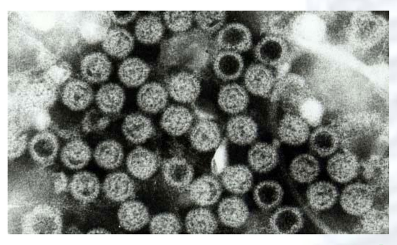
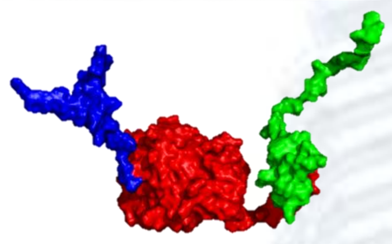
About BioValence

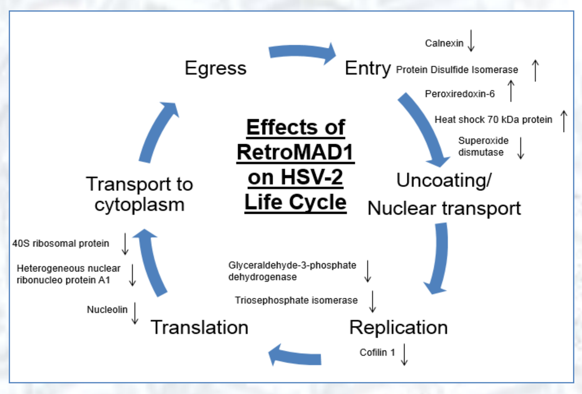
Antiviral & Anticancer Technologies

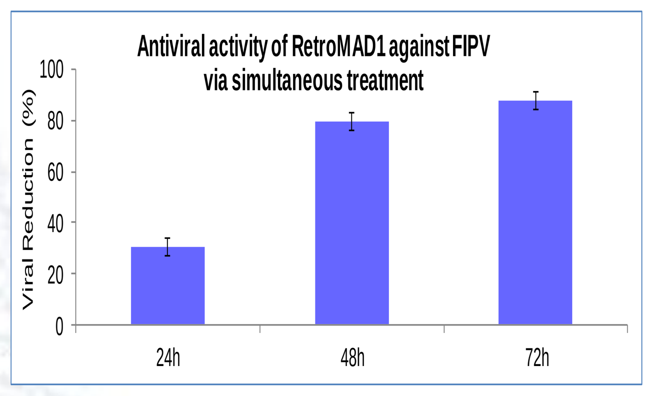
Viruses

Viruses impact our quality of life, our pets and our food production systems. Biovalence has novel technology platforms that focus on making broad-spectrum antiviral proteins that are can be orally administered. These proteins can enter cells and even the bone marrow. RetroMAD1 is our lead drug. We have *in vitro* and *in vivo* results for many unrelated viruses in a range of animal models.

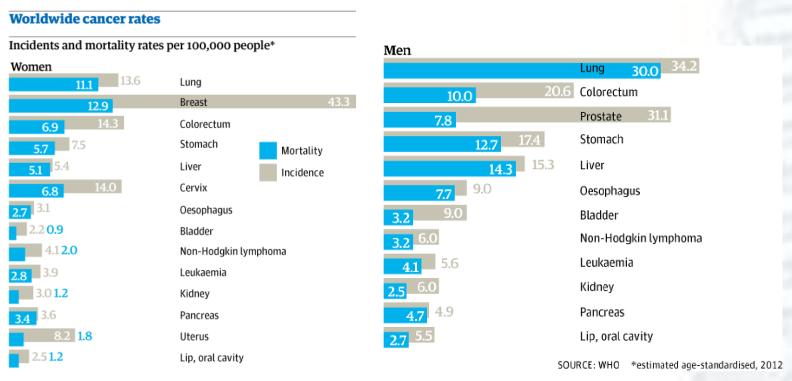


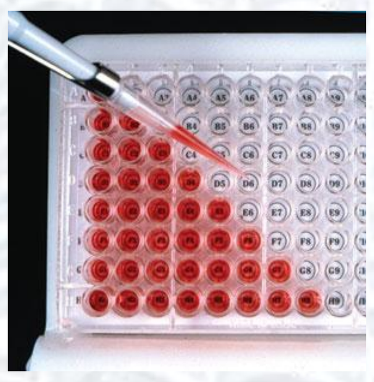


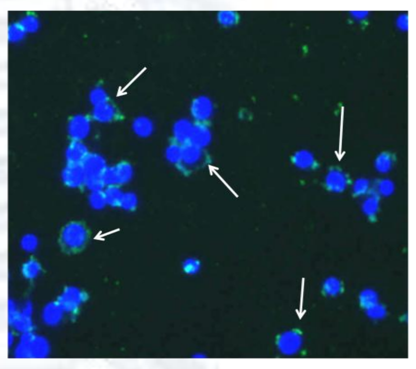




Cancer



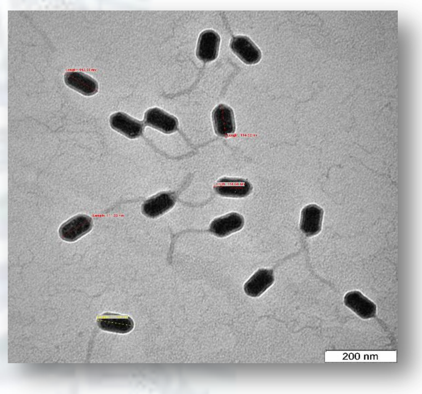




Cancer is so widespread that almost everyone has lost at least one family member to this terrible disease. Cures are still few and far between and the majority of cancer drugs only prolong life expectancy by months or at the most years. We are developing new anticancer therapeutic proteins that have so far showed promise in cell line assays.

Aquaculture Diseases

Aquaculture accounts for half of all the seafood consumed today. It is the fastest growing animal production system but one that is constantly plagued by diseases that spread with relative ease in the aquatic environment. We are developing immune-stimulants, bacteriophages and antiviral proteins that can protect these fragile but highly necessary food production systems.

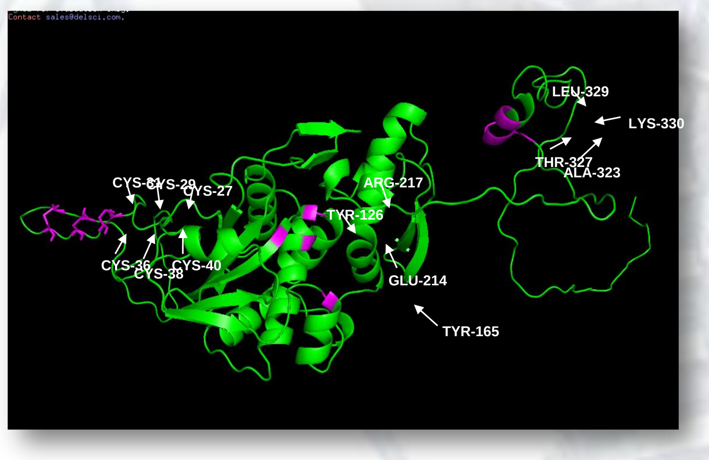


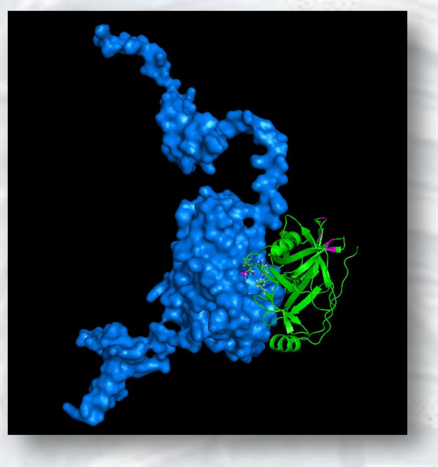
Research Infrastructure

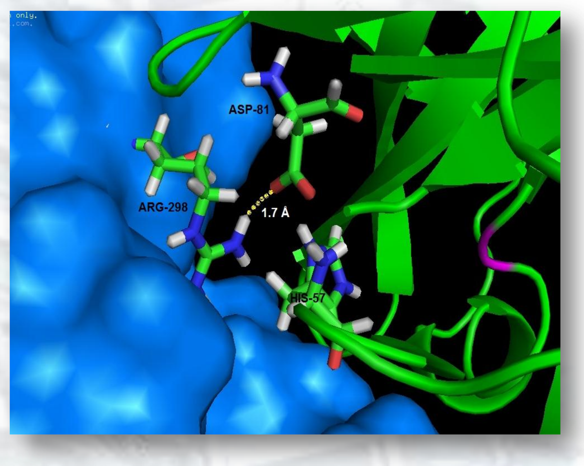
We have a Discovery Lab for bench top research and an Upscale Optimization Lab for solving various problems commonly encountered in recombinant protein upscale. We have also invested in a GMP Clinical Batch production facility that is expected to be PIC/S certified sometime in 2014. State-of-the-Art research infrastructure ensures predictability and repeatability for our platform research.

Bio-Informatics

As we carry out gene-mining for antiviral and anticancer applications, we employ leading edge Bio-Informatics to assist us in Drug Design and Candidate Selection. Bio-Informatics also helps us answer questions regarding mechanism of action and helps to suggest new disease targets for us to consider using our novel Platform Technologies.







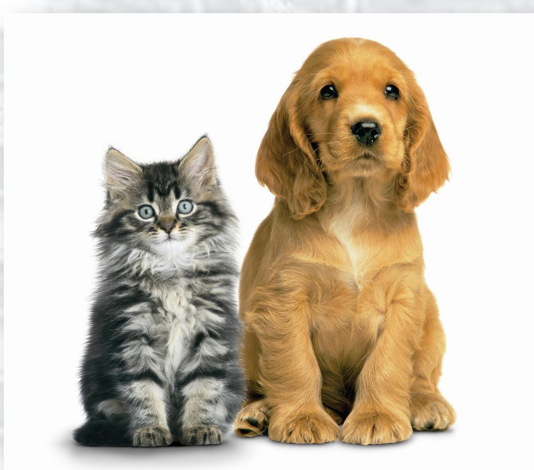
DNA/RNA Extraction Technologies

We have been using our own DNA/RNA extraction kits for the last 3 years and our results are equivalent with the leading brands found within the market. We are now exploring OEM production for a client in the EU and are open to exploring similar arrangements with potential clients anywhere. Send an email to tmo@biovalence.com.my, if you have any interest.

Key Personel

Biovalence is led by Ung Eng Huan who is the Chief Technology Officer and acting CEO together with Prof. Shamala Devi as CSO. Other key Principal Investigators are Awang Mohammed Sagaf (Recombinant Proteins), Dr. Hussin Alwan (Molecular Medicine) and Dr. Teoh Teow Chong (Bio-Informatics). They are supported by capable teams each tasked with solving particular issues.

Business Interest



We wish to target ‘low hanging fruit’ first such as the Aquaculture and Companion Animal markets while developing Human applications for out-licensing to reputable Pharmaceutical. companies. We are also collaborating with other companies that wish to see their protein-based therapeutic made into an oral delivery drug. Supply of DNA/RNA extraction kits on OEM basis is another new business interest.

Please forward any enquiries to tmo@biovalence.com.my

Facilities Geared Toward Becoming A Drug Discovery Engine

BioValence Antiviral & Anticancer Technologies

BioValence has 3 facilities in Kuala Lumpur:

- ✓Research and development laboratory
- ✓Process optimization laboratory
- ✓GMP manufacturing facility (audits & validation to complete in 2014)

GMP Manufacturing Facility


Clean Corridor


Process Room 1


Process Room 2


QC Lab


Microbiology Lab


Media Preparation Room

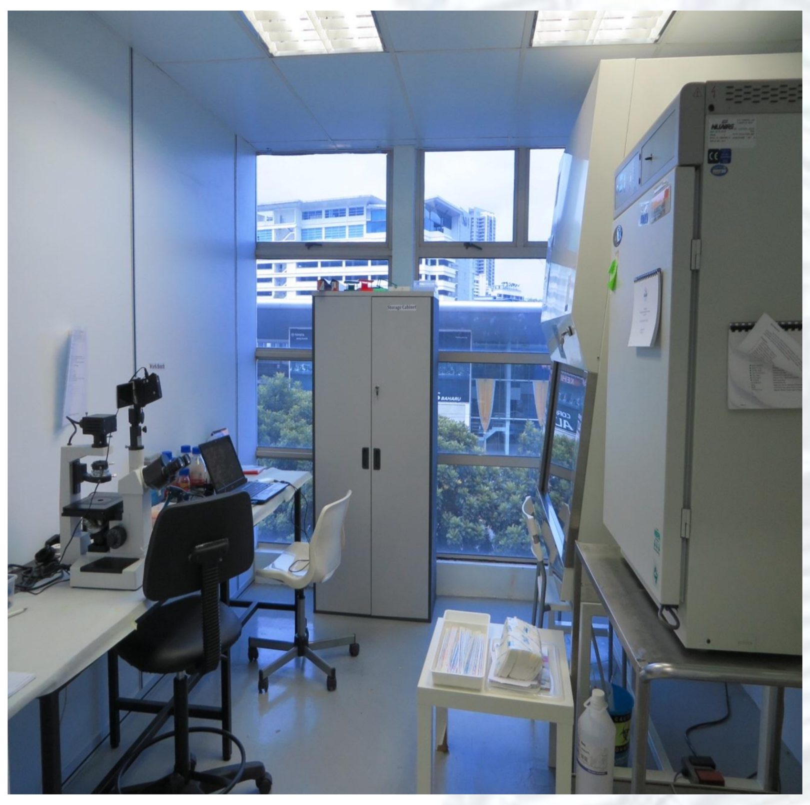

General Corridor

SYNERTEC

QUEST TECHNOLOGY

SeerPharma® CONFIDENCE IN COMPLIANCE

Research & Development Laboratory


Cell Culture Lab


Microbiology Lab


Common Lab


Common Lab

Process Optimization Laboratory


QC Lab


Bioreactor Room


Centrifuge Room


Main Lab

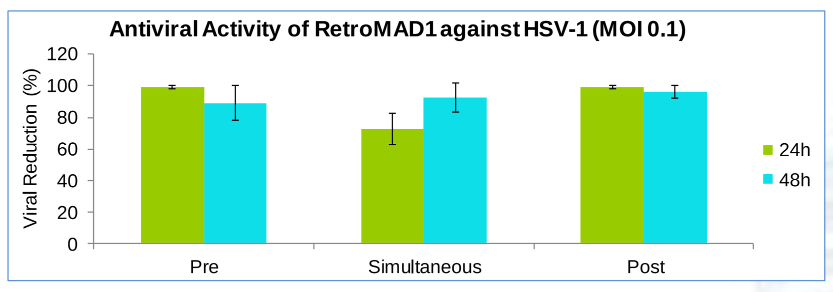
RetroMAD1 Our Lead Drug

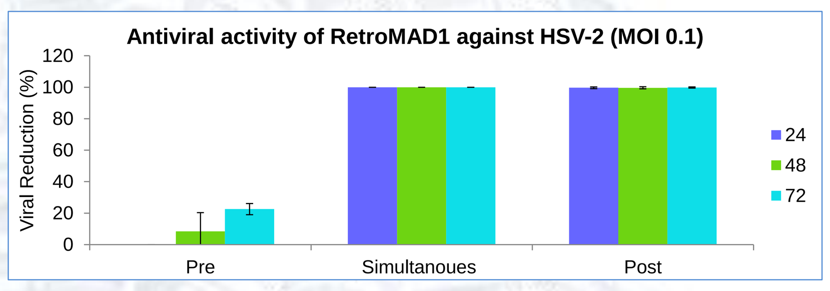
BioValence
Antiviral & Anticancer Technologies

