus gertschi, P0C8W5) and putative scorpine-like peptide (Superstitionia donensis, A0A1V1WC79).



ACKNOWLEDGEMENTS



Isabela Gobbo Ferreira, PhD
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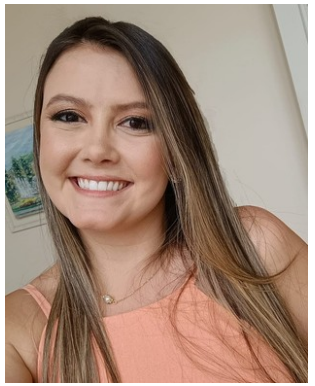
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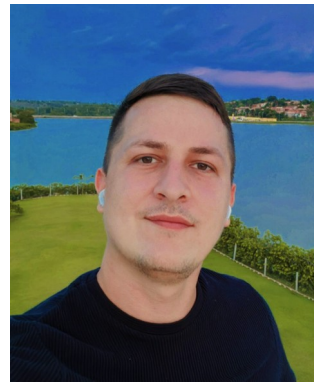
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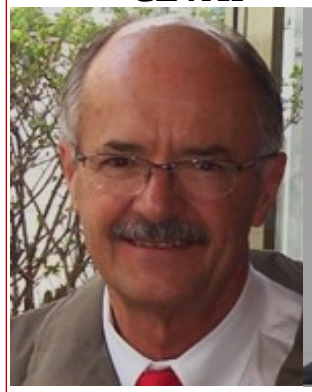


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England



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Thank you for your attention



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