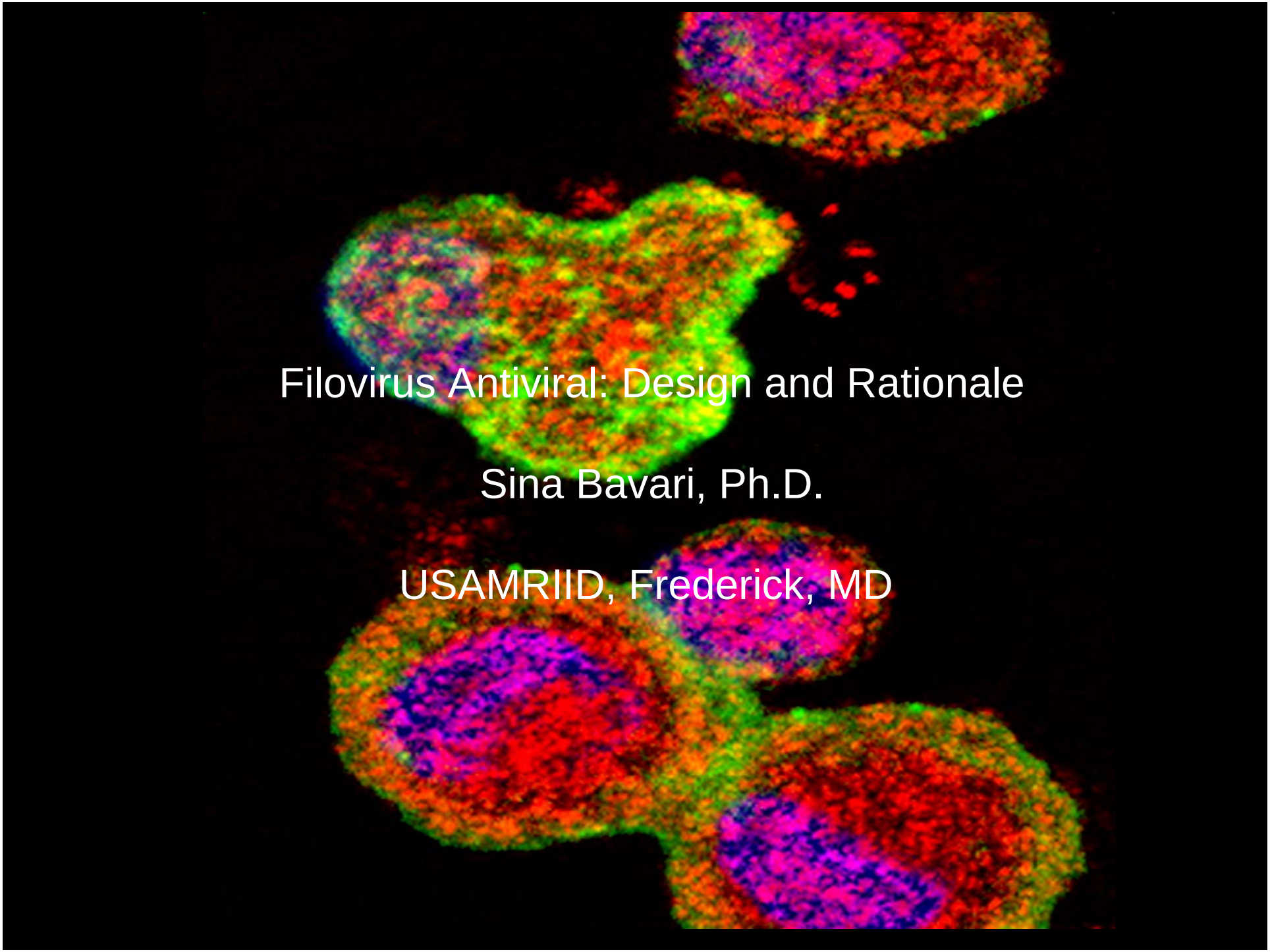


Filovirus Antiviral: Design and Rationale

Sina Bavari, Ph.D.

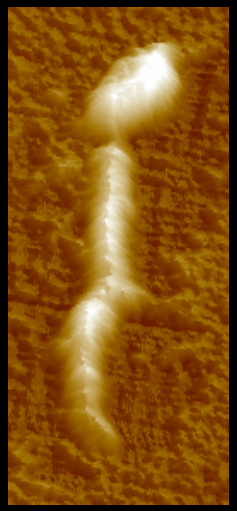
USAMRIID, Frederick, MD

Our General Philosophy: Countermeasures

Dissecting the biology of filoviruses: therapeutic and vaccine development

- Utilize known properties of the viruses
  - Requirements for viral assembly guided us to discovery of virus-like particles as vaccine candidates
  - Sequence information to design antisense and siRNA-based therapeutics
- Understand how viral proteins work
  - Target them with small molecule “druggable” compounds
- Understand how immune system responds, especially innate responses
  - Use it to benefit the host
- Understand how cellular proteins help the virus
  - Target them with small molecule inhibitors or gene-specific approaches
- Develop tools and make them available
  - Development and characterization of rodent models

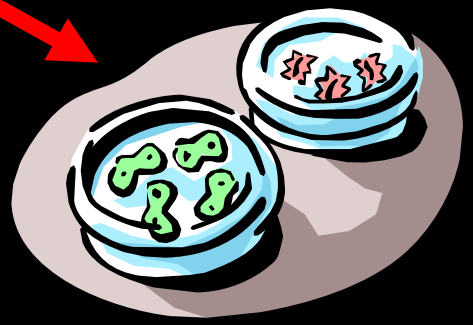
Virus-like Particles (VLPs)

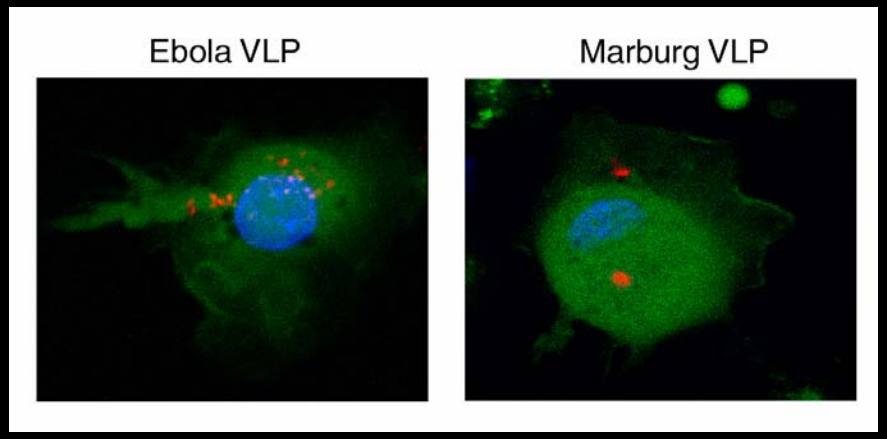


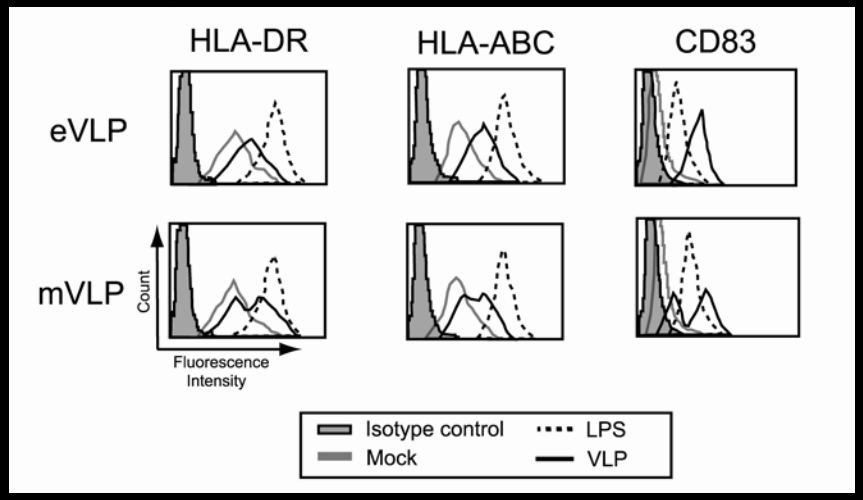
- Similar morphology to live viruses
  - Fully enveloped
  - Non-replicating
- Generated in mammalian or insect cell lines
  - Can be produced in large quantities
- No vector or vector immunity
- Generate innate, humoral and cellular immunity
- Safely and effectively administered in humans
  - Hepatitis B, Norwalk and papillomavirus
- Platform for delivery of other cargo (multivalent VLPs, i.e. carrying other viral/bacterial antigens and have documented protection against multiple BTAs)
- Tools to dissect immunology of filoviruses