Maximum Non-toxic Dose (MNTD) of Retromad1 against Various Normal Cells Lines

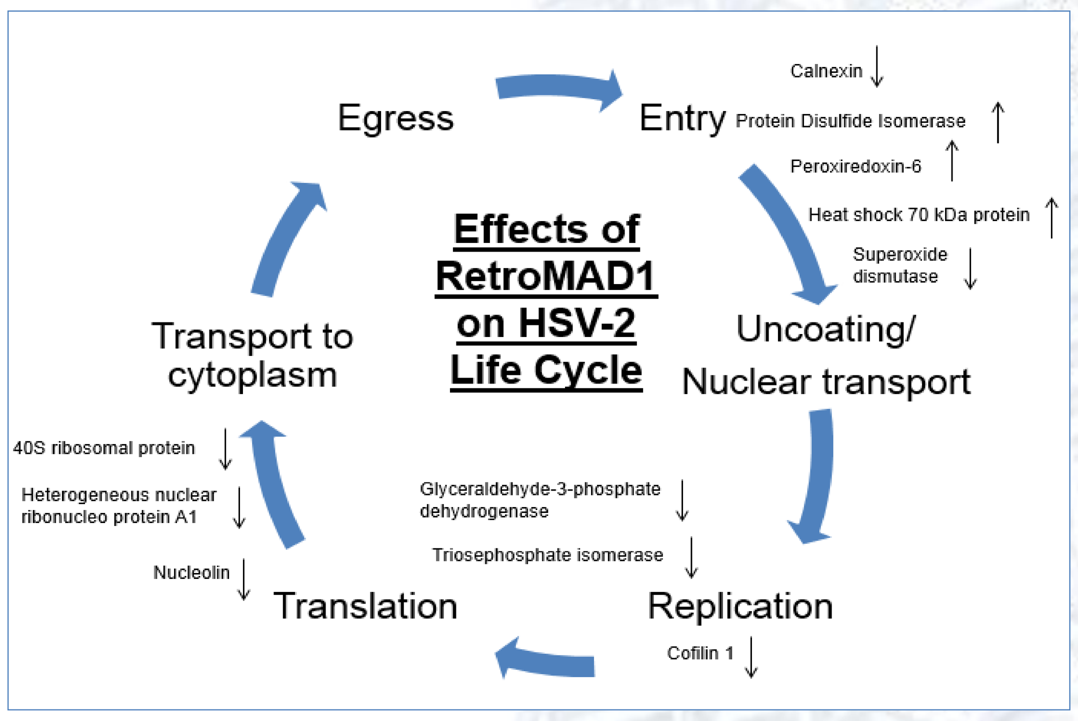
Cell Lines / Time		Vero	LLC-MK2	BHK-21	RWPE-1	Chang's Liver	NL-20	CCD-1127SK
MNTD (µg/mL)	24h	100	100	200	100	100	200	100
	48h	100	100	100	100	100	100	100
	72h	100	50	100	100	100	100	100

Antiviral Activity of RetroMAD1 against HSV-1 and HSV-2



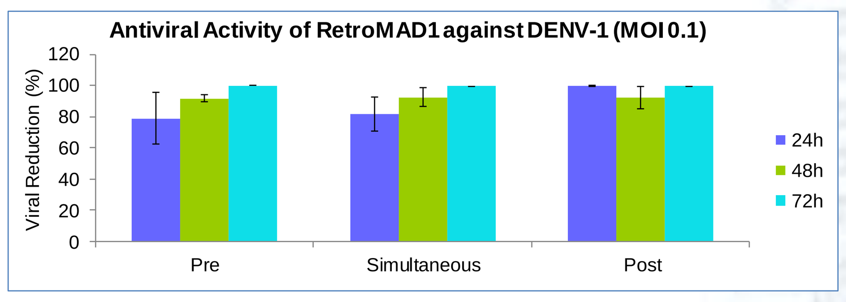


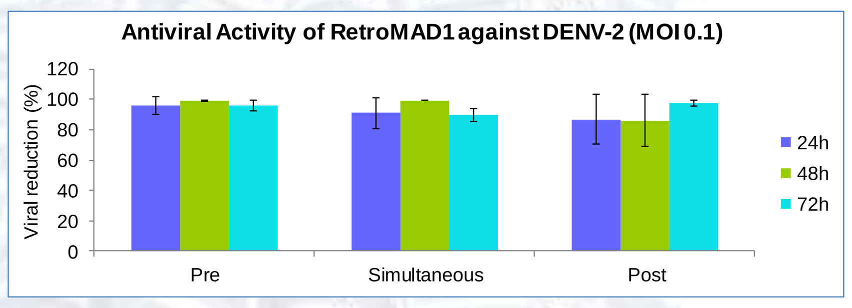
Mechanism of Action based on Proteomics for HSV-2

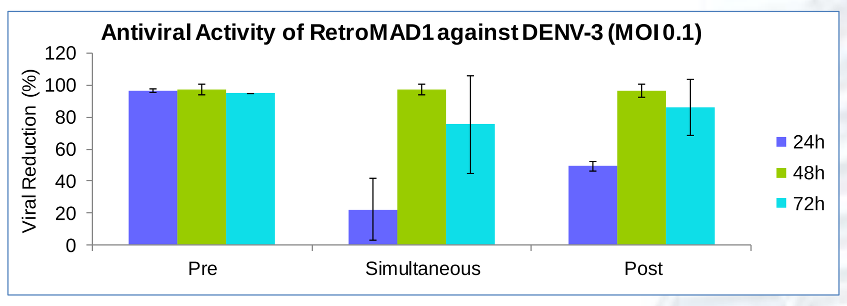


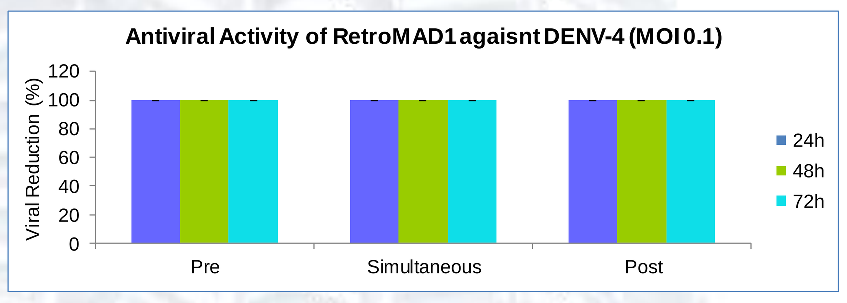
Upregulated / Downregulated Proteins	Cells + RetroMAD1	Cells + Virus (HSV2)	Cells + Virus (HSV2)+ RetroMAD1
Protein Folding			
Protein disulfide-isomerase	+1.87	-5.03	+2.03
Calnexin	-2.51	+3.77	- 6.17
Heat shock 70 kDa protein	-1.80	-9.07	+1.84
Energy, Transport, Metabolism			
Nucleoside diphosphate kinase	+1.55	-1.11	+2.48
Glyceraldehyde-3-phosphate dehydrogenase	-1.27	+2.90	-1.24
Triosephosphate isomerase	+2.60	+2.41	+1.47
Oxidative Proteins			
Superoxide dismutase	+1.14	-3.38	+3.52
Peroxiredoxin-6	+1.82	-1.30	+1.98
Transcription/Translation			
40S ribosomal protein	+4.7	+2.78	-1.73
Heterogeneous nuclear ribonucleo protein A1	-1.07	-2.14	-1.08
Nucleolin	-1.55	-10.04	+17.89
Cytoskeleton			
Cofilin-1	+1.01	+2.94	+1.27

Antiviral Activity of RetroMAD1 against DENV-1, DENV-2, DENV-3 and DENV-4

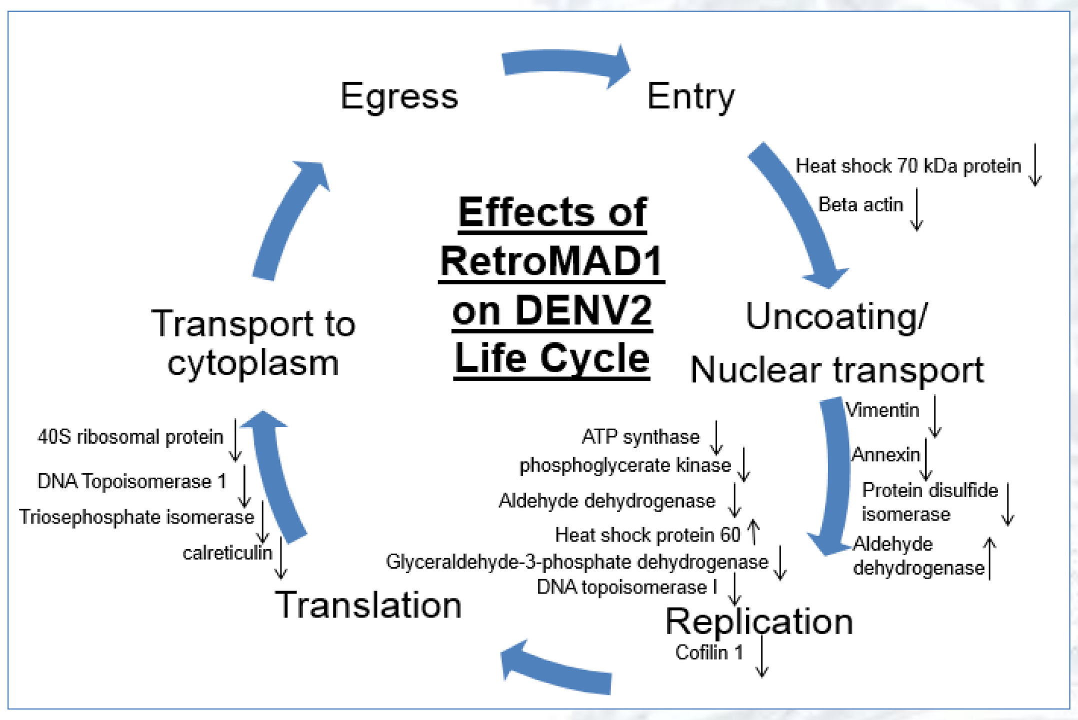






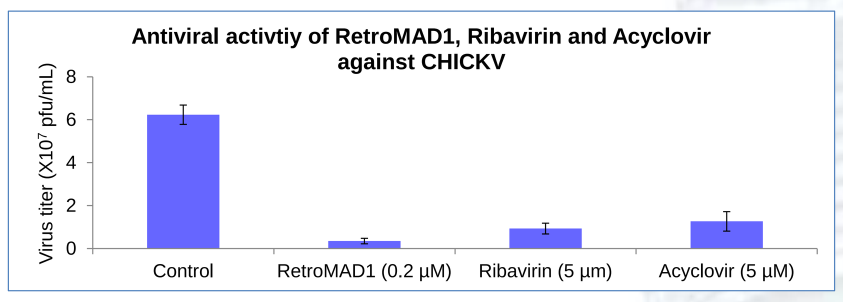


Mechanism of Action based on Proteomics for DENV-2

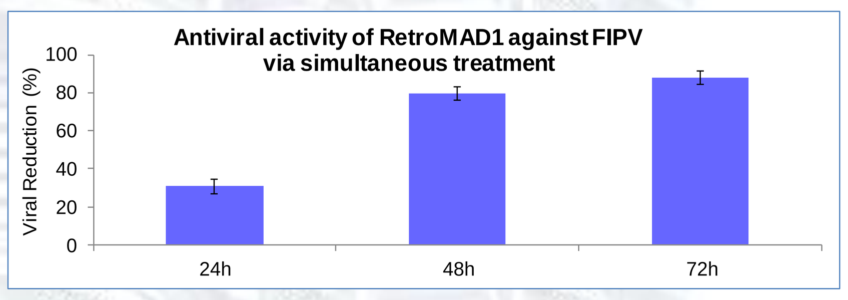


Upregulated / downregulated Proteins	Cells + RetroMAD1	Cells + Virus (DENV2)	Cells + Virus (DENV2)+ RetroMAD1
Protein Folding			
DNA Topoisomerase 1	+ 1.91	+ 3.13	- 4.80
Heat shock 70 kDa protein	- 4.49	+ 2.11	- 3.93
Calreticulin	+ 2.21	- 1.50	+ 2.01
Protein disulfide isomerase	+ 2.18	-2.03	+ 2.65
Endoplasmin	+ 2.89	- 1.66	+ 3.84
Cellular Metabolism and ATP Synthesis			
Phosphoglycerate kinase	+ 4.36	- 1.61	- 3.19
ATP synthase	- 2.05	+ 5.30	- 2.19
Calmodulin	- 3.09	+ 1.20	- 2.06
Aldose reductase	+3.33	- 2.02	- 4.87
Glyceraldehyde-3-phosphate dehydrogenase	+ 6.25	+ 8.66	- 3.01
Transcription/Translation			
Triosephosphate isomerase	+ 2.59	+ 3.56	- 1.21
nucleolin	+1.03	- 2.22	+ 4.62
40S ribosomal protein	- 3.66	+ 2.37	- 2.44
Component of Cytoskeleton/ Cytosol			
Actin, beta	- 3.12	+ 4.29	- 3.51
Actin, cytoplasmic 2	+ 10.32	+ 3.30	- 1.61
non-muscle myosin IIA	- 1.89	+ 2.41	- 2.44
Vimentin	- 3.07	+ 11.65	- 8.06
Oxidative Proteins			
Aldehyde dehydrogenase	+ 2.06	- 3.16	+ 2.32
Component of Cytoskeleton/ Cytosol			
Cofilin-1	+ 1.01	+ 2.94	- 2.27
Annexin A2	- 3.55	+ 2.12	- 2.72

