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PVX, A Host-Targeting Broad-Spectrum Antiviral Therapeutic Derived from Cobra Venom Toxin

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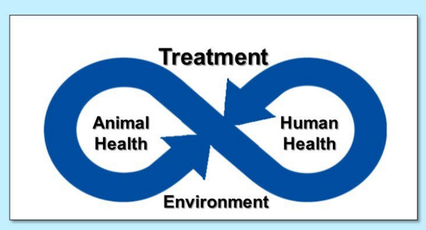
INTRODUCTION & AIM

CYGNOS BioTech is a biotechnology company specializing in innovative antimicrobial biologic therapeutics targeting zoonotic infectious disease in animals and humans, with expertise in translational research, regulatory compliance, and pre-clinical and clinical trials.

CYGNOS BioTech

One Health



- "**One Health**" means that human health is intimately connected to the health of animals and influenced by our shared environments.
- **"Zoonosis"** is the process by which infectious diseases are transmitted between species: from animals to humans and humans to animals.

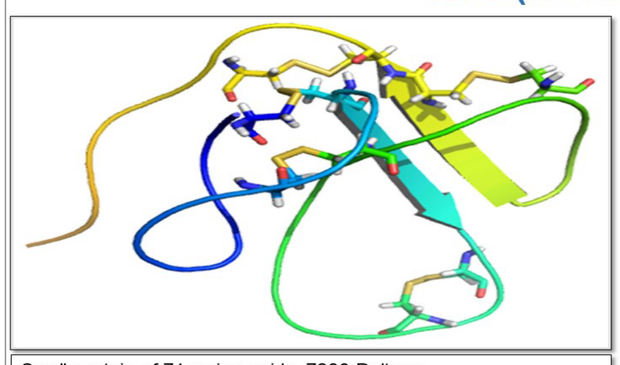
The incidence of epidemics is increasing due to new viruses emerging across the globe

- Ebola
- Chikungunya
- Dengue
- mPox
- Lassa
- Nipah
- MERS CoV
- SARS-COV-2
- Marburg
- Zika0
- Hendra
- Avian Influenza

- Recent viral epidemics has involved <u>zoonotic</u> transmission (animal to human).
- For most, vaccines and therapeutics have either not been developed or are not widely available.
- Existing vaccines and therapeutics are not 100% effective.
- **There is a need for new and more effective antiviral therapeutics and vaccines.**

METHOD

NT3 (α-Neurotoxin)



Small protein of 71 amino acids, 7280 Daltons

- NT3 purified from whole cobra venom

Ile-Arg-Cys-Phe-Ile-Thr-Pro-Asp-Ile-Thr-Ser-Lys-
Asp-Cys-Pro-Asn-Gly-His-Val-Cys-Tyr-Thr-Lys-Thr-
Trp-Cys-Asp-Ala-Phe-Cys-Ser-Ile-Arg-Gly-Lys-Arg-
Val-Asp-Leu-Gly-Cys-Ala-Ala-Thr-Cys-Pro-Thr-Val-
Lys-Thr-Gly-Val-Asp-Ile-Gln-Cys-Cys-Ser-Thr-Asp-
Asn-Cys-Asn-Pro-Phe-Pro-Thr-Arg-Lys-Arg-Pro

References
- Tu, A., Venoms: Chemistry and Molecular Biology. John Wiley & Sons, 1977.
- Martin, B.M., Chibber, B.A., and Maelieke, A., The sites of neurotoxicity in α-cobratoxin. J Biol Chem, 1983. 258(14): p. 8714-8722.