Filovirus-like particles

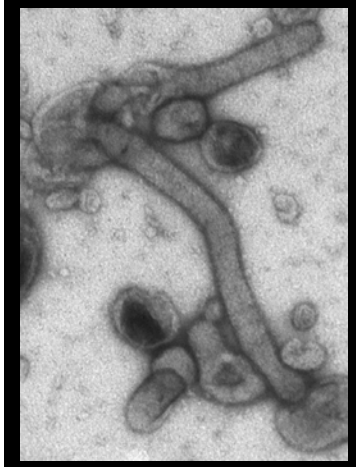
Transfect GP, NP, and VP40

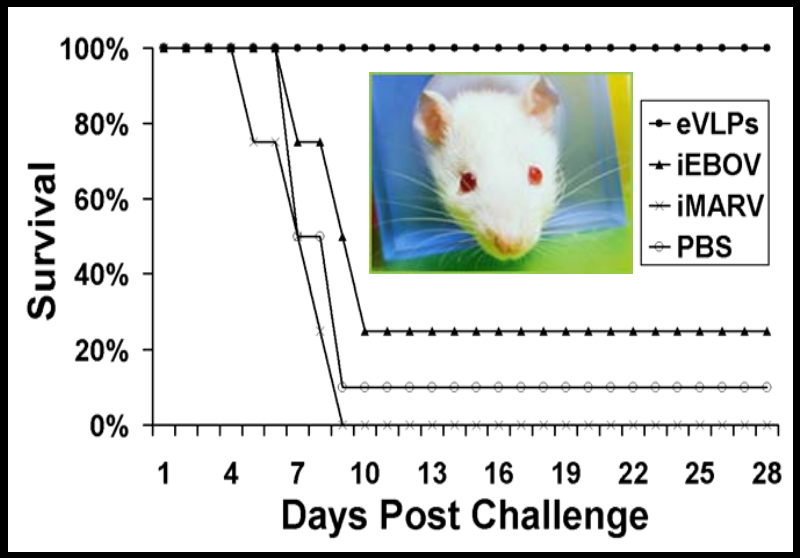


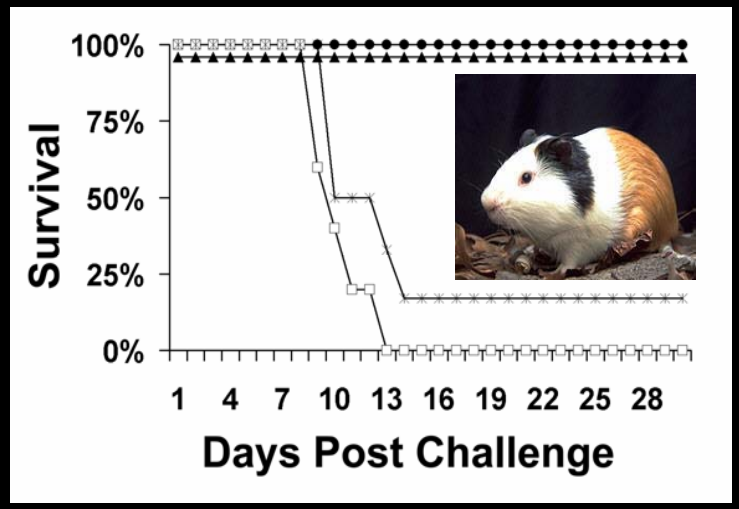




Ebolavirus-like particles







B and T cells are required for eVLP-mediated protection against Ebola

Table 1. Immune responses and survival of eVLP-vaccinated knockout mice

Genotype[a]	Immune deficiencies	Vaccine[b]	Geometric mean titer [c]	Survivors/ Total [d]	MTD [e]
C57Bl/6	wild-type	eVLPs	31711	18/20	N/A
		PBS	<33	0/20	7.8 +/- 0.69
BALB/c	wild-type	eVLPs	32346	9/10	N/A
		PBS	<33	0/10	6.1+/- 0.85
scid (BALB/c)	B and T cells	eVLPs	<33	0/10	5.5 +/- 0.52
		PBS	<33	0/10	5.6 +/- 0.53
Jh (BALB/c)	B cells	eVLPs	<33	0/10	6.2 +/- 0.44
		PBS	<33	0/10	6.1 +/- 0.32
β/δ TCR (C57)	T cells	eVLPs	460	0/10	7.2 +/- 0.42
		PBS	<33	0/10	6.9 +/- 0.57
CD4 (C57)	CD4 T cells	eVLPs	5196	5/10	8.4 +/- 0.54
		PBS	<33	0/10	7.0 +/- 0.47
β2m (C57)	CD8 T cells	eVLPs	15924	0/10	10.2 +/- 1.4
		PBS	<33	0/10	7.1 +/- 0.32
IFN-γ (C57)	IFN-γ	eVLPs	11523	2/15	9.6 +/- 1.1
		PBS	<33	0/15	7.0 +/- 0
perforin (C57)	perforin	eVLPs	36955	15/15	N/A
		PBS	<33	0/15	6.3 +/- .0.73

[a] Genotype of mice used are indicated along with the strain background in paratheses

[b] Ten micrograms of eVLPs or PBS mixed with QS-21 adjuvant were administered on days 0 and 21

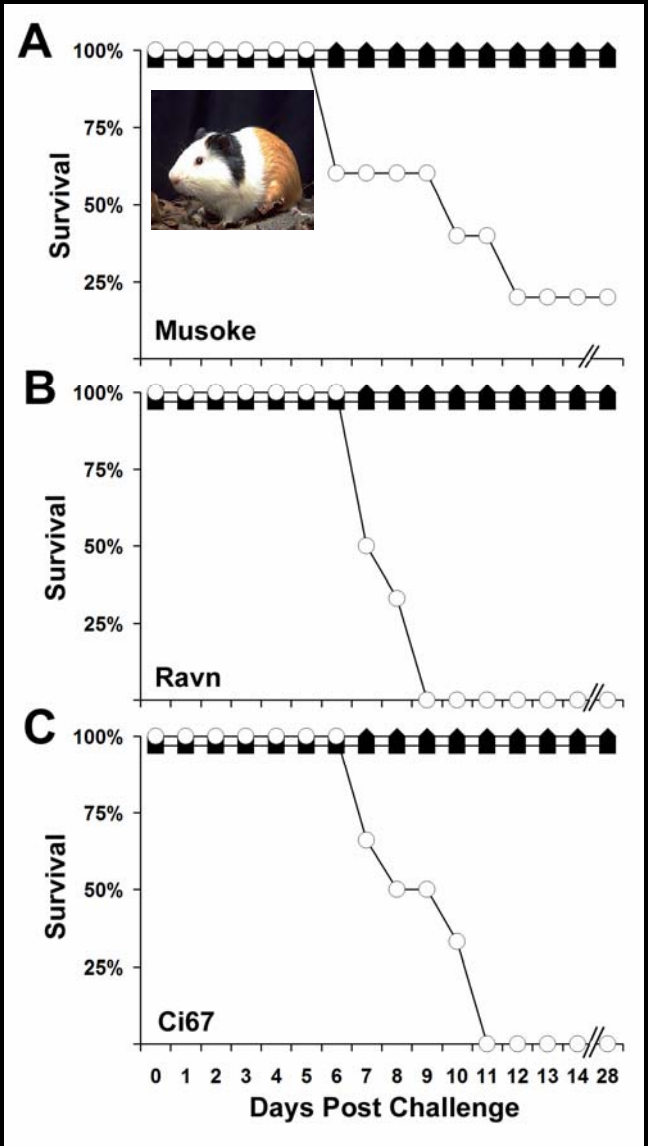
[c] Geometric mean titer of EBOV-specific antibodies as measured by ELISA

[d] After challenge with 1000 pfu of mouse-adapted EBOV 6 weeks following the last vaccination

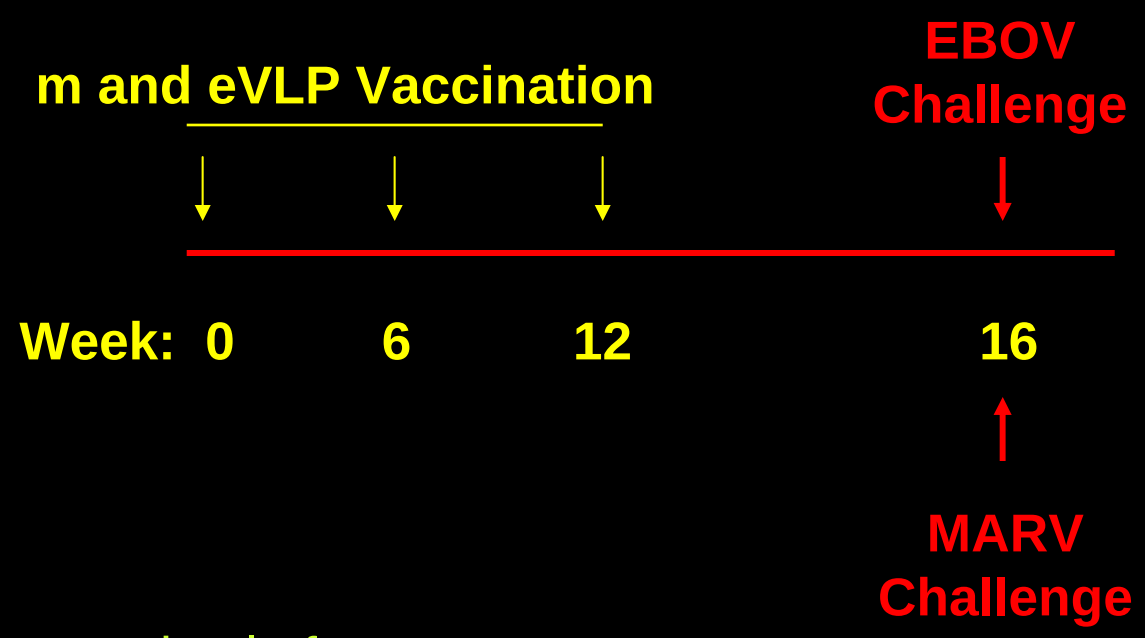
[e] Mean time to death (MTD) following EBOV challenge in days +/- standard deviation, N/A = not applicable