Antiviral Activity of RetroMAD1 against CHIKV

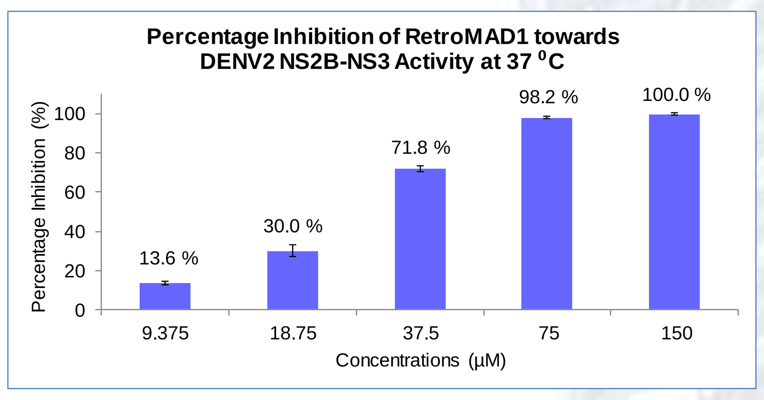


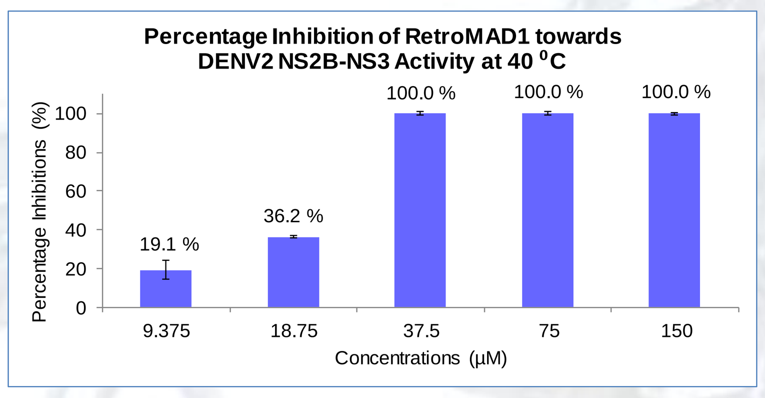
Antiviral Activity of RetroMAD1 against FIPV



- ❖ RetroMAD1 was found to be effective against all the HSV, Dengue, Chikungunya and FIPV viruses over an incubation period of 24, 48 and 72h.
- ❖ The inhibitory effect of RetroMAD1 could be via blocking of viral absorption, replication and also via virucidal effects.

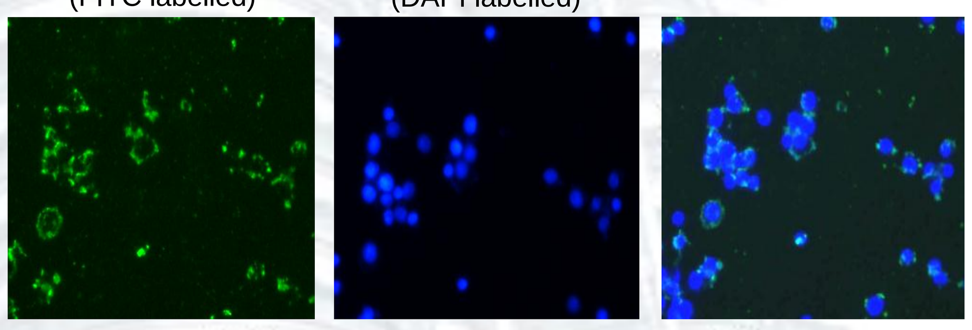
Inhibition of Dengue NS2B-NS3 by RetroMAD1





Entry of RetroMAD1 into cells

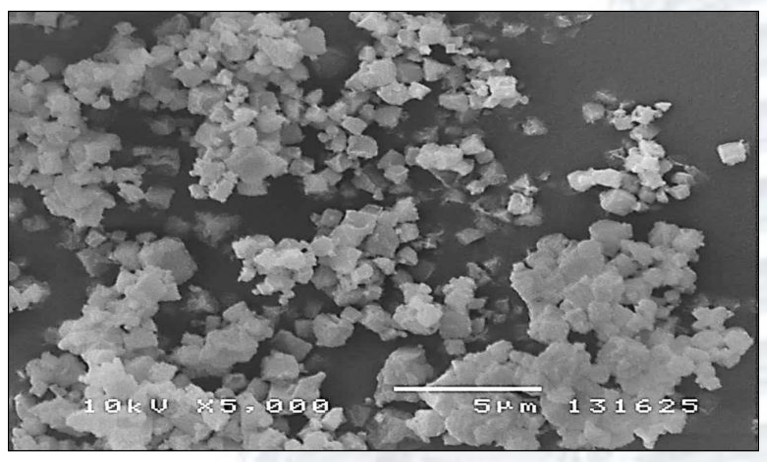
Recombinant protein (FITC labelled)　Cell nuclei (DAPI labelled)　Merged image

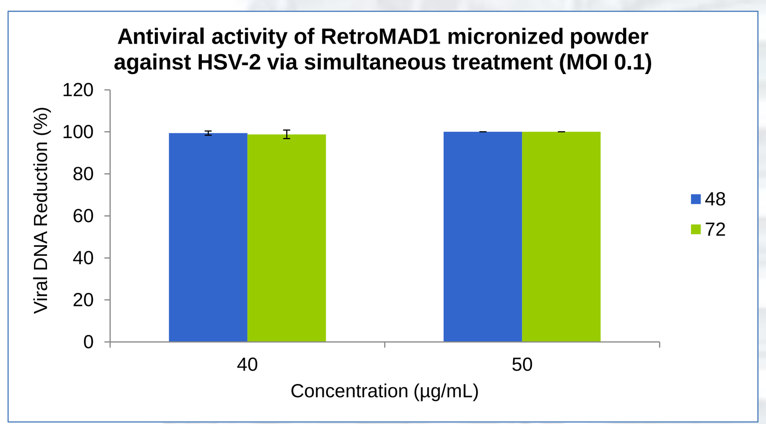


FITC tagged to His-tag on RetroMAD1 shows it congregates around the nucleus after entering the cell

FITC = Fluorescein isothiocyanate
DAPI 4',6-diamidino-2-phenylindole

Micronized Powder of RetroMAD1



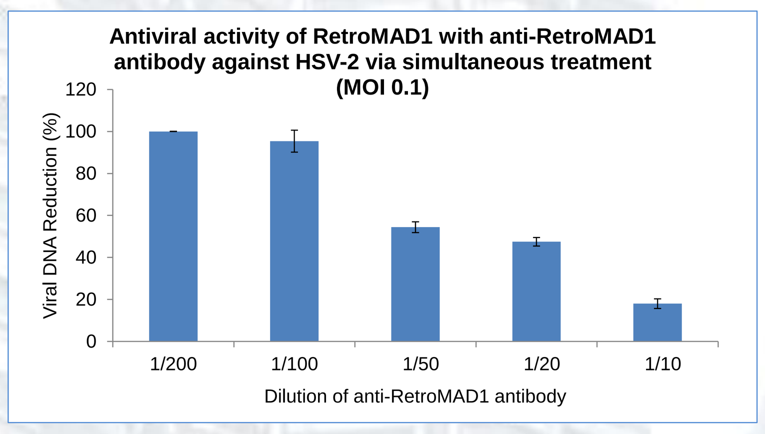


- ❖ RetroMAD1 in powder form exhibited strong inhibitory activity against HSV-2 via simultaneous treatment giving more than 85% of inhibition

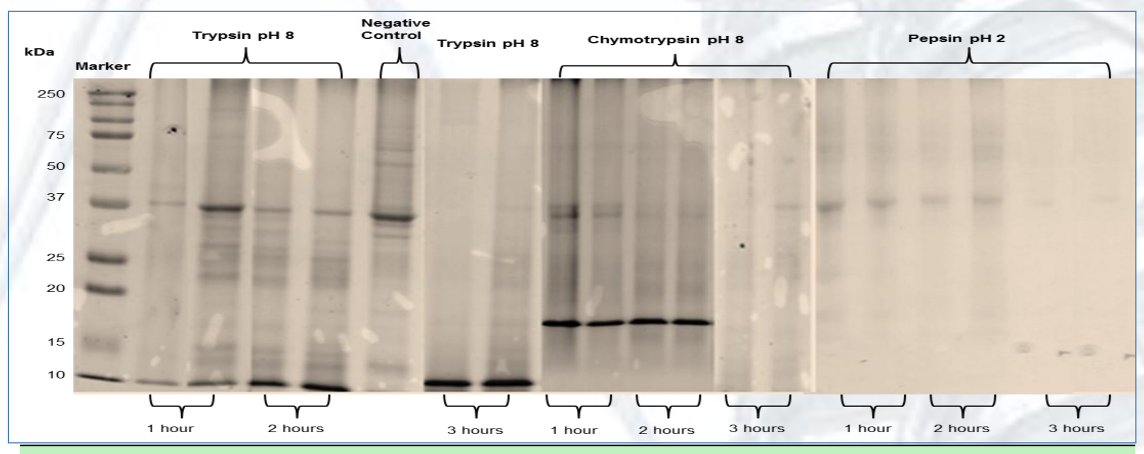
Effects of RetroMAD1 on Feline and Canine Viruses

	Disease / Infection	Sample No.	Symptomatic Recovery Rate (%)
Feline viruses	Feline Immunodeficiency Virus (FIV)	25	76.0
	Feline Leukemia Virus (FeLV)	28	67.9
	Feline Panleukopenia Virus (FPV)	10	90.0
	Feline Calicivirus (FCV)	8	75.0
Canine viruses	Canine Parvovirus (CPV2)	143	80.4
	Canine Coronavirus (CCV)	3	100.0

Effects of Neutralizing Antibody on RetroMAD1

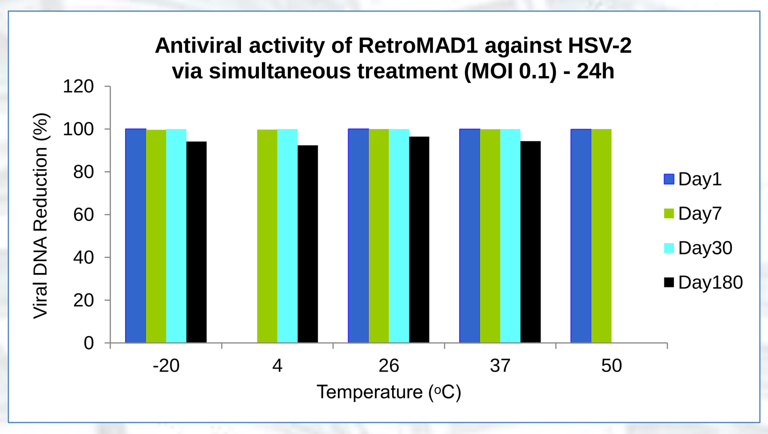


Protease stability of RetroMAD1



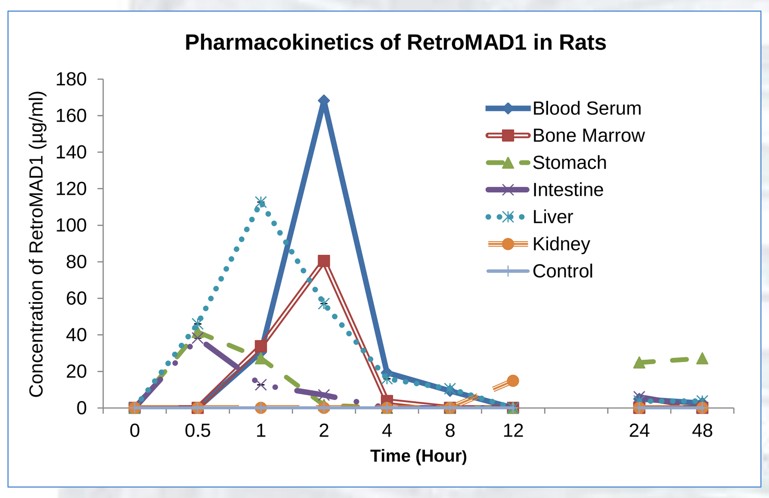
Time	Pepsin (pH2)	Trypsin (pH8)	Chymotrypsin (pH8)
1 hour	Not Digested	Not Digested	Not Digested
2 hours	Not Digested	Not Digested	Not Digested
3 hours	Not Digested	Partially Digested	Partially Digested