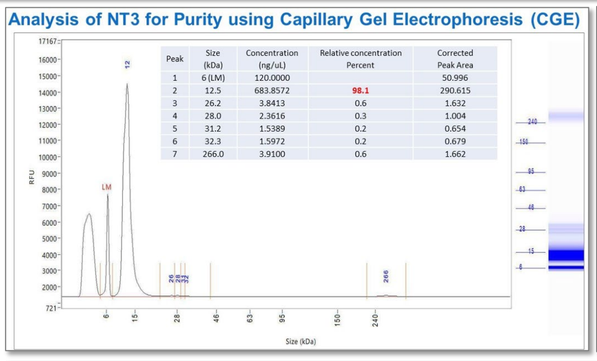
- Scorpion toxin and defensins share a conserved three-dimensional structure and related biological activities.
- A small alteration in protein sequence of the defensin changing two key amino acid residues converts the insect defensin into a neurotoxin.
- The NT3 amino acid sequence is similar to the scorpion defensin in their active sites.

References
- Zhu, S., Peigneur, S., Gao, B., Umetsu, Y., Ohki, S., and Tytgat, J., *Experimental Conversion of a defensin into a neurotoxin: implications for origin of toxic function*. Mol Biol Evol, 2014. 31(3): p. 546-549.
- Zhu, J., Meng, W., Wan, X. and Wang, H.R. *Broad-Spectrum Antiviral Agents*. Front. Microb. 2015. 6:517, p. 1-15.

PVX Production: The neurotoxin, NT3, is the precursor of PVX, derived from the Naja naja kaouthia snake species. It is detoxified by irreversible chemical modification by oxone oxidation Complete NT3 detoxification and conversion to highly active anti-viral PVX is achieved by oxidation of at least three of its five disulfide bonds.
Purification: FPLC HiTrap Chromatography is used to isolate NT3 from whole cobra venom and PVX from oxidization reactions.
Characterization: FPLC Chromatography and Capillary Gel Electrophoresis (CGE) are used to analyze NT3 and PVX samples for purity. CGE analysis was performed using Agilent's Proteo-analyzer at their test facility in Linthicum, MD.
LCMS analysis determined the molecular mass of oxidized PVX as ~8,300-8,400 Daltons (Data not shown).
Activity Testing: PVX's protein kinase A inhibitory activity was assessed using the Promega PKA Kinase Enzyme System using NT3 as a negative control.
Anti-viral Testing: Addition of PVX to mammalian cell lines 24 hrs prior to addition of replicating viruses demonstrated activity in the low micromolar range against each virus tested.
Pilot Efficacy studies in Felines and Canines: Animals were treated for up to two months with PVX at daily systemic dosing of approximately 400 micrograms/Kg with no observed toxicity.

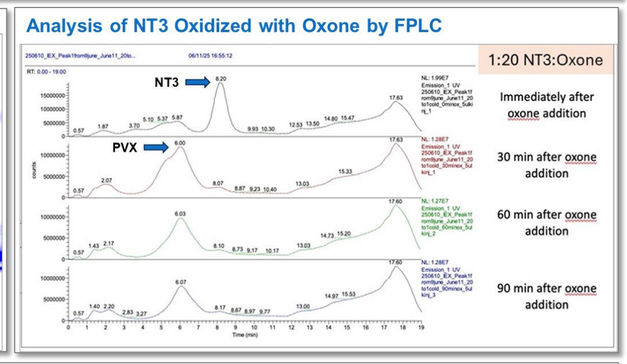
RESULTS & DISCUSSION

Analysis of NT3 for Purity using Capillary Gel Electrophoresis (CGE)