Warfield, *et al* J. Immunology 2005

Musoke-based VLP protects against multiple MARVs

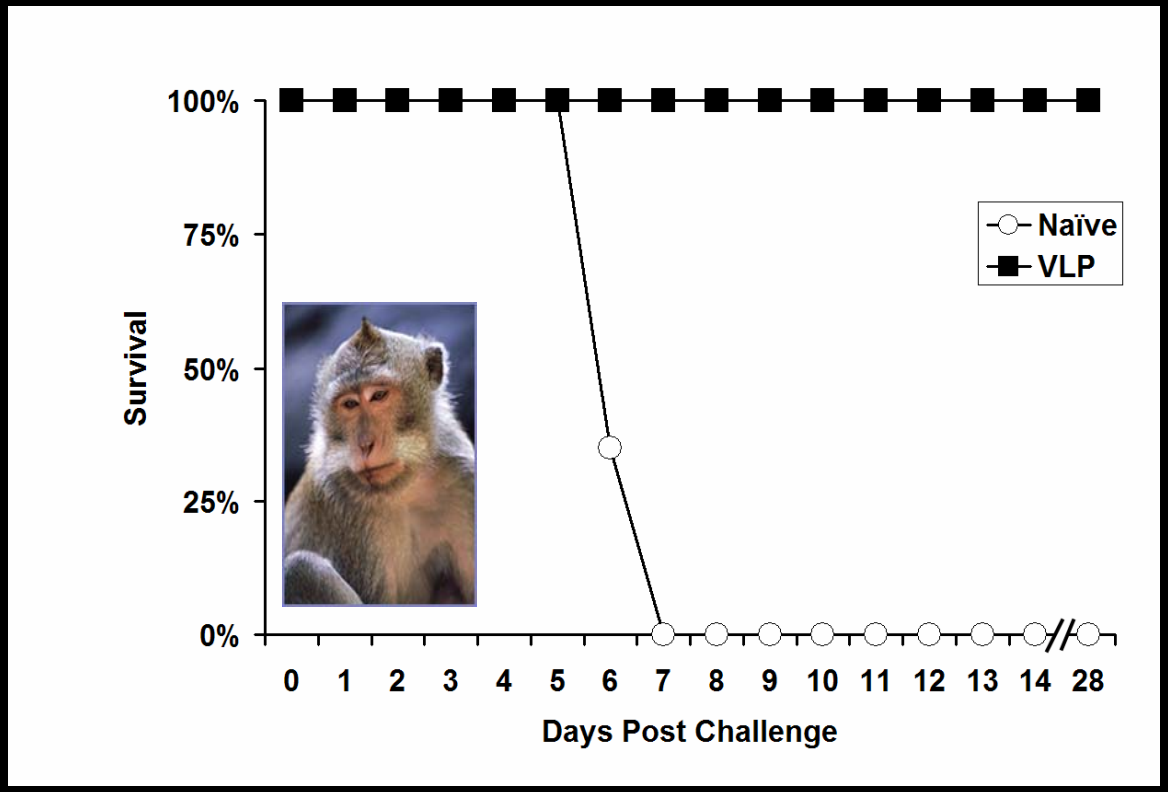


Experimental Design: Pan-filovirus VLP Vaccination



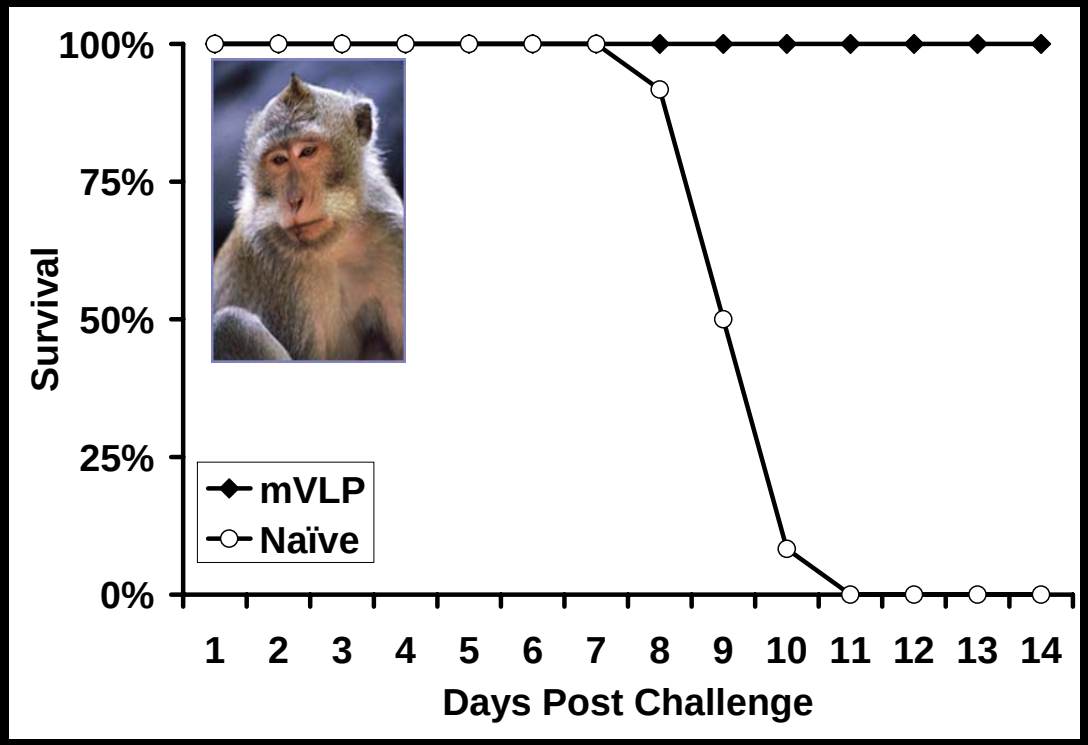
Ab titers were maximal after two Vaccines.

Ebolavirus-like particles fully protects NHPs against EBOV challenge



5 NHP/group
Vaccinated with eVLP and mVLPs
Challenged with 1000 pfu ZEBOV

Marburgvirus-like particles fully protects NHPs against MARV challenge



6 NHP/group
Vaccinated with eVLP and mVLPs
Challenged with 1000 pfu MARV

Summary of VLP vaccination studies

- Vaccination with marburg or ebola VLPs protect: Mice and Guinea Pigs
- Vaccination with Musoke VLPs protects against multiple MARVs
- NHP vaccination with ebola and marburg VLPs elicit maximal Ab titers after 2 vaccines and 100% protection. Demonstrates no interference.
- Robust CD8 epitope specific responses are observed in NHPs following VLP vaccination