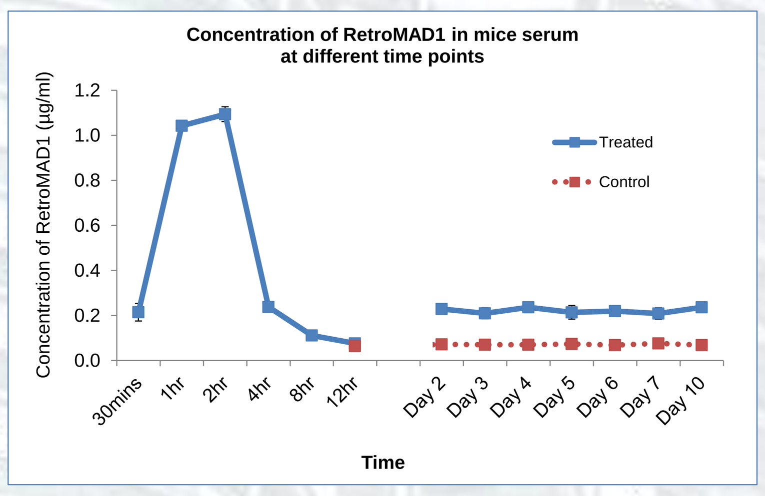
Thermostability of RetroMAD1



- ❖ RetroMAD1 is very heat stable. RetroMAD1 exposed to various temperatures for 1, 7, 30 and 180 days showed strong inhibitory activity against HSV-2 via simultaneous treatment

Pharmacokinetics of RetroMAD1 in rats and mice





Serum bioavailability in rats

- ❖ Of 9000 µg RetroMAD1 fed, 370.47 µg were detected by capture ELISA over a 12h period indicating 4% of bioavailability

Serum bioavailability in guinea pigs

- ❖ Of 1050 µg RetroMAD1 fed, 893.28 µg were detected by capture ELISA over a 12h period indicating 85% of bioavailability

Acute toxicity of RetroMAD1 on rats

Acute toxicity study showed that RetroMAD1 did not exhibit any adverse reaction (mortality / histopathological effects) on rats up to 500 times the therapeutic dose (0.2 mg/kg)

Liver Profile

	Dosage	ALT U/L	AST U/L
Male	Control	49.33 ± 10.33	158.28 ± 19.01
	Low Dose	44.70 ± 8.75	159.40 ± 29.45
	High Dose	50.10 ± 7.36	163.7 3± 60.03
Female	Control	45.45 ± 5.15	164.15 ± 24.18
	Low Dose	32.32 ± 5.71	146.52 ± 7.22
	High Dose	41.58 ± 6.06	156.12 ± 17.35

The lower level of Alanine Aminotransferase (ALT) suggests that change in liver function had not occurred. This reading indicates that orally administered RetroMAD1 has no toxic effect on the liver function of rats.

Kidney Profile

	Dosage	Creatine Umol/L	Urea Mmol/L
Male	Control	40.83 ± 3.54	8.17±1.16
	Low Dose	43.67 ± 5.57	6.65 ± 0.73*
	High Dose	40.33 ± 8.36	6.37 ± 0.91*
Female	Control	44.00 ± 2.94	10.03 ± 1.53
	Low Dose	46.83 ± 3.82	8.15 ± 1.73
	High Dose	49.17 ± 4.62	10.08 ± 1.00

The lower levels of Blood Urea Nitrogen (BUN) does not indicate renal malfunction. Moreover the values of creatine levels when compared to control suggest that RetroMAD1 did not induce renal abnormalities.

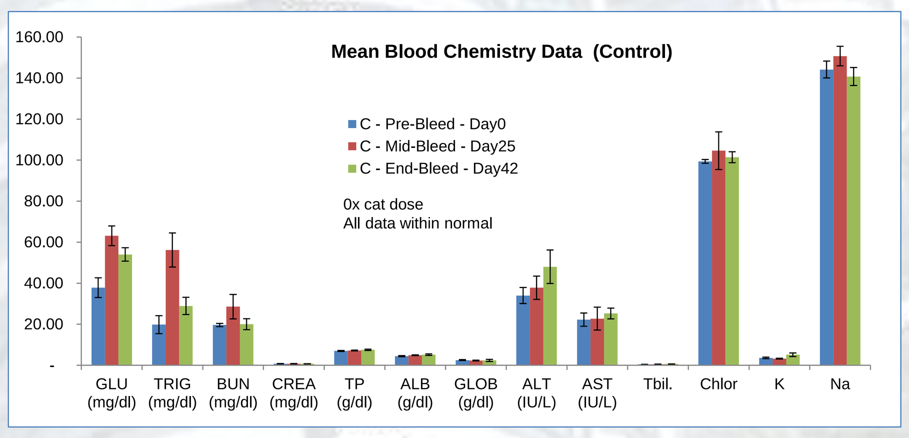
Survivorship of monkeys infected with Simian Rotavirus A after treatment with RetroMAD1

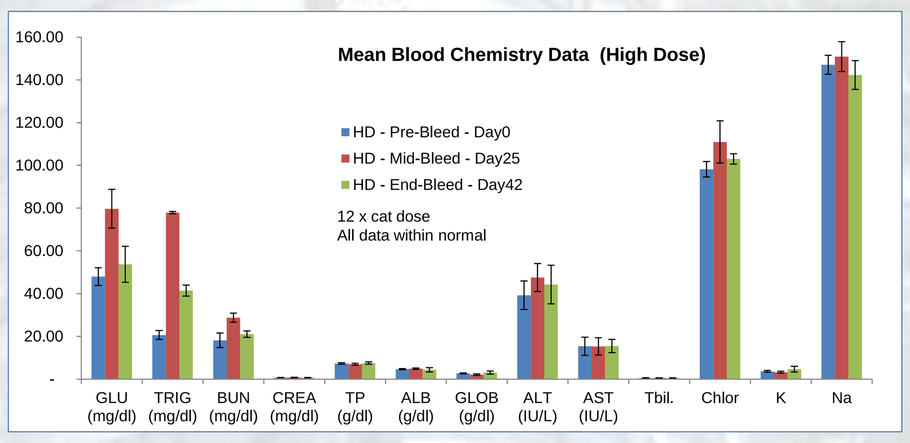
Effect of RetroMAD1 on Normal and Sick Post-Weaned Juvenile Monkeys affected by Simian Rotavirus A
SUMMARY OF RESULTS

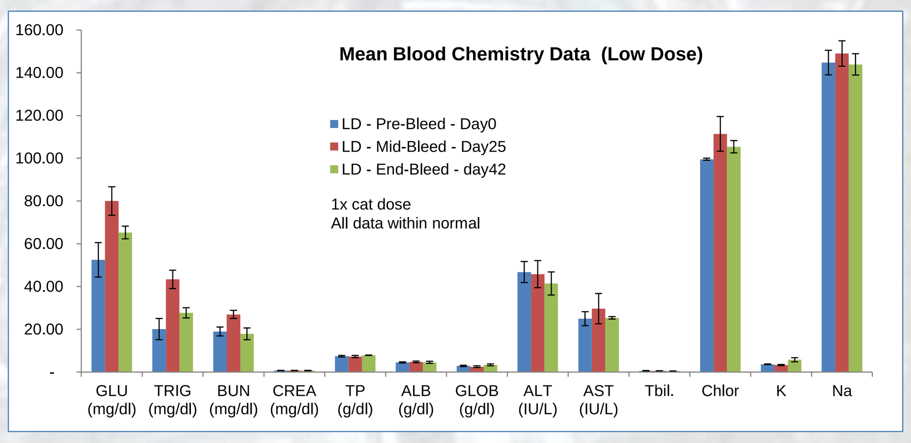
Batch		Healthy			Sick	
Group		HIGH DOSE	LOW DOSE	CONTROL	TREATMENT	CONTROL
Dose		12 x (2.4 mg)	1 x (0.2 mg)	None	1 x (0.2 mg)	None
No. of animals		5	5	5	5	5 (2 survivors)
Fecal Consistency	Beginning		Formed		Watery (5)	Watery (5)
	End		Formed		Pasty-formed (3) Pasty-formed (2)	Watery (2)
Appetite	Beginning		Fair-good		Poor	Poor
	End		Fair-good		Fair-good	Fair-good
Feed consumption (%)		84.10	80.90	77.00	87.50	73.9
					Cerelac – 20 mL (inclusive)	
Wt. Gain/Loss in Grams (%)		-10 (-0.6)	-20 (-1.2)	-40 (-2.4)	120 (7.0)	159 (20.0)
Mortality (hds)			None		None	3

Toxicology study was carried out at a primate centre SICONBREC, an AAALAC facility near Manila.

- ❖ The main observation was a slight increase in blood triglycerides in the high-dose group. All other blood profile parameters were within expected ranges.
- ❖ Hematology, histopathology and blood chemistry were noted to be within the standard range. No lesions were observed in the low dose group.
- ❖ 5/5 of treated monkeys survived while in the untreated control group 2/5 survived. Simian Rotavirus was confirmed to be present only in the faeces of sick monkeys.







Molecular Docking for RetroMAD1

BioValence
Antiviral & Anticancer Technologies

Molecular Docking

- ✓ Docked energy, Van der Waals, electrostatic, biding site confirmation, pi-pi interactions
- ✓ Two main types:

I. Protein-protein docking
   - Extensive computational power and time
   - Example: HadDock, FireDock

II. Ligand-protein docking
   - Moderate computational requirement
   - Example: AutoDock 4, AutoDock Vina

Current Study

- ✓ Protein-protein docking
- ✓ Both NS2B_NS3 and NS3_4A are serine proteases
- ✓ NS3_4A: His-57, Asp-81 and Serine-139; chain A/C Protease/Helicase
- ✓ NS2B_NS3: His-51, Asp-75 and Serine-135; chain B
- ✓ First comparative docking studies of NS2B_NS3 and NS3_4A by RetroMAD1 antiviral chimeric peptide using HadDock and FireDock

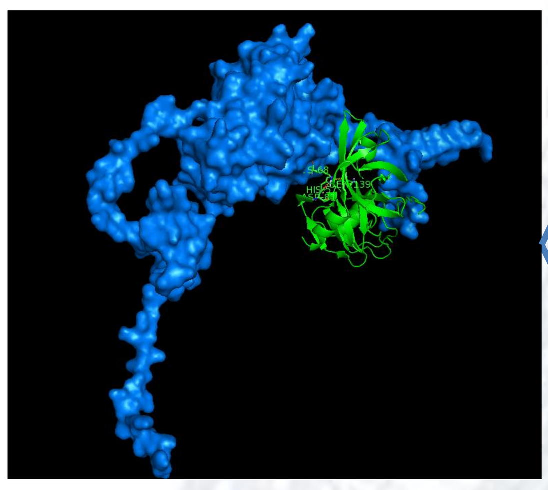
Lock-and-key docking of RetroMAD1 to NS3_4A/NS2B_NS3

HadDock Receptor/ligand	Docked Energy (kJ/mol)	Van der Waals (kJ/mol)	Electrostatic (kJ/mol)
RetroMAD1(receptor)			
ligand			
NS3_4A-Lys68*	-79.2 ± 6.2	-53.2 ± 6.0	-299.0 ± 69.3
NS2B_NS3-Ile30_leu31^	-70.3 ± 2.1	-39.7 ± 2.7	-238.2 ± 22.3
NS3_4A*(receptor)			
ligand			
RetroMAD1-Lys68	-108.0 ± 12.6	-59.9 ± 13.2	-330.4 ± 40.9
RetroMAD1-His85	-99.0 ± 4.7	-72.4 ± 4.9	-191.3 ± 25.6
NS2B_NS3^ (receptor)			
ligand			
RetroMAD1-Arg101	-93.4 ± 8.7	-70.9 ± 7.0	-247.3 ± 38.0

Active site-active site docking of RetroMAD1 to NS3_4A/NS2B_NS3

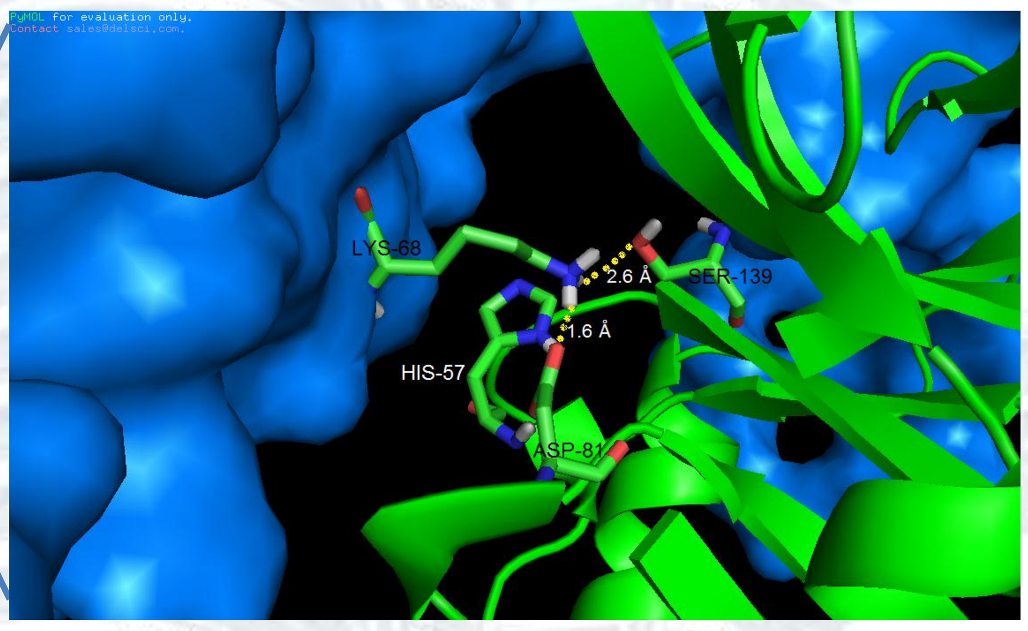
Docking software	Docked Energy (kJ/mol)	Van der Waals (kJ/mol)	Electrostatic (kJ/mol)	Binding site
HadDock				
(targeted)				
NS3_4A*	-78.7 ± 14.1	-44.0 ± 10.1	-399.6 ± 32.8	Yes
NS2B_NS3^	-67.3 ± 5.3	-60.7 ± 5.7	-225.6 ± 9.2	Yes
FireDock				
(blind)				
NS3_4A*	-18.12	-4.82	-3.76	No
NS3_4A+	-57. 87	-31. 71	-19.68	No
NS2B_NS3+	-9.72	-19.88	-40.71	No

NS3_4A



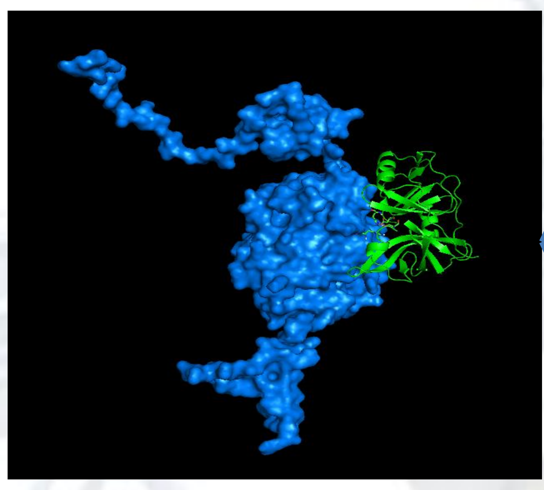
Lys-68 on RetroMAD1 inhibits NS3_4A HIS-57, ASP-81, SER-139 active site.