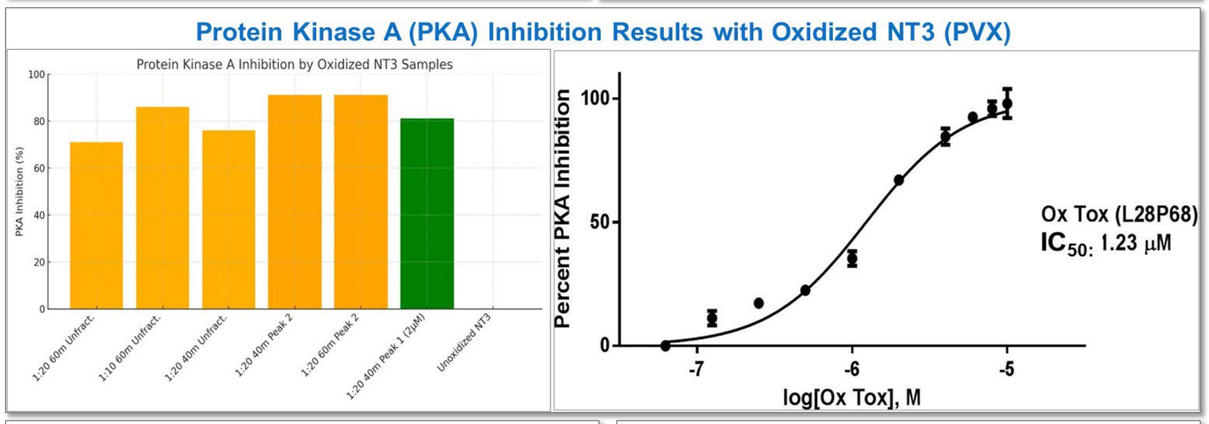


Peak	Size (kDa)	Concentration (ng/uL)	Relative concentration Percent	Corrected Peak Area
1	6 (LM)	120.0000		50.996
2	12.5	683.8572	98.1	290.615
3	26.2	3.8413	0.6	1.632
4	28.0	2.3616	0.3	1.004
5	31.2	1.5389	0.2	0.654
6	32.3	1.5972	0.2	0.679
7	266.0	3.9100	0.6	1.662

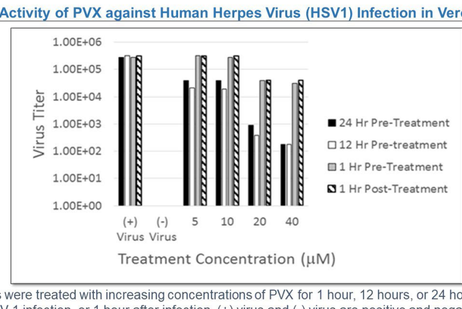
Analysis of NT3 Oxidized with Oxone by FPLC



Protein Kinase A (PKA) Inhibition Results with Oxidized NT3 (PVX)

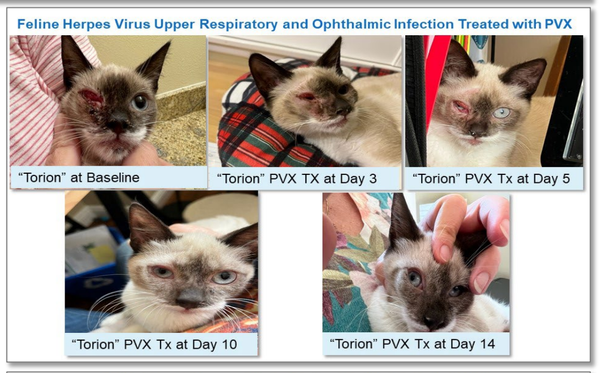


Antiviral Activity of PVX against Human Herpes Virus (HSV1) Infection in Vero Cells



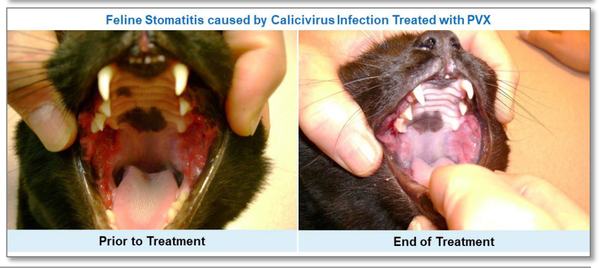
Vero Cells were treated with increasing concentrations of PVX for 1 hour, 12 hours, or 24 hours before HSV-1 infection, or 1 hour after infection. (+) virus and (-) virus are positive and negative controls for the virus, respectively, and were not treated with PVX