Developing Therapeutics

Therapeutic Targets

Host

- Identify druggable innate response pathways

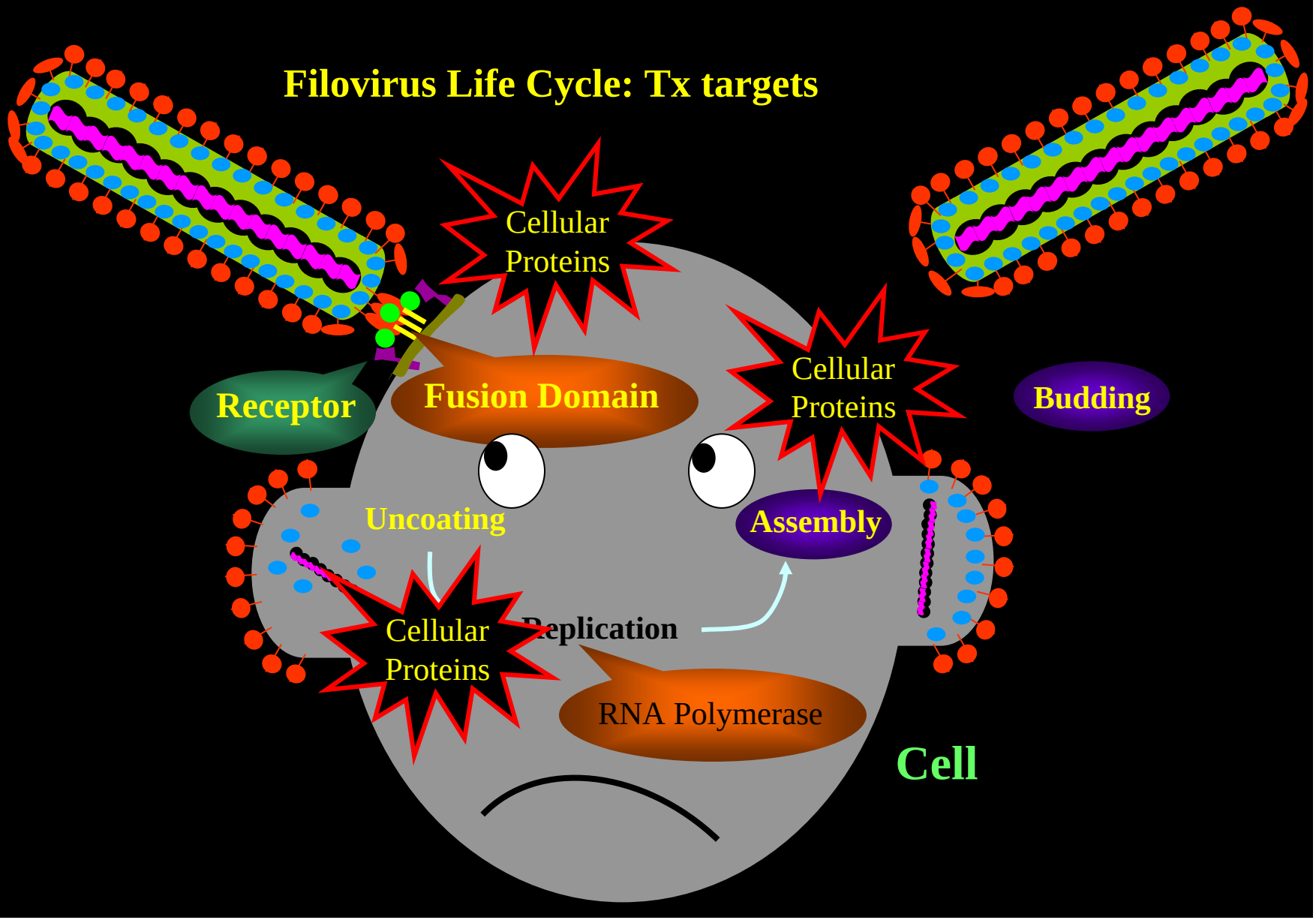
- Define effector molecules
- Meganomics
- Immune evasion mechanisms
- Druggable genetic components
- Target common microbial virulence mechanisms

response to define novel adjunctive treatment regimens.

Pathogen

Therapeutics:
siRNA, Antisense, and
Small Molecules non-peptidic

Targeted approach



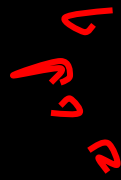
Our General Philosophy

Dissecting the biology of filoviruses: therapeutic and vaccine development

- Utilize known properties of the viruses
  - Requirements for viral assembly lead to virus-like particles as vaccine candidate
  - Sequence information to design antisense and siRNA-based therapeutics
- Understand how viral proteins work
  - Target them with small molecule “druggable” compounds
- Understand how immune system responds, especially innate responses
  - Use it to benefit the host
- Understand how cellular proteins help the virus
  - Target them with small molecule inhibitors or gene-specific approaches
- Develop tools and make them available
  - Development and characterization of rodent models

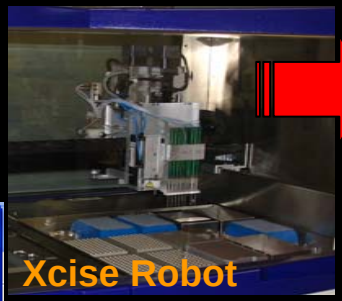
Identify EBOV and MARV total proteome

protein

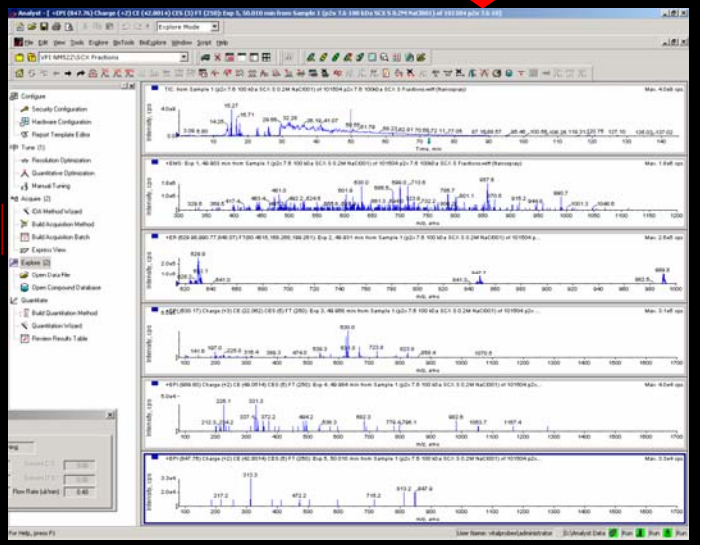


peptides

2D



LC-MS/MS



Microsoft Excel - VPI Ebola Marburg Virus Proteomics 121405

File Edit View Insert Format Tools Data ApproveIt Window Help Adobe PDF

Reply with Changes... End Review...