NS3_4A (HIS-57,ASP-81,SER-139) -108.0 ± 12.6 kJ/mol



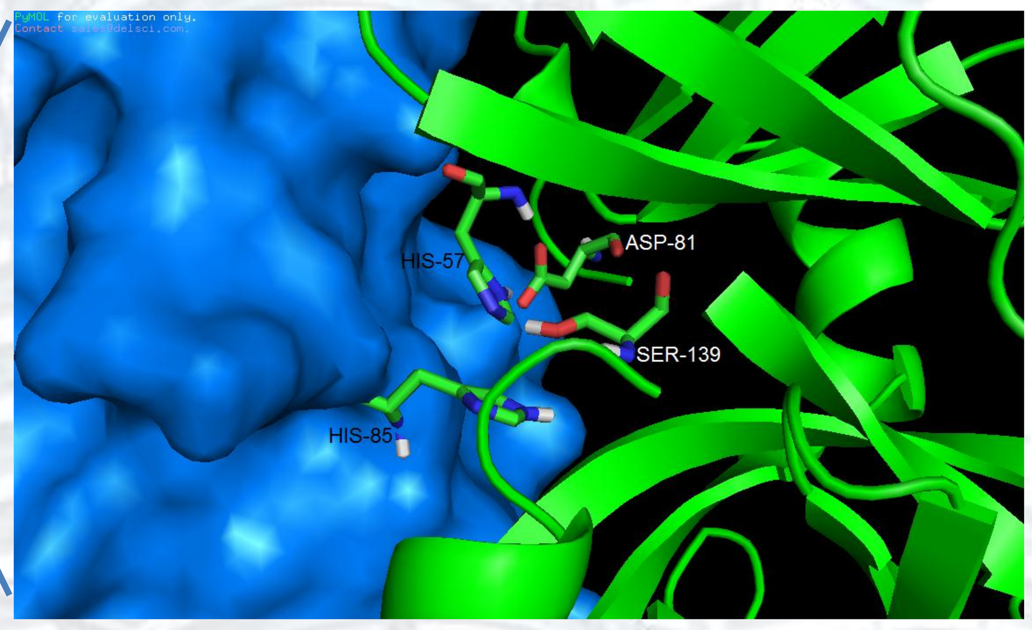
Hydrogen bond 1.6 Å between LYS-68 (RetroMAD1) and ASP-81 (NS3_4A putative binding site); Hydrogen bond 2.6 Å between LYS-68 (RetroMAD1) and SER-139 (NS3_4A putative binding site)
Green: C; white: H; red: O; blue: N; blue molecule: RetroMAD1; green molecule: NS3_4A; yellow dotted line: hydrogen bond.

NS3_4A



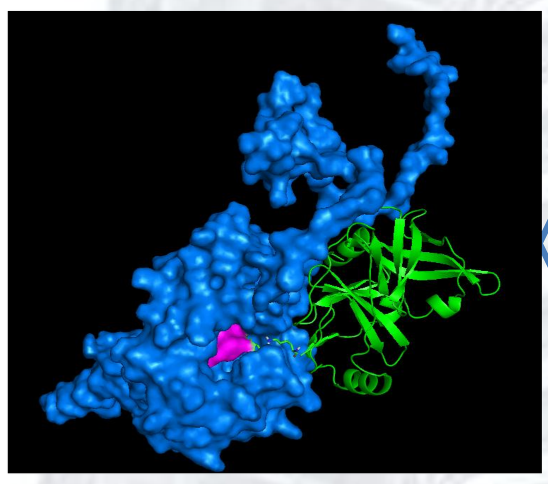
HIS-85 on RetroMAD1 inhibits NS3_4A HIS-57, ASP-81, SER-139 active site.

NS3_4A (HIS-57,ASP-81,SER-139) -99.0 ± 4.7 kJ/mol



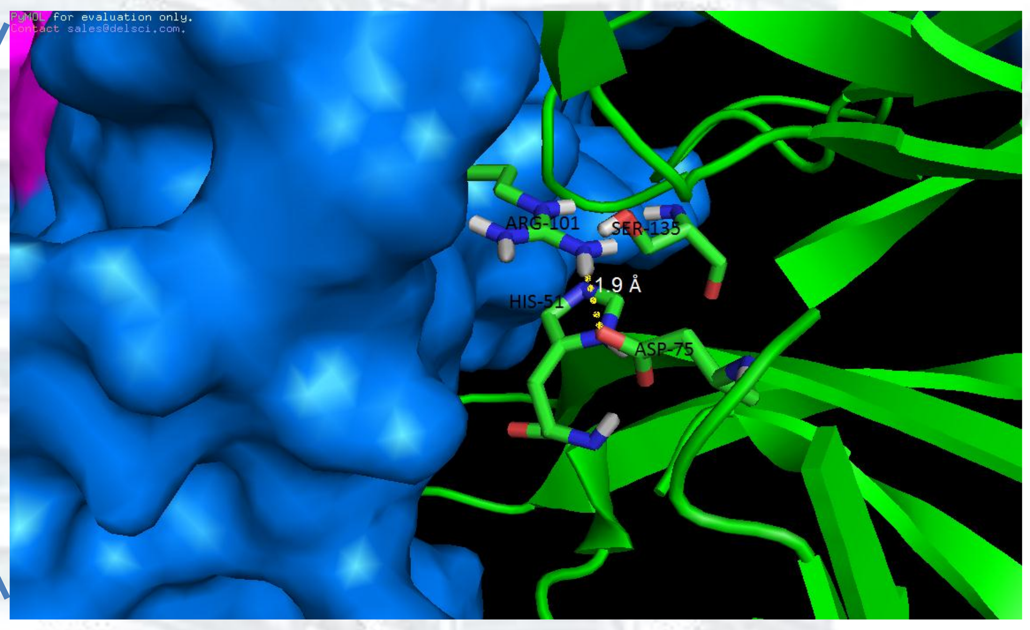
No hydrogen bond. Green: C; white: H; red: O; blue: N; blue molecule: RetroMAD1; green molecule: NS3_4A.

RetroMAD1



RetroMAD1 TYR-126,TYR-165,GLU-214,ARG-217 active site binds to LYS-68 on NS3_4A
.

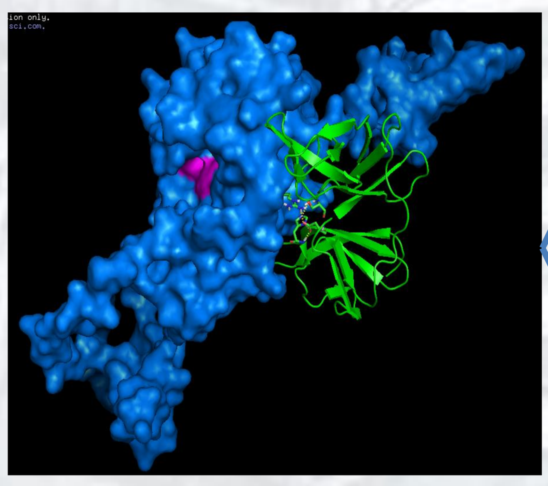
RetroMAD1 (TYR-126,TYR-165,GLU-214,ARG-217) -79.2 ± 6.2kJ/mol



Hydrogen bond 1.6 Å between LYS-68 (NS3_4A) and GLU-166 (near RetroMAD1 binding site);
Green: C; white: H; red: O; blue: N; blue molecule: RetroMAD1; green molecule: NS3_4A; yellow dotted line: hydrogen bond.

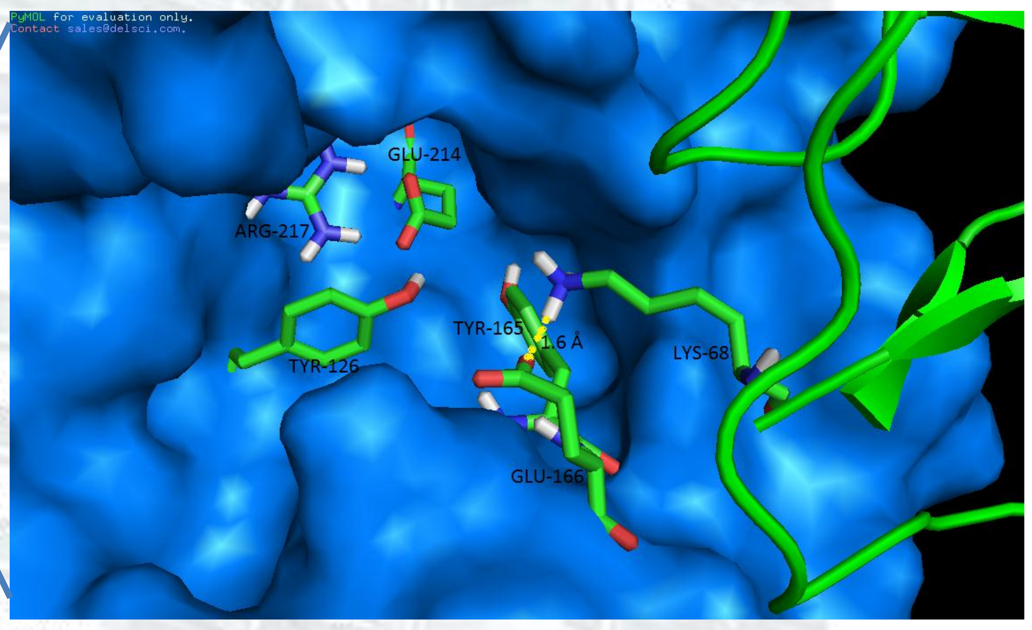
- HadDock produced more consistent results
- RetroMAD1 showed comparable inhibition to both HCV NS3_4A and Dengue NS2B_NS3 proteases
- Lock-and-key docking produced better results
- RetroMAD1 as a ligand, RetroMAD1-Lys68 showed best inhibition to NS3_4A at -108.0 ± 12.6 kJ/mol, followed by RetroMAD1-Arg101 to NS2B_NS3 at -93.4 ± 8.7 kJ/mol
- RetroMAD1 as a receptor, moderate inhibition was obtained
  - Indeed, RetroMAD1 is more specific as a receptor for adenosine in eukaryotic ribosome and viral polynucleotide
- Molecular interactions mainly due to electrostatic and Van der Waals interactions

NS2B_NS3



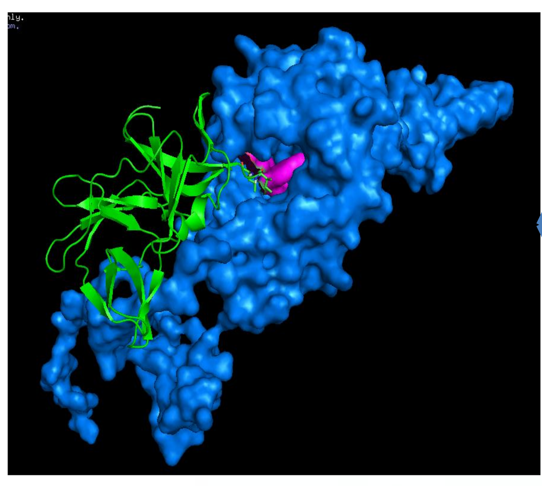
ARG-101 on RetroMAD1 inhibits NS2B_NS3 HIS-51,ASP-75,SER-135 active site

NS2B_NS3 (HIS-51,ASP-75,SER-135) -93.4 ± 8.7 kJ/mol



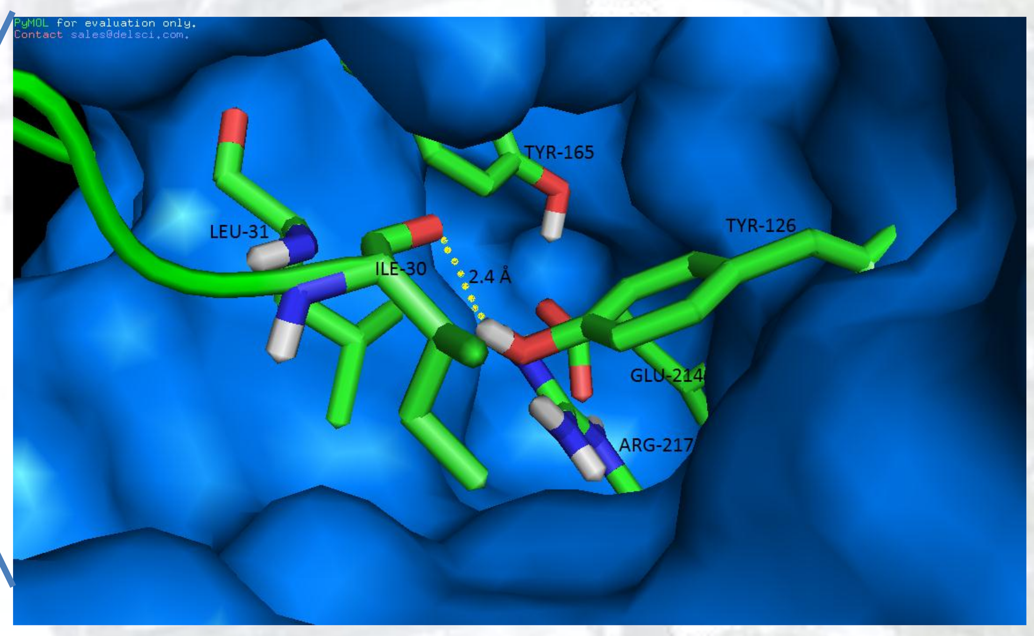
Hydrogen bond 1.9 Å between ARG-101 (RetroMAD1) and ASP-75 (NS2B_NS3 putative binding site)
Green: C; white: H; Red: O; blue: N; magenta patch: putative binding site; blue molecule: RetroMAD1; green molecule: NS2B_NS3; yellow dotted line: hydrogen bond.