Feline Herpes Virus Upper Respiratory and Ophthalmic Infection Treated with PVX

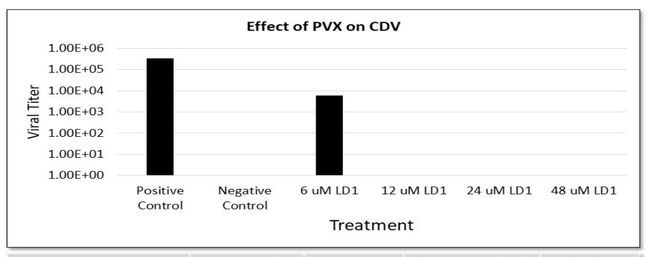


Feline Peritoneal, Upper Respiratory and Ophthalmic Corona Virus Infection Treated with PVX

Feline Stomatitis caused by Calicivirus Infection Treated with PVX

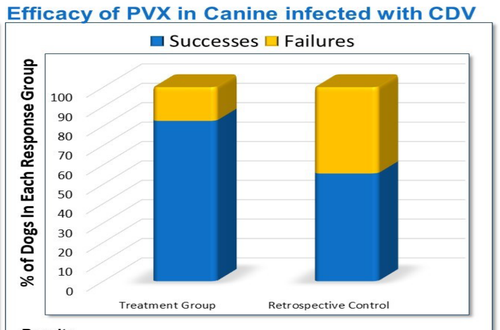


Anti-viral Activity of PVX against CDV infection in Vero Cells



Virus	Pre-Treatment	Treatment	Concentration (uM)	Virus Titer
Canine Distemper Virus	-	Control		3.20E+05
-	-	Control		0
Canine Distemper Virus	24 hours	LD1	6	5.60E+03
Canine Distemper Virus	24 hours	LD1	12	0
Canine Distemper Virus	24 hours	LD1	24	0
Canine Distemper Virus	24 hours	LD1	48	0

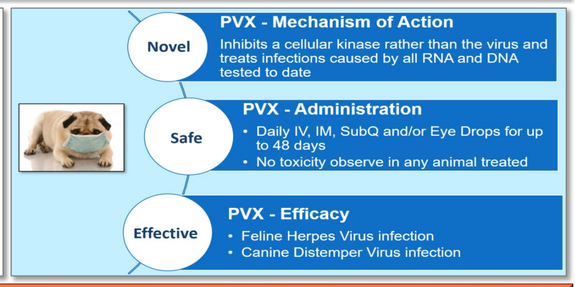
Efficacy of PVX in Canine infected with CDV



Results
- Treatment Group – A survival rate of 83% or 62 out of 75 animals which was significant compared to the control
- All treated puppies aged 12 months or less survived – Puppies aged 12 months or less do not typically survive infection.

CONCLUSION

- **PVX** is a host-directed therapeutic with broad spectrum antiviral activity
- **NT3** like other toxins have evolved from defensins that are innate immune factors
- **NT3** isolated from cobra venom is chemically modified to produce therapeutically active PVX.
- **PVX** effectively treats companion animals infected with RNA and DNA viruses
- **PVX** could be used to combat viral outbreaks and epidemics in humans



FUTURE WORK

Recombinant PVX (r-PVX) Development

- Production and testing of native PVX (n-PVX) for biologic activity as a reference will enable development of recombinant form for production *E. coli* to make enough r-PVX for IND enabling activities, a Phase I human study and extended stability testing.
- At pilot scale of production both will be compared for purity and tested *in vitro* and *in vivo* against the relevant veterinary and human viral pathogens.

Optimizing PVX Activity using AI

- PVX will be recombinantly expressed and evaluated using the latest AI and imaging technologies to improve protein kinase inhibitory activity.
- Modifying PVX's protein sequence may increase its activity and influence downstream signaling in the cell.