Delete Comment

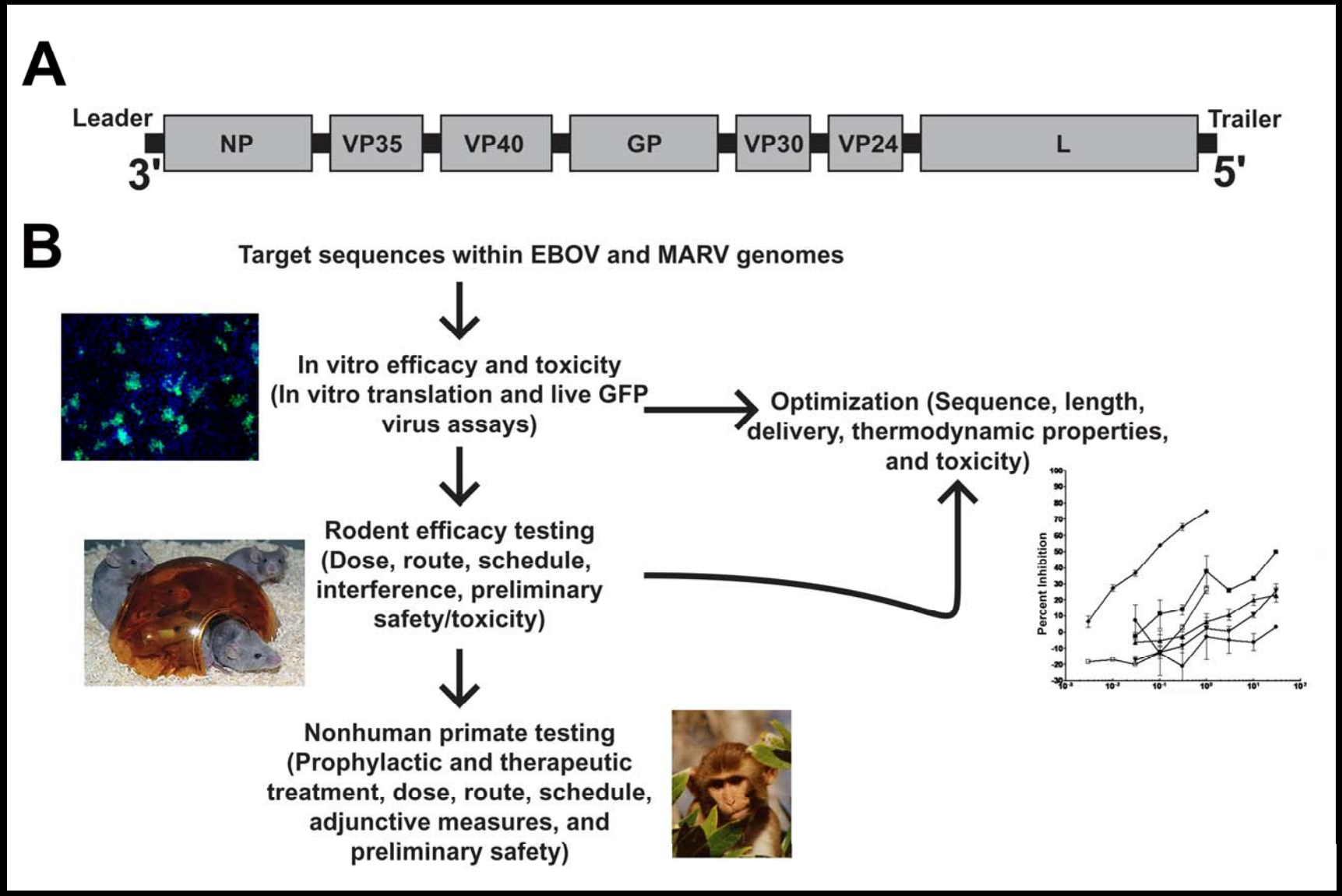
H10

Ebola Proteins identified against Zaire Ebola genome database

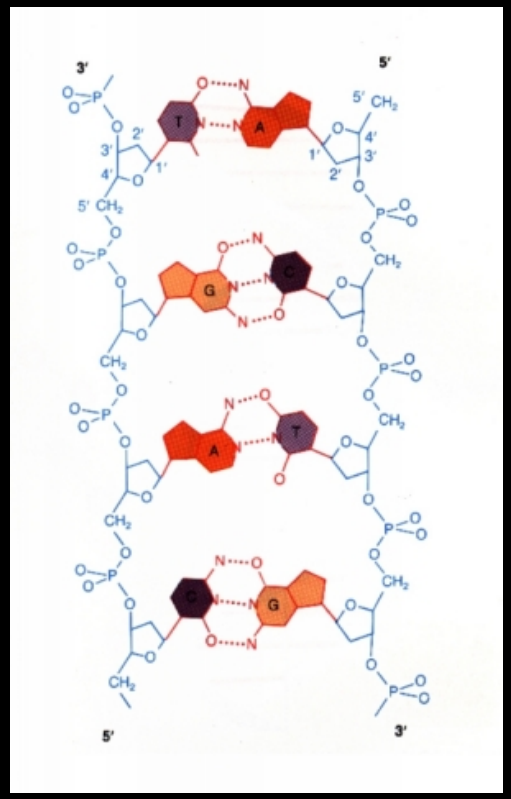
slice #	Slice MW	virus	score	MW	accession #	protein
2	200-250	Ebola	63	252564	gi|10313999|ref|NP_066251.1|	polymerase; L
5	100-125	Ebola	258	83235	gi|10314000|ref|NP_066243.1|	nucleoprotein; NP
5		Ebola	99	41149	gi|10313994|ref|NP_066247.1|	sGP
5		Ebola	80	252564	gi|10313999|ref|NP_066251.1|	polymerase; L
6	100-125	Ebola	444	83235	gi|10314000|ref|NP_066243.1|	**nucleoprotein; NP**
6		Ebola	172	252564	gi|10313999|ref|NP_066251.1|	polymerase; L
6		Ebola	95	41149	gi|10313994|ref|NP_066247.1|	sGP
7	60-90	Ebola	395	252564	gi|10313999|ref|NP_066251.1|	**polymerase; L**
7		Ebola	346	83235	gi|10314000|ref|NP_066243.1|	nucleoprotein; NP
7		Ebola	118	32501	gi|10313997|ref|NP_066249.1|	polymerase complex protein; minor nucleoprotein; VP30
7		Ebola	102	74418	gi|10313995|ref|NP_066246.1|	virion spike glycoprotein precursor
8	45-55	Ebola	404	83235	gi|10314000|ref|NP_066243.1|	nucleoprotein; NP
8		Ebola	129	252564	gi|10313999|ref|NP_066251.1|	polymerase; L
8		Ebola	98	74418	gi|10313995|ref|NP_066246.1|	virion spike glycoprotein precursor
8		Ebola	80	32501	gi|10313997|ref|NP_066249.1|	polymerase complex protein; minor nucleoprotein; VP30
8		Ebola	78	35161	gi|10313993|ref|NP_066245.1|	**matrix protein; VP40**
9	36-45	Ebola	419	83235	gi|10314000|ref|NP_066243.1|	nucleoprotein; NP
9		Ebola	128	41149	gi|10313994|ref|NP_066247.1|	sGP
10	30-36	Ebola	312	83235	gi|10314000|ref|NP_066243.1|	nucleoprotein; NP
10		Ebola	133	41149	gi|10313994|ref|NP_066247.1|	sGP
10		Ebola	73	252564	gi|10313999|ref|NP_066251.1|	polymerase; L
11	26-30	Ebola	445	83235	gi|10314000|ref|NP_066243.1|	nucleoprotein; NP
11		Ebola	81	35161	gi|10313993|ref|NP_066245.1|	matrix protein; VP40
12	20-25	Ebola	418	35161	gi|10313993|ref|NP_066245.1|	matrix protein; VP40
12		Ebola	309	83235	gi|10314000|ref|NP_066243.1|	nucleoprotein; NP
12		Ebola	145	74418	gi|10313995|ref|NP_066246.1|	virion spike glycoprotein precursor
12		Ebola	108	37339	gi|10313992|ref|NP_066244.1|	**VP35**
14	20-25	Ebola	244	35161	gi|10313993|ref|NP_066245.1|	matrix protein; VP40

Ebola Viral Proteins / Marburg Viral Proteins / Ebola_Hi

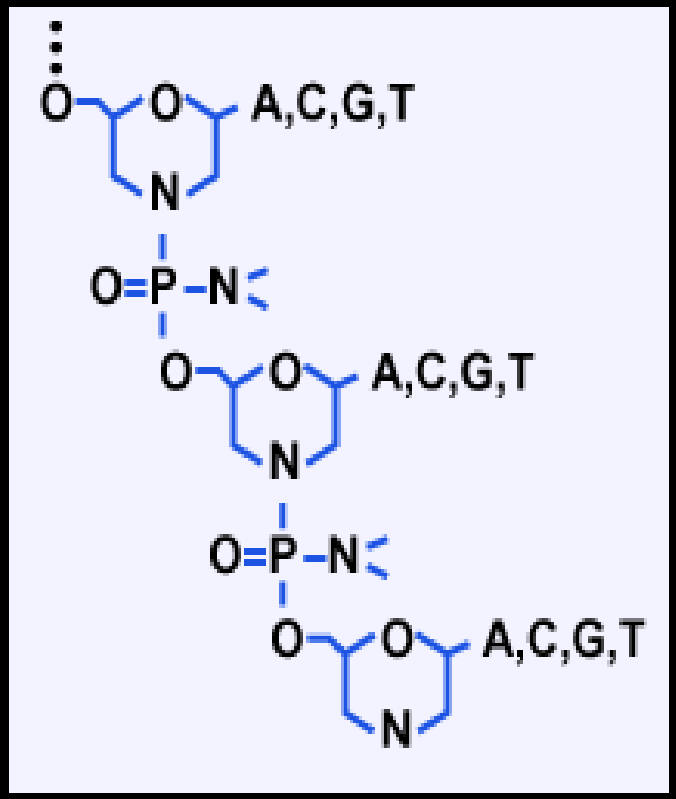
Section 1: 'Designer' drugs for Filoviruses