NS2B_NS3



RetroMAD1 HIS-51,ASP-75,SER-135 active site binds to Ile30_leu31 on NS2B_NS3.

NS2B_NS3 (HIS-51,ASP-75,SER-135) -70.3 ± 2.1 kJ/mol

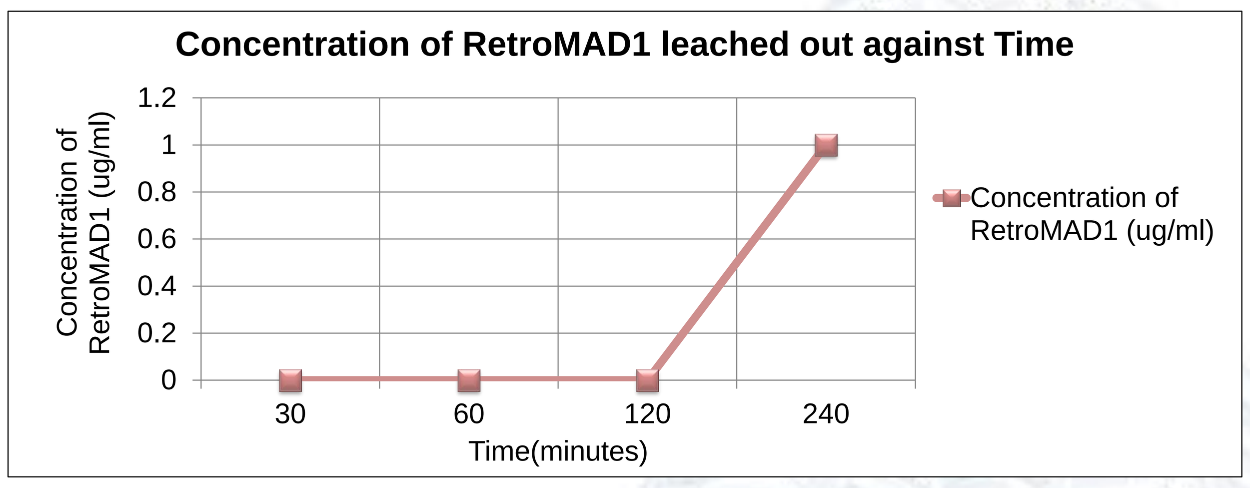


Hydrogen bond 2.4 Å between ILE-30 (NS2B_NS3) and TYR-126 (RetroMAD1 putative binding site) Green: C; white: H; Red: O; blue: N; blue molecule: RetroMAD1; green molecule: NS2B_NS3; yellow dotted line: hydrogen bond.

Aquaculture

BioValence Antiviral & Anticancer Technologies

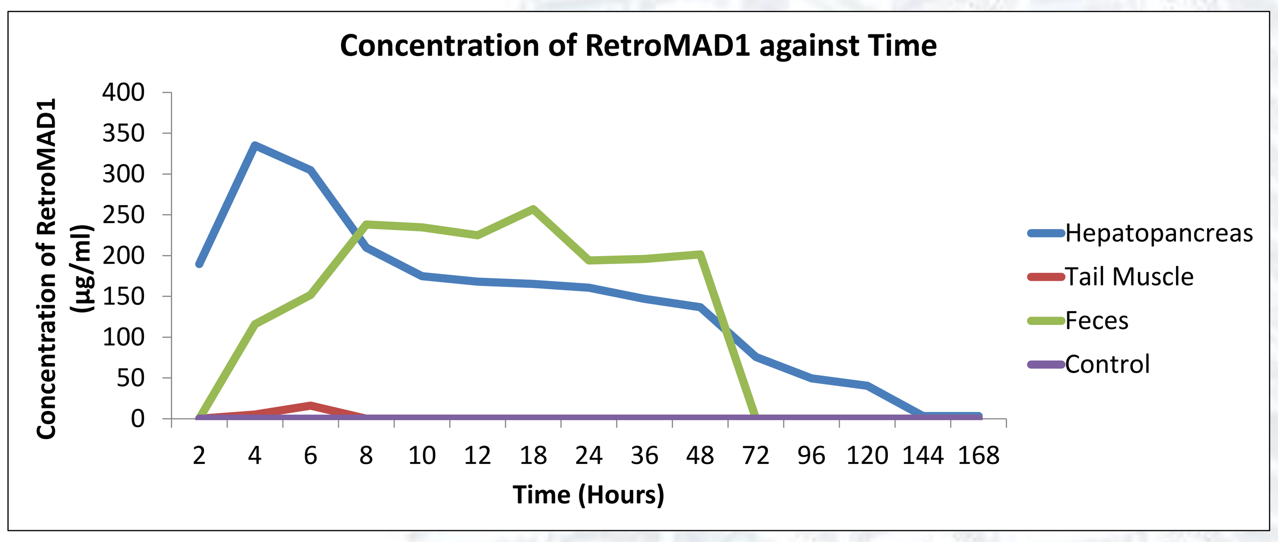
Low leaching rate demonstrated from feed pellets



- ❖ Concentration of RetroMAD1 that has leached out of the feed pellet is determined using capture ELISA
- ❖ RetroMAD1 can be retained in feed wafer for up to 2 hours which is sufficient time for the prawn or fish to consume with minimal loss of the drug.

This experiment shows that RetroMAD1 can be coated into feed pellets for aquaculture animals.

Retention of RetroMAD1 in Shrimp after long term feeding



Graph of concentration of RetroMAD1 in hepatopancrease, tail muscle and feces against time.

- ❖ Each shrimp was fed with 0.2g for shrimp feed containing RetroMAD1 of 300mg/kg
- ❖ Absorption , retention and excretion were studied for up to 7 days
- ❖ 2-4 hours RetroMAD1 was absorbed into the hepatopancreas then showing a decrease
- ❖ 4-8 hours RetroMAD1 was absorbed into the tail muscle
- ❖ 8 hours onwards RetroMAD1 is excreted into the feces

This study suggests that RetroMAD1 can be orally delivered to the shrimp and will not be retained in the body and organs of shrimp within a week of consumption.

Monodon Baculovirus (MBV)

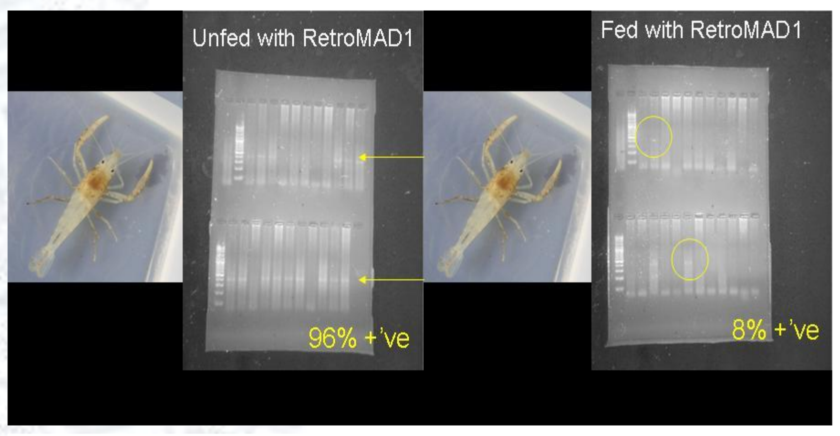
PCR Results			
P.vannamei	Day 0	Day 7	Day 14
Animal 1	Positive	Positive	Negative
Animal 2	Positive	Positive	Negative
Animal 3	Positive	Positive	Negative
Animal 4	Positive	Positive	Negative

Table: Results of PCR for MBV post RetroMAD1 treatment. Time needed for sero- reversal to occur in MBV shrimp when treated with RetroMAD1.

- ❖ PCR results shows that after week 1 shrimp were negative for the Monodon Baculovirus.
- ❖ RetroMAD1 efficiently eliminated MBV from shrimp. Larger sample size will be conducted in the future.

RetroMAD1 can be used for MBV virus clean up in shrimp

Shrimp Hepatopancreatic Parvovirus (HPV)



- ❖ *Palaemonetes kadakensis* with HPV were fed RetroMAD1 for 4days
- ❖ PCR for the highly conserved 441 bp coat-protein of the virus wad done and compared against unfed control.

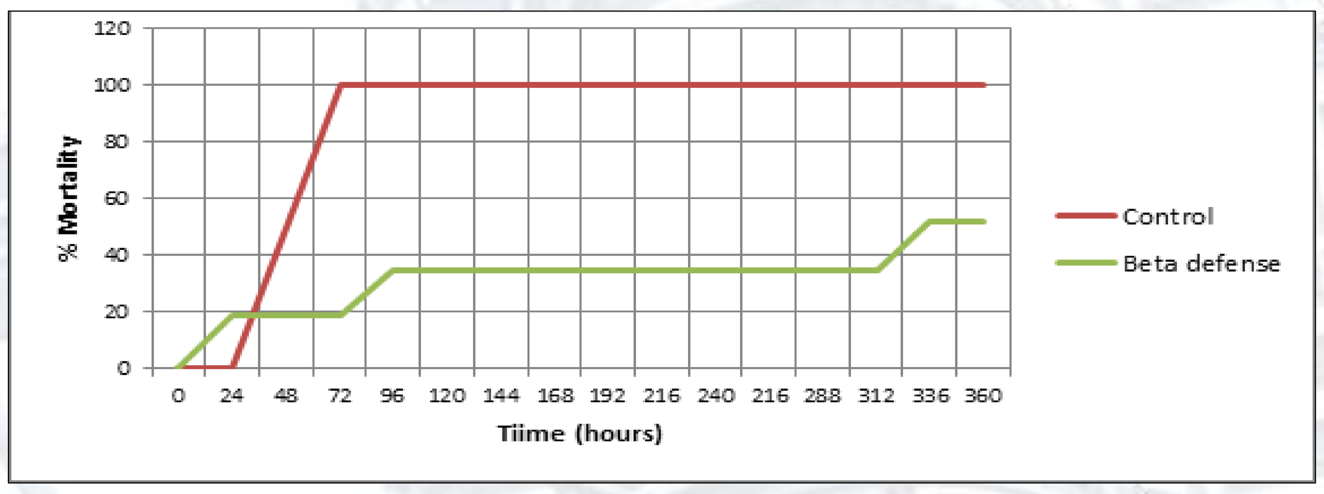
RetroMAD1 effectively eliminate HPV from shrimp in 4 days.

Results of WSSV Challenge assay using RetroMAD1

Tanks	Mortality (%)						
	24 hours	48 hours	72 hours	96 hours	120 hours	144 hours	168 hours
Control (+) 1	0	60	90	100	100	100	100
Control (+) 2	0	20	100	100	100	100	100
RetroMAD1-1	0	0	0	0	0	0	0
RetroMAD1-2	0	0	0	0	0	0	0
Control (-) 1	0	0	0	0	0	0	0
Control (-) 2	0	0	0	0	0	0	0

- ❖ Positive control shrimp were all killed by 96 hours.
- ❖ Orally infected PCR positive WSSV shrimp are converted to PCR negative shrimp with 100% survival using RetroMAD1.

Improved survival against White Spot Syndrome Virus (WSSV) using Beta-Defense



- ❖ Prawns cultured for 40 days in the farm before being subjected to WSSV oral challenge.
- ❖ Beta Defense provides significant protection against WSSV .

Field Trial with Beta-Defense & RetroMAD1

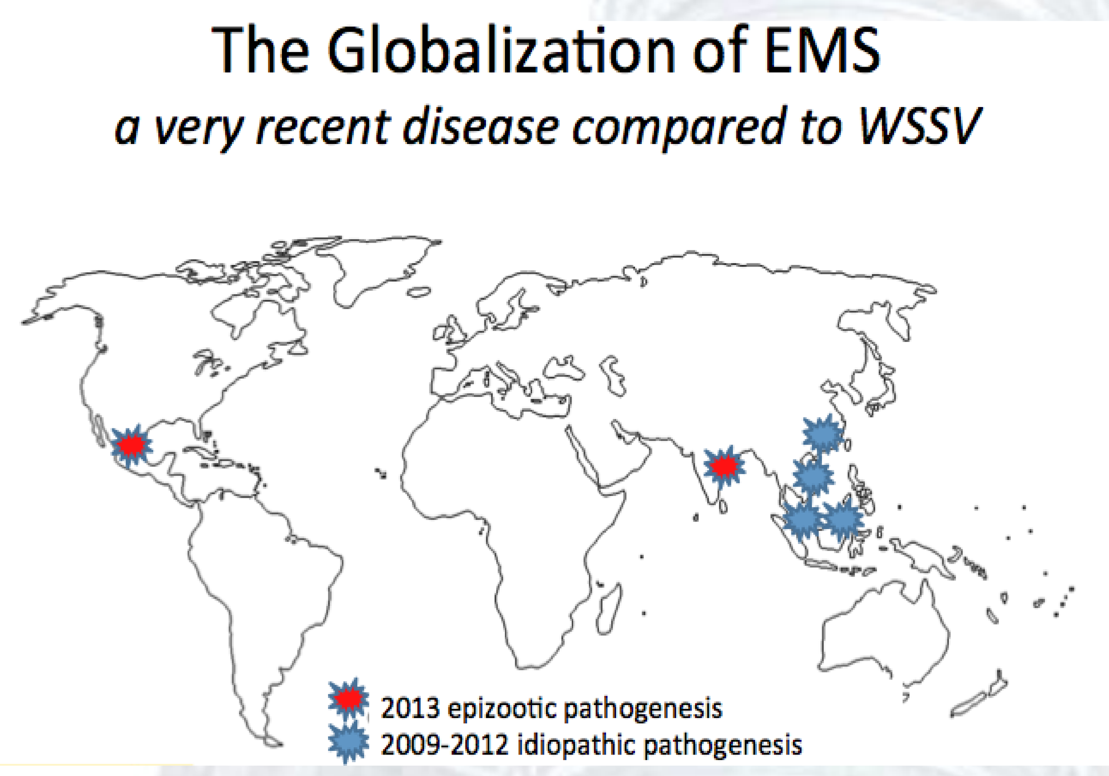
Treatment	Pond	Size(Ha)	Stocking	No. pcs.	Pcs/m2	Harvest	DOC	size (g)	harvest(kg)	feed used	FCR	MT/ha	survival%
Z+E+BS	C3	0.60	09/11/13	500000	83	20/01/14	73	12.68	5,632.00	7,819.00	1.39	9.39	88.83
Z+E+BS	C4	0.60	09/11/13	500000	83	25/01/14	77	14.57	7,299.95	8,623.00	1.18	12.17	100.21
Z+S+BS	C1	0.60	04/11/13	500000	83	24/01/14	81	15.80	7,078.00	9,176.00	1.3	11.80	89.59
Z+S+BS	C2	0.60	10/11/13	500000	83	26/01/14	77	13.58	6,932.00	8,925.00	1.29	11.55	102.09
BS	C6	0.60	16/11/13	500000	83	27/01/14	72	14.40	6,424.54	7,306.00	1.14	10.71	89.23
BS	C5	0.60	16/11/13	500000	83	28/01/14	73	13.95	5,916.65	7,232.00	1.22	9.86	84.83
BS	C7	0.60	16/11/13	500000	83	29/01/14	74	14.96	6,243.02	7,546.00	1.21	10.41	83.46

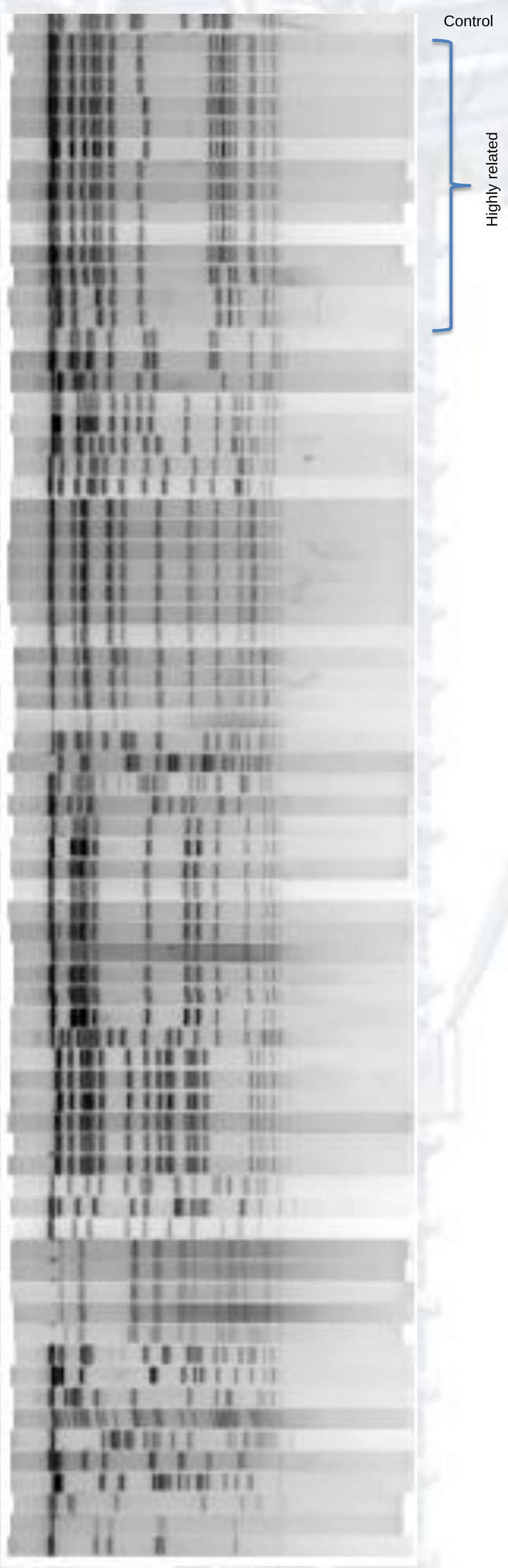
Treatment	ponds	DOC	size (g)	MT/ha	FCR	survival
Z+E+BS	2	75	13.63	10.78	1.285	94.52
Z+S+BS	2	79	14.69	11.68	1.295	95.84
BS	3	73	14.44	10.33	1.19	85.84

Z = Zymedin
E = Easy Tab
S = Superbiotic
BS = Beta Defense+ , Beta Defense , Sludgebuster
Beta Defense+ = Beta Defense + RetroMAD1

RetroMAD1 along with Beta-defense have at least 91% survival rate as compared to the neighboring farms where mass mortality occurred during the same culture period.

Early Mortality Syndrome (EMS) leads to huge price increase

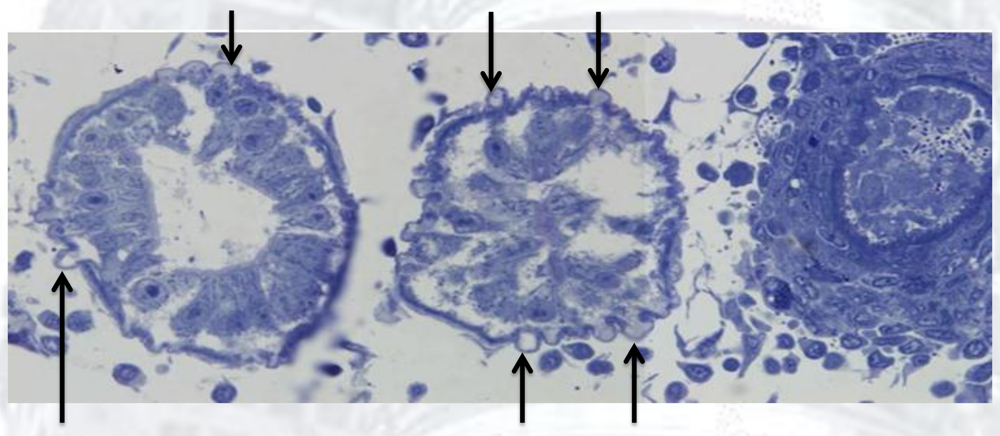




Early Mortality Syndrome (EMS) is an emerging disease caused by bacteria. This disease is often fatal to shrimp. Infected shrimp ponds can experience loss rates as high as 100 percent.

Since EMS was first reported in China in 2009, it has spread to Vietnam, Malaysia and Thailand, and now causes annual losses more than U.S. $2 billion. EMS outbreaks typically occur within the first 30 days after stocking a newly prepared shrimp pond, and mortality can exceed 70%. There is now a global shortfall of 1 million MT/year of shrimp supply due to EMS.

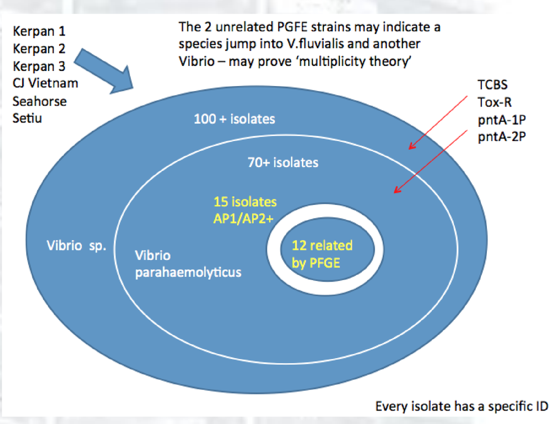
BioValence is researching on various mitigation strategies to overcome this disease.

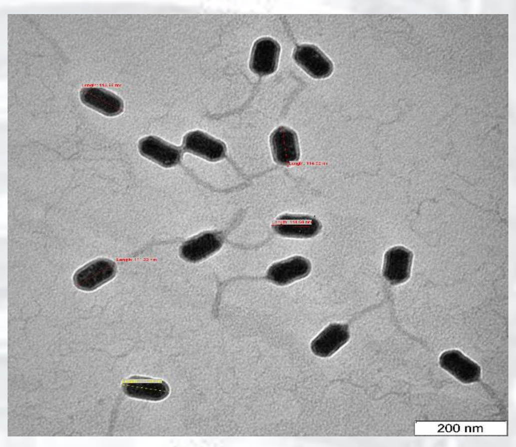


- ❖ Blebbing was observed in the hepatopancreas of an EMS infected shrimp.
- ❖ Blebbing is when a cell swells and bursts – this also happens to bacteria when a Cationic Antimicrobial Peptide (CAP) is involved.

Therefore, we hypothesize that there could be a CAP production by the pathogenic bacteria.

Pulsed-field Gel Electrophoresis (PFGE) of pathogenic *Vibrio parahaemolyticus*





Transmission Electron Microscope of a phage found to be able to kill Vietnamese EMS strain of *Vibrio parahaemolyticus*

- ❖ Screening of *Vibrio parahaemolyticus* from EMS infected shrimp.
- ❖ From the PFGE : 12 related V. *parahaemolyticus* is found.

Effects of RetroMAD1 on Asian Seabass fingerling fishes infected with VNN

Lates calcarifer survivor juveniles	Survival Rate (%)					Weight gain and FCR		
Treatment	Day 1	Day 6	Day 14	Day 22	Day 28	Initial Weight	Final Weight	FCR
Control – feed only	100	0	0	0	0	5.7	7.53	n.a.
Feed+BD	100	0	0	0	0	5.71	7.22	n.a.
Feed+RetroMAD1	100	100	100	48	48	5.65	15.8	1.8
Feed+BD+RetroMAD1	100	100	100	78	78	5.67	17.9	0.5

- ❖ Further evidence of oral delivery as drug was absorbed onto fish feed
- ❖ Superior survival, FCR and weight gain when treated with both Beta-Defense and RetroMAD1.

VNN = Viral Nervous Necrosis
FCR = Food Conversion Ratio i.e. kg feed : kg weight gain
BD = Beta-Defense ; an immuno-stimulant type adjuvant

RetroMAD1 together with Beta-defense gave a better survival of Seabass suffering form VNN than just RetroMAD1 alone. Therefore RetroMAD 1 can be used as a combinatorial.

Koi Herpes Virus

In vitro assay on Cell Lines infected with Koi Herpes Virus (KHV).

	Treatment	
Cell Line	**100µg RetroMAD1 Day 1 Post infection**	**100µg RetroMAD1 Day 7 Post infection**
CCB	No virus growth	No virus growth
KF	No virus growth	No virus growth

CCB= Common Carp Brain Cells
KF= Koi Fin
* All experiments were done 4 times

Dr. Manfred Weidmann
University of Stirling
Institute of Aquaculture

Targeting Cancer:
Using Oral Delivery Peptides

BioValence
Antiviral & Anticancer Technologies

Unique features of our fusion compounds:

- ✓ Our drugs are designed for oral delivery
- ✓ Proven to survive the Gastro-Intestinal Tract
- ✓ Demonstrated to be thermostable at various conditions
- ✓ Screening assays have shown good Therapeutic Index

Schematic Representation of Development of Anti-Cancer Peptide

Searching for Anticancer peptide candidates

↓

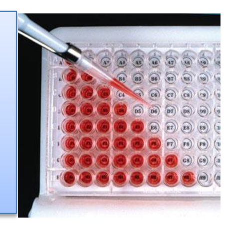
Expression and confirmation of chimeric peptides