Targeting EBOV using Antisense Oligomers



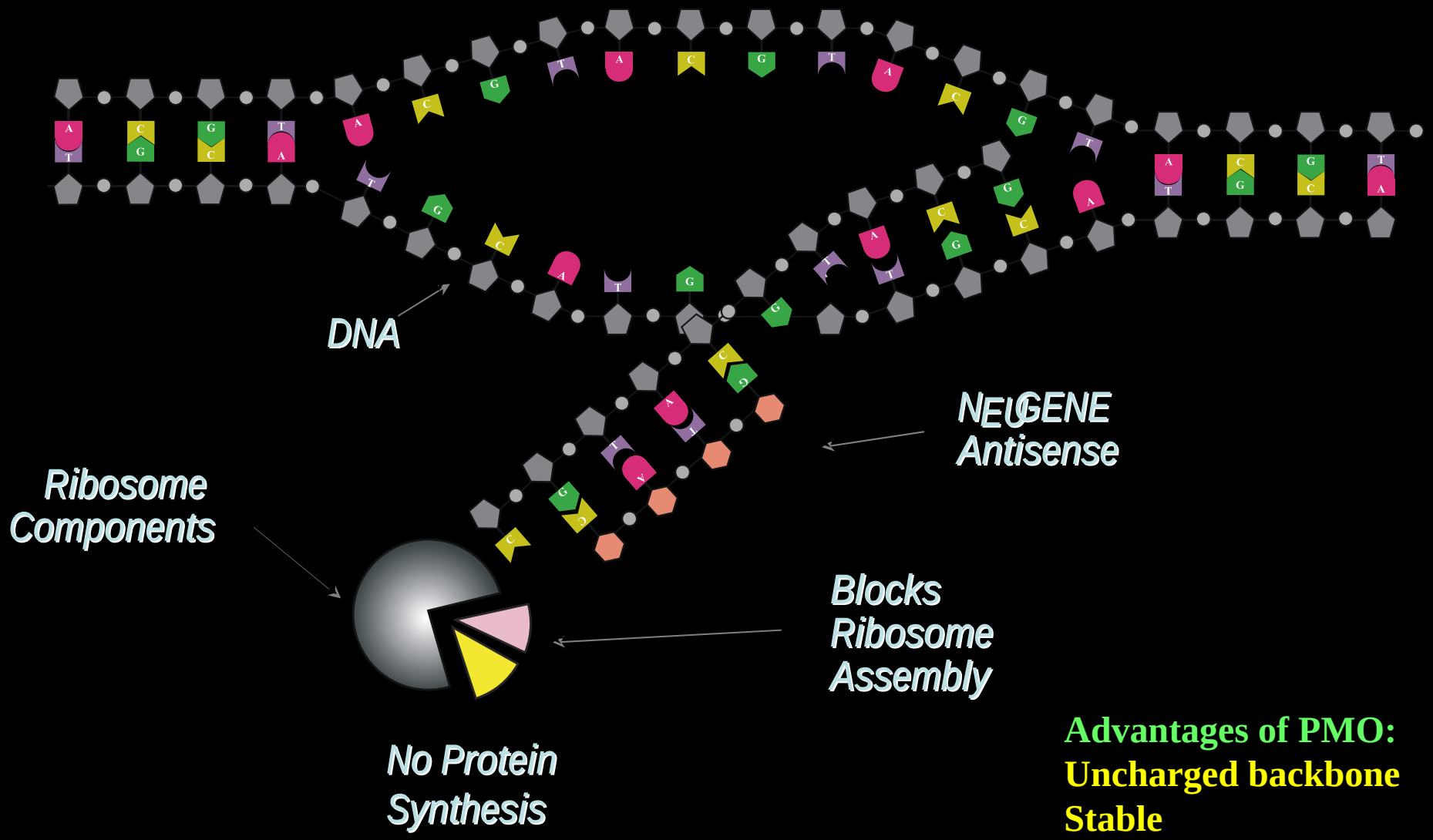
Natural DNA



Phosphordiamadite Morpholino-Oligonucleotides (PMOs)

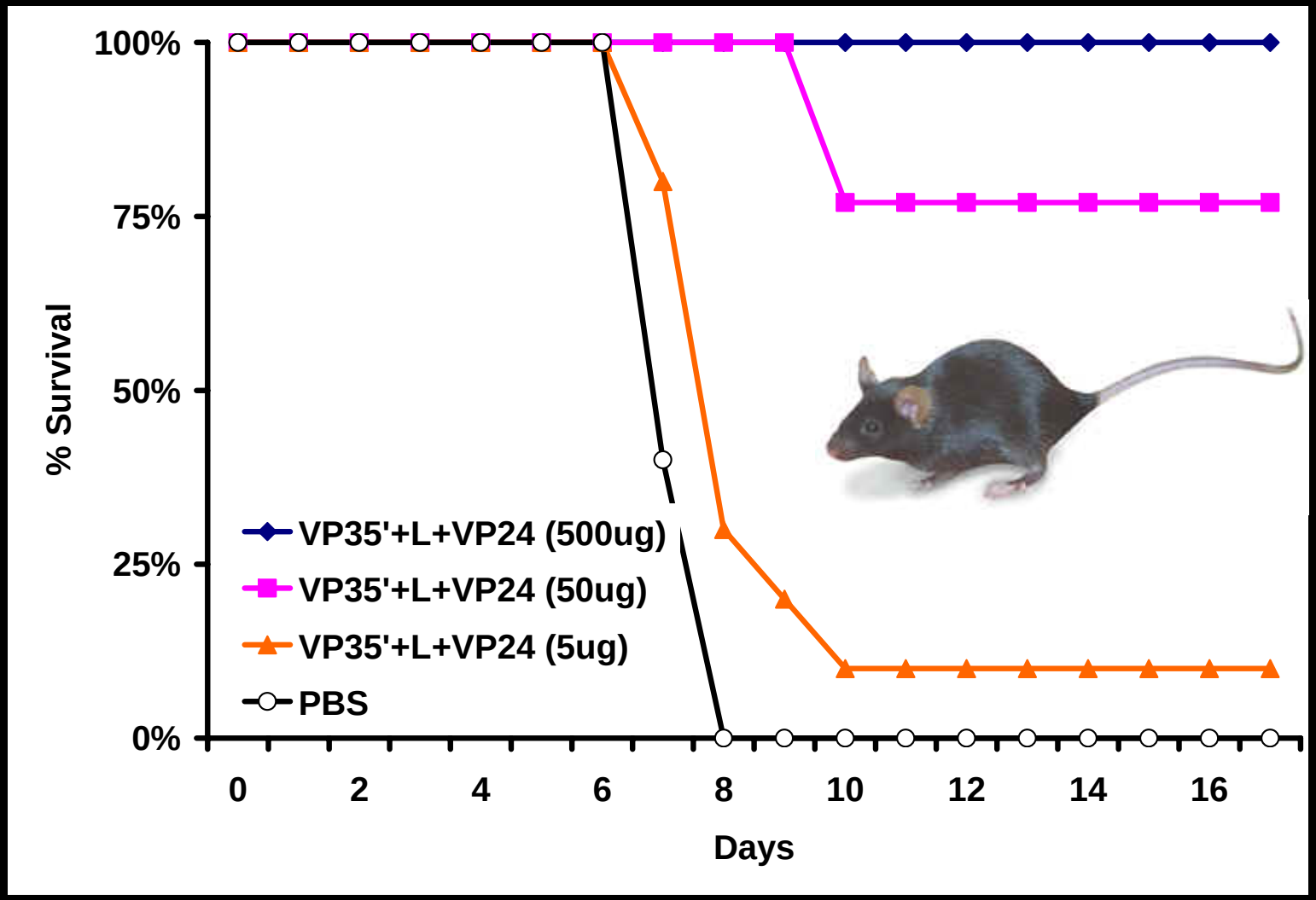
Partner: AVI BioPharma, Corvallis, OR

PMO Mechanism of Action



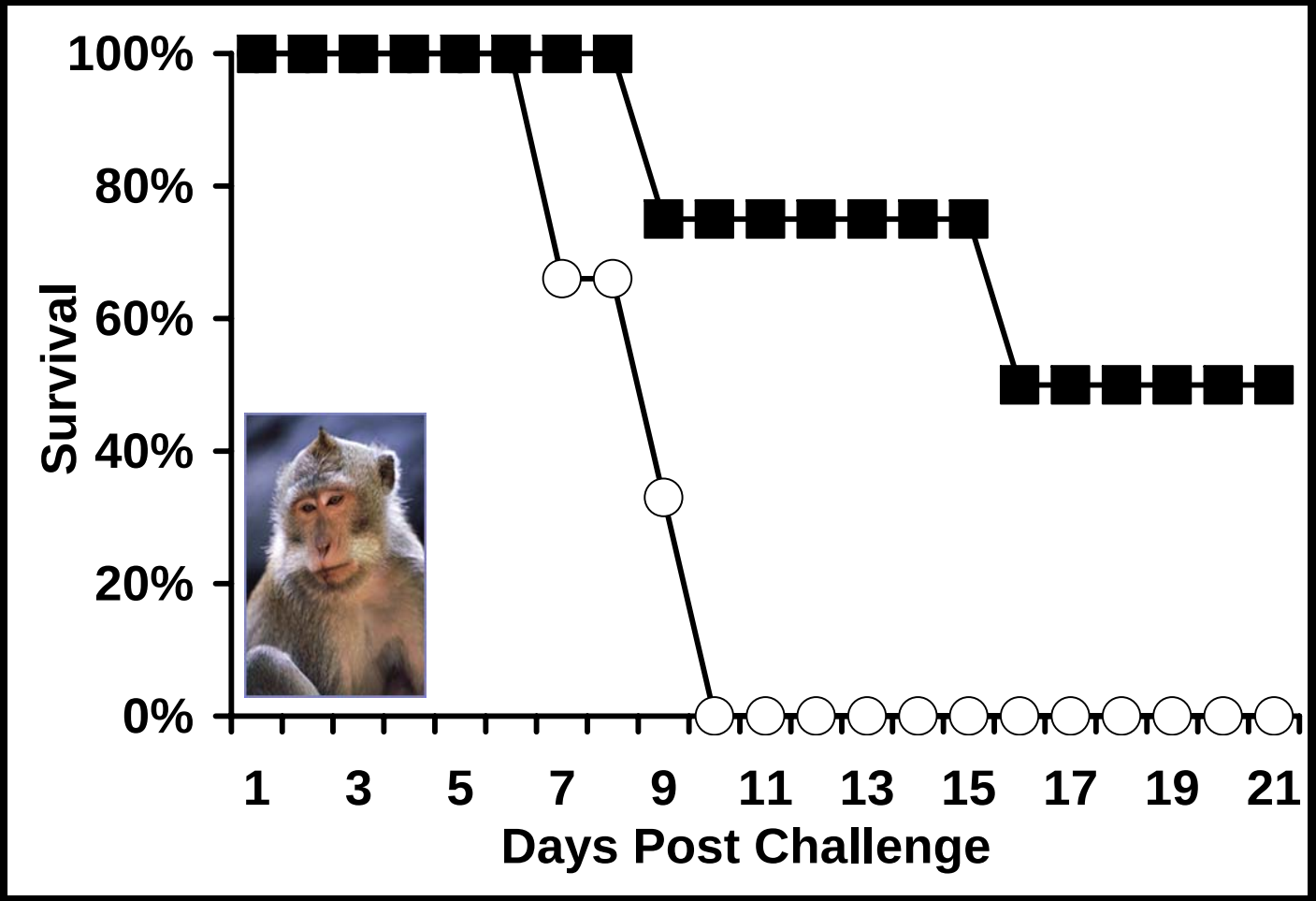
Advantages of PMO:
Uncharged backbone
Stable
No IFN response