↓

Computational Biology to study active sites within the structure

↓

Cell based assays: Phenotypic endpoint, MTS and LDH assay



⇩ **Analysis of Pathway**

Analysis of signaling pathway: FACS and PCR

↓

Proteomics 2D gel assay and Thermostability study

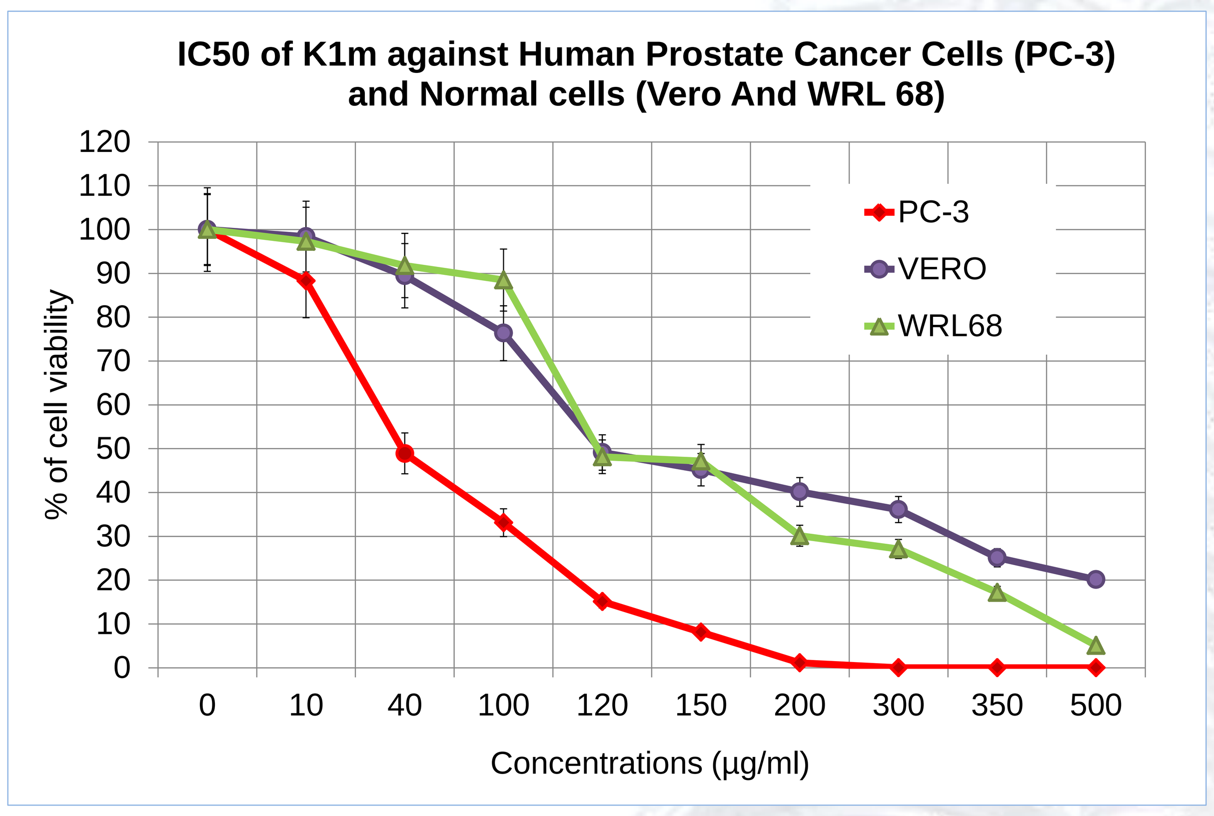
↓

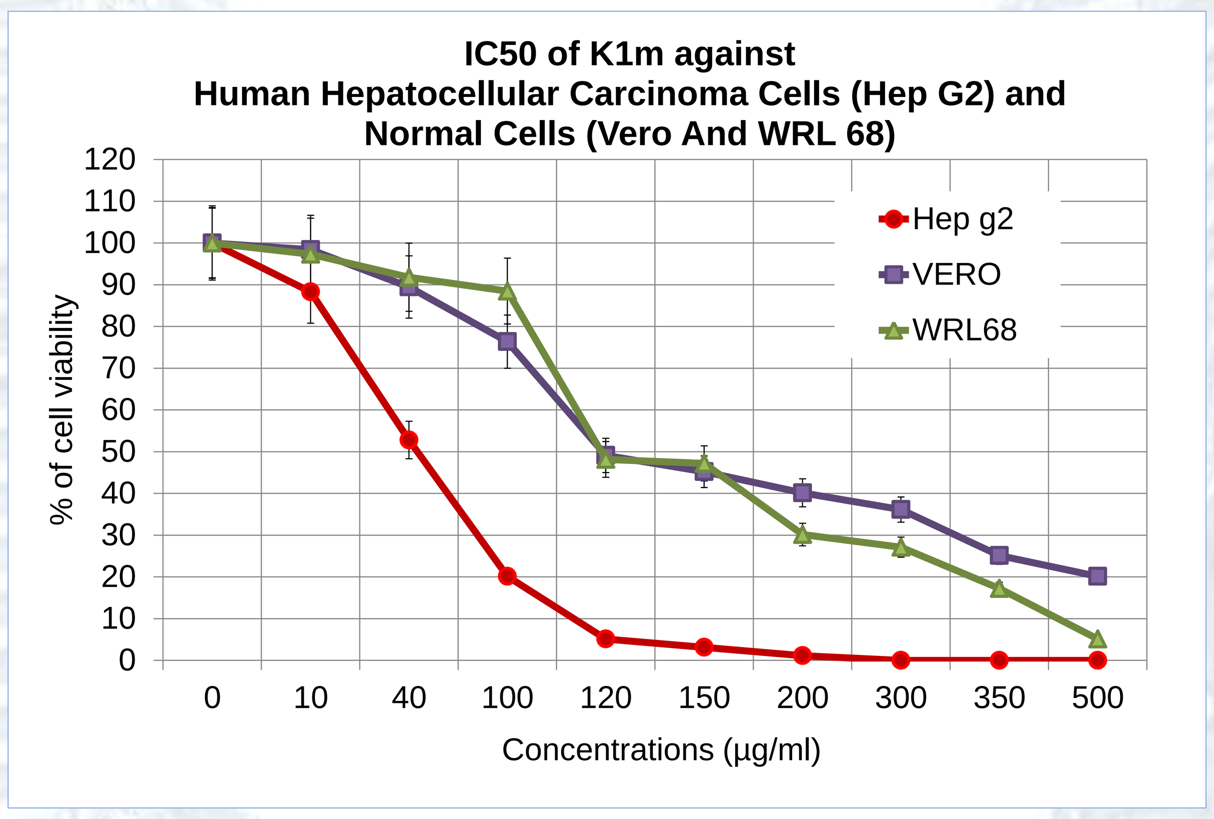
pK/pD studies in animal models

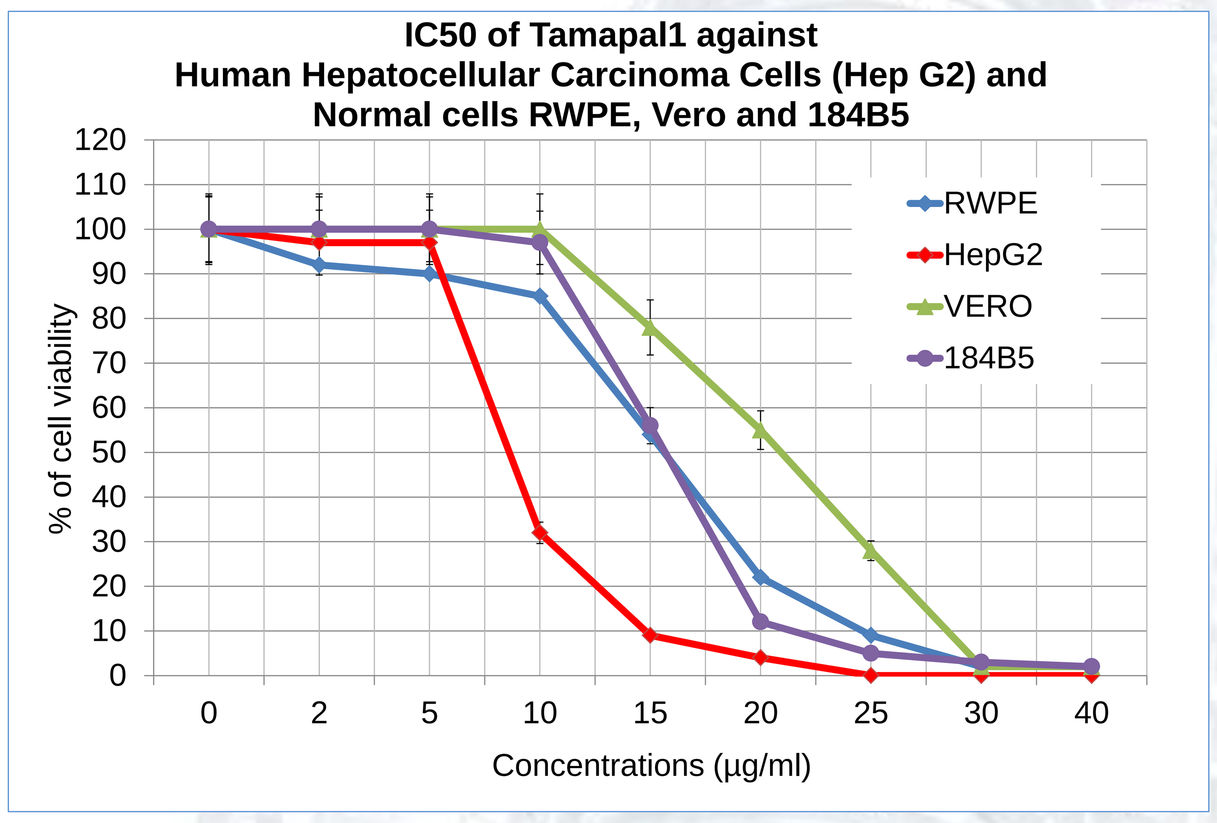
↓

Transcriptomics analysis

Action of Candidate Anticancer Peptide Drug





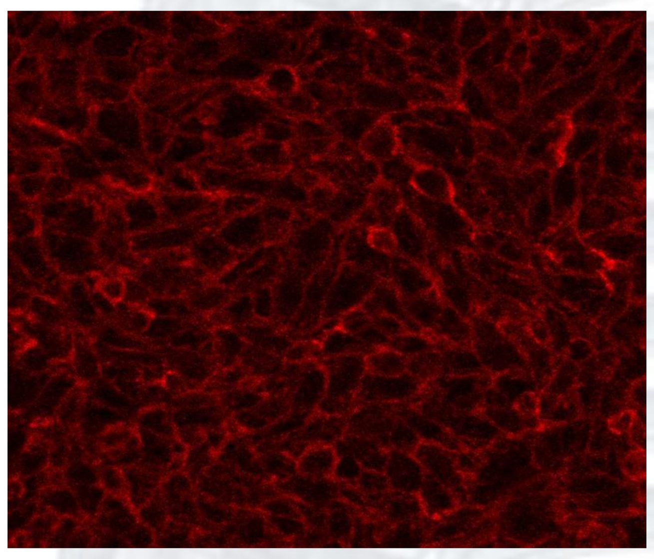


Enzyme Stability of Tamapal1

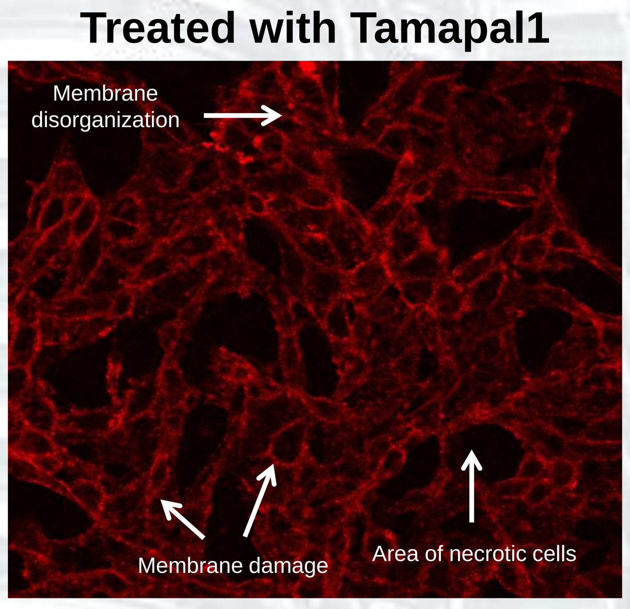
Time	Pepsin (pH2)	Trypsin (pH8)	Chymotrypsin (pH8)
1 hour	Not Digested	Not Digested	Partially Digested
2 hours	Not Digested	Not Digested	Partially Digested
3 hours	Not Digested	Not Digested	Partially Digested
4 hours	Not Digested	Not Digested	Digested

Confocal Microscopy Images of Hep G2 treated with Tamapal1

Control

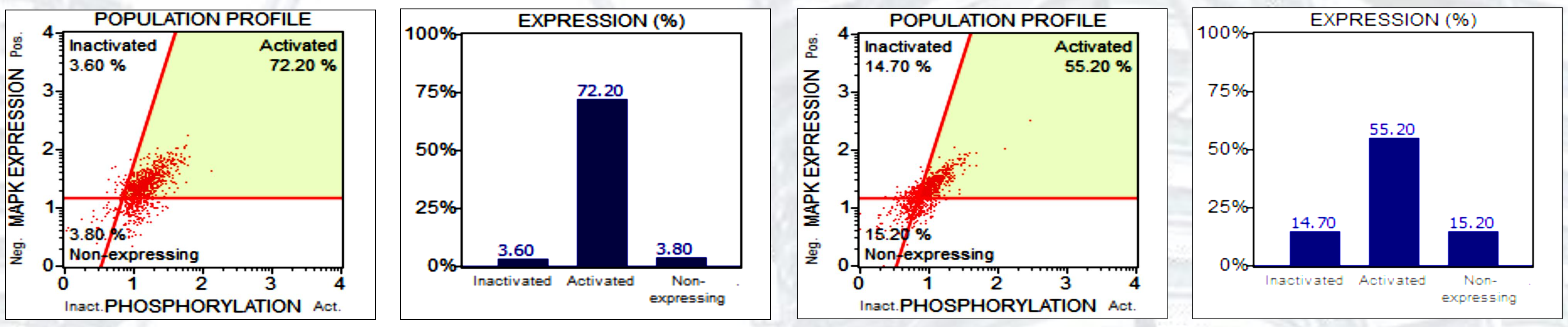


Treated with Tamapal1



FACS Analysis of Peptide K1m on MAPK Pathway in HepG2 cell lines

Control **Treated with K1m**



Transcriptomics Analysis: Action Of Tamapal1 on Hep G2

Downregulated Genes	Log2 (fold change)	Major Function	Pathways
RND3	-4.33704	Rho related GTP binding protein RhoE	Rho-GTPase
RHOF	-4.2016	Rho related GTP binding protein RhoF. Key regulator of Actin cytoskeleton. Gene transcription and adhesion	Ras
IQ GAP3	-4.084	Regulation of RAS pathway	Ras
DHRS2	-3.024	Dehydrogenase, reductase SDR membrane 2. Role in mitochondrial activity	MAPK Ras
PMEPA1	-3.014	Transmembrane prostrate androgen-induced protein Smad interacting motif (SIM)	TGF-beta Transcriptional activity of SMAD2/SMAD3:SMAD4 heterotrimer

Action of Tamapal1 and its Putative Action on Down Regulation of Genes

Blocking Of Differentiation

GMCSFR
GCSFR
MCSFR
GCSFR
IL6
E2F
C-MYC
MCSFR
TCR

PU1 Target gene
C/EBP gene
AML1 target gene

PU1
C/EBP gene
C/EBP gene

Action of Tamapal1 on Hep G2