Prechallenge administration of PMO protects mice from EBOV

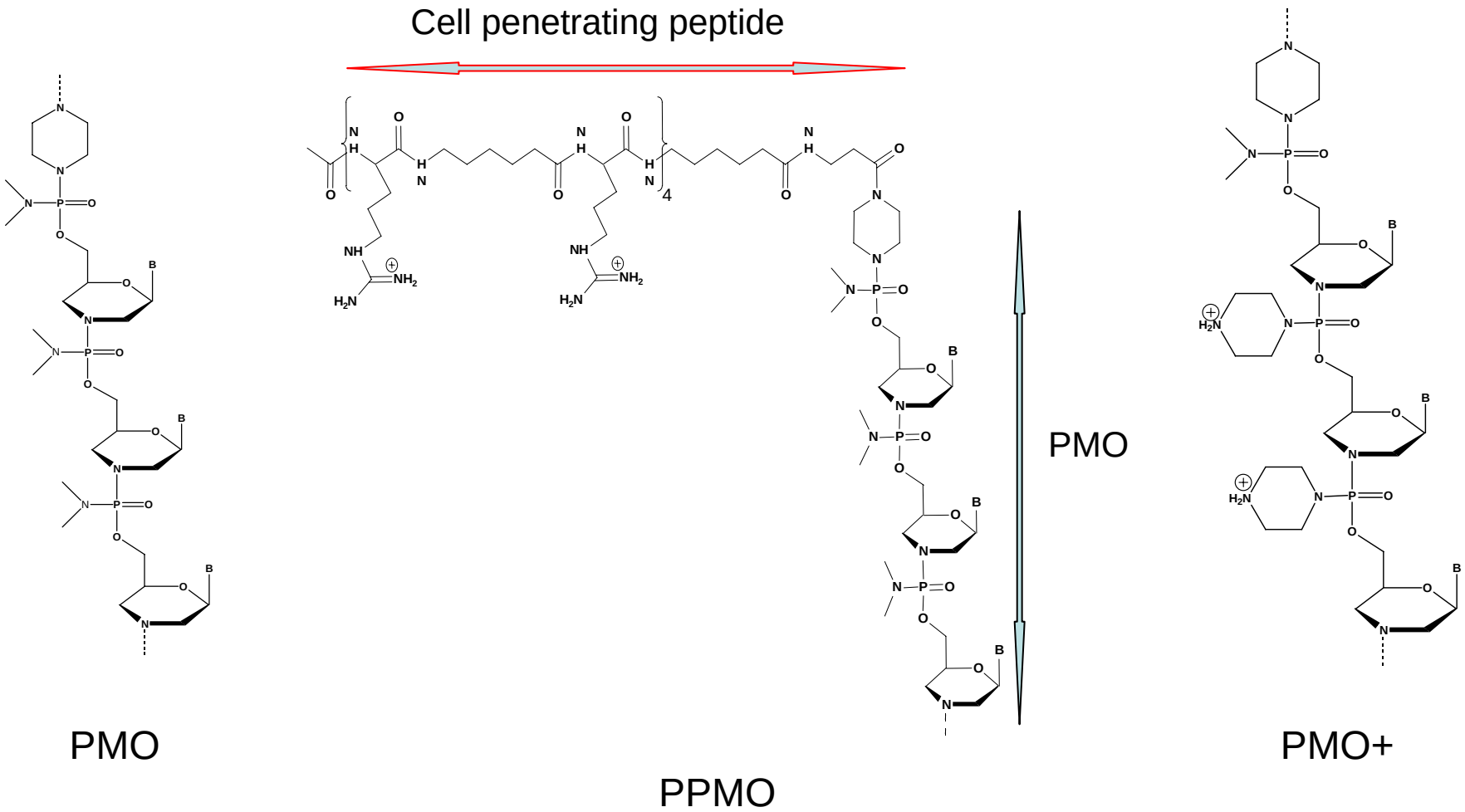


n=10 C57BL/6 mice/group treated at -24 and -4hpc

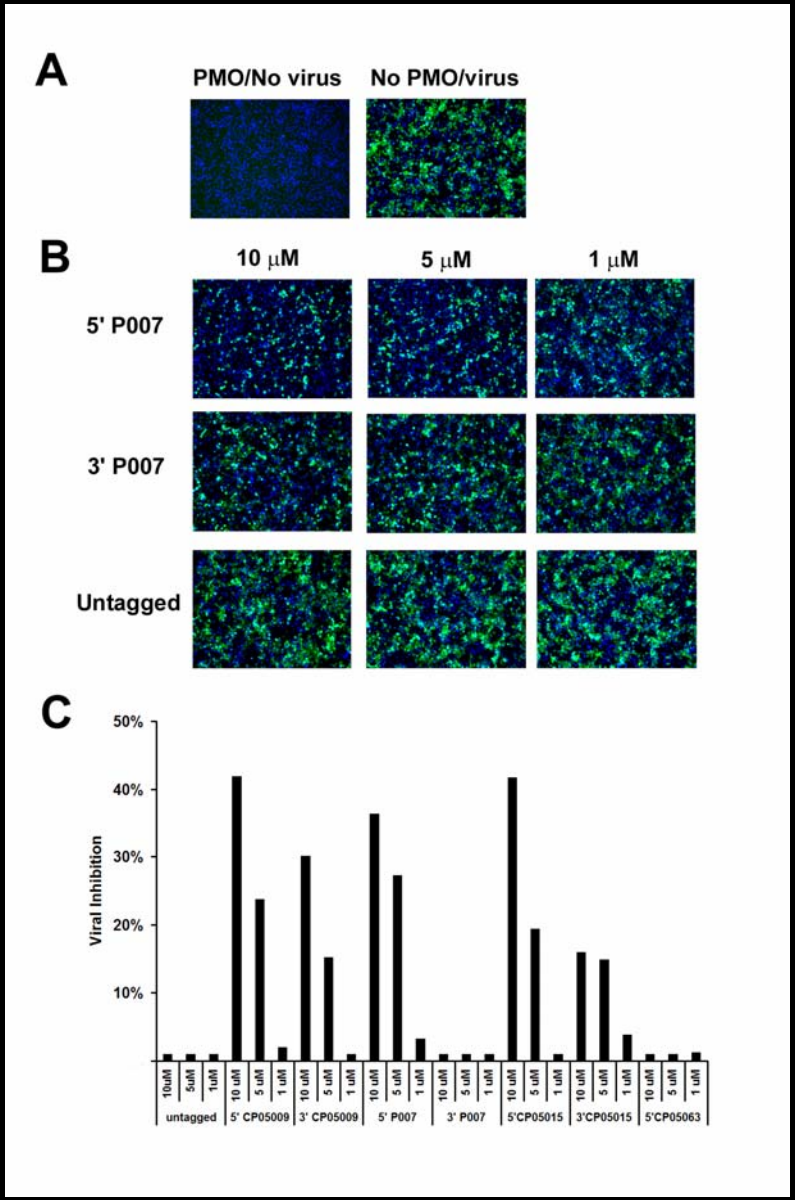
Pre-challenge administration of EBOV-specific PMO protects NHPs



Chemical modifications to increase PMO delivery



Chemical modifications increase PMO efficacy



Summary of Antisense data (PMO)

- Pre-treatment and therapeutic treatment of Ebola and MARV infected mice and guinea pigs protected
- Mice and guinea pigs which were treated with antisenses and survived EBOV or MARV challenge showed antiviral antibodies and were fully protected upon re-challenging with the same virus but not with heterologous virus.
- Pretreatment of NHP with PMO protected 50% of the NHP
- Peptide-tagged PMOs have higher tendency to enter cells and disrupt EBOV and MARV infections. pPMOs also protected mice against EBOV.

Post-challenge treatment of NHP with PMO+

- Challenge 5 rhesus macaques with 1000pfu of EBOV-Zaire
- Treat 4 rhesus macaques with a combination of two PMO+ starting at 1hour after infection
- Continue treatment daily for 10-14 days via SC and IP injection at 20mg/kg

Summary of Development: Antisense PMO+

- Originally identified 3 unmodified PMO compounds that were effective in prophylactic regimen

- Further development led us to narrow to 2 PMO+ compounds targeting EBOV genome that are effective in post-challenge treatment regimen. These are the best virally-directed post-exposure therapeutics in NHPs.

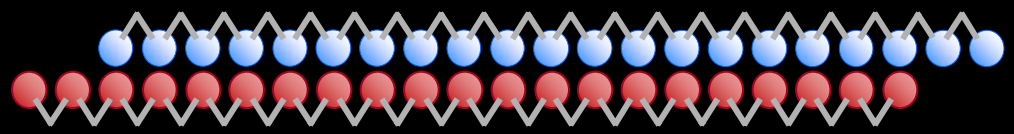
- Identified 2 compounds targeting MARV genome that we are currently testing for efficacy in NHP.

- EBOV PMO+ are safe and well-tolerated in mice. A single 50X dose used in NHP studies showed no overt toxicity after 24 hrs

- Future:
  - GMP, GLP tox studies
  - Efficacy in NHP of delayed treatment

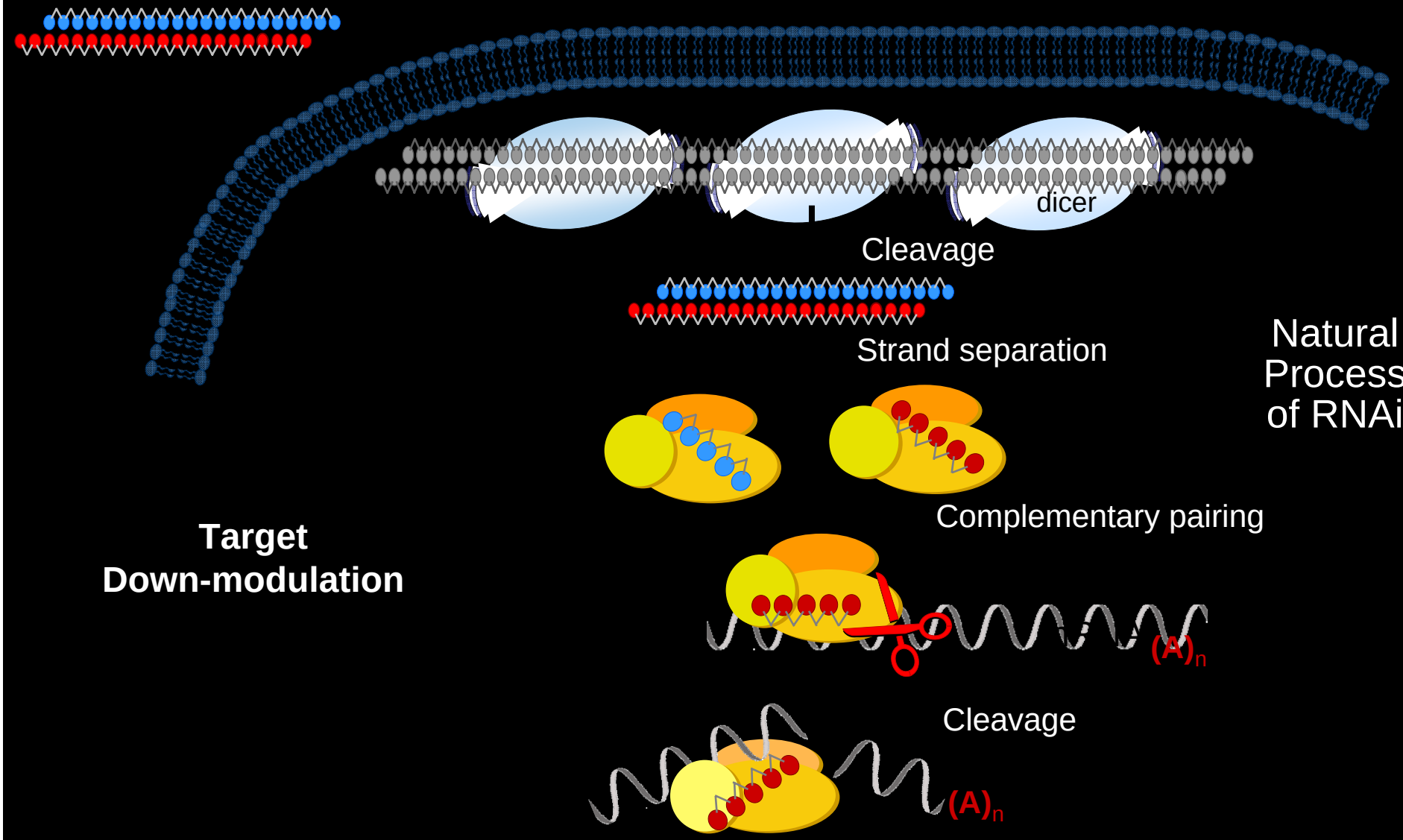
RNA Interference (RNAi)

- Potential for a whole new class of drugs
  - Harnessing natural pathway
  - Therapeutic gene silencing
    - Upstream of today's medicines
    - Any gene in the genome
- Natural process for gene silencing
  - Catalytic mechanism
    - Potent and selective
  - Mediated by 'small interfering RNAs' or 'siRNAs'
- 2006 Nobel Prize awarded for RNAi discovery



Partner: Alnylam, Cambridge, MA

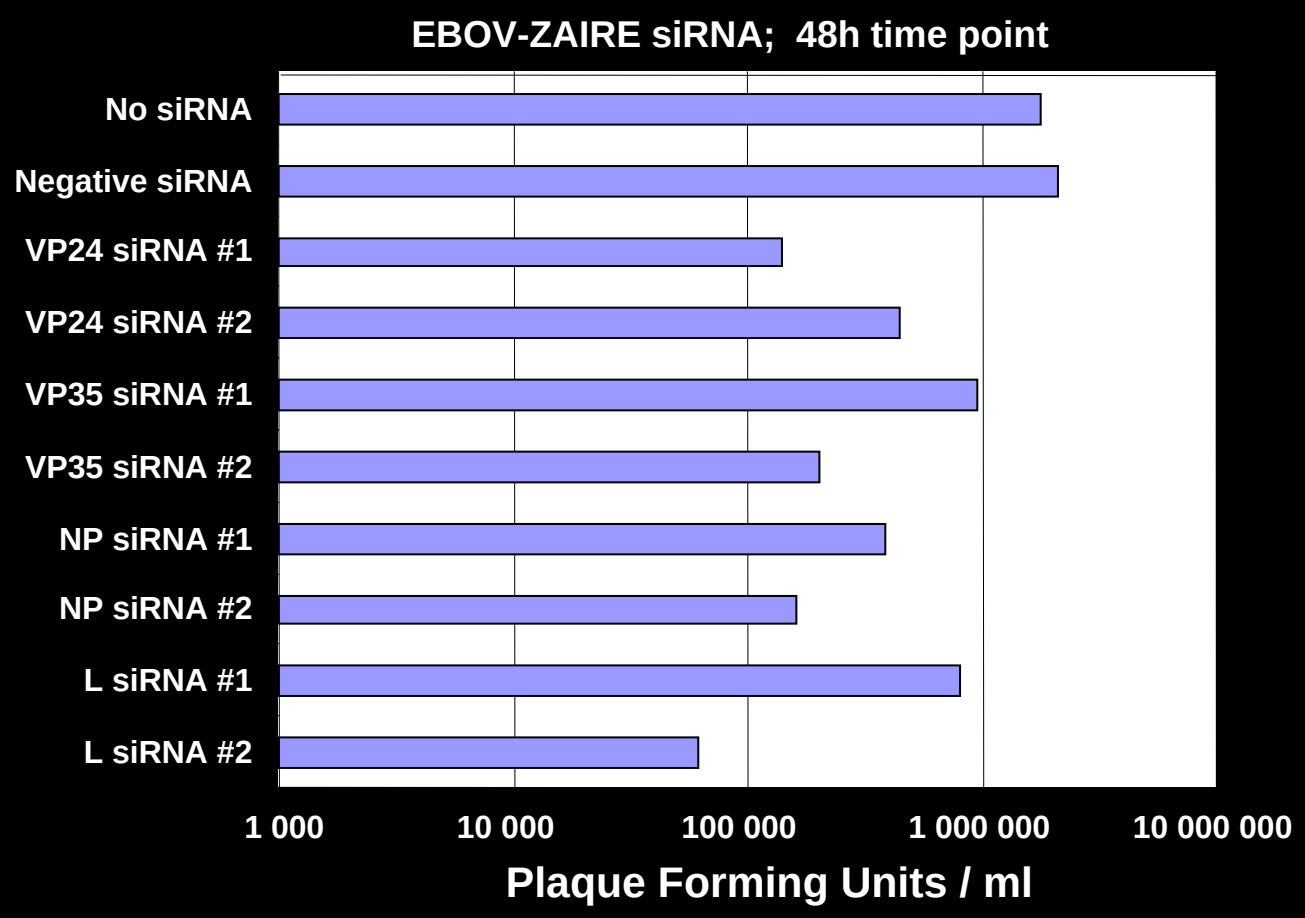
RNA Interference



Natural Process of RNAi

Target Down-modulation

Ebola siRNA reduces viral titer by over 90%



1 log reduction in viral titer with unmodified siRNA

Protocol: siRNA was transfected into 293T cells and cells were infected next day. Viral titer was measured after 48h in the sup by plaque assay.

Section 2: Identification of small molecule inhibitors of EBOV in a whole cell assay

•**Hypothesis:** **Can we identify small molecule drug-like compounds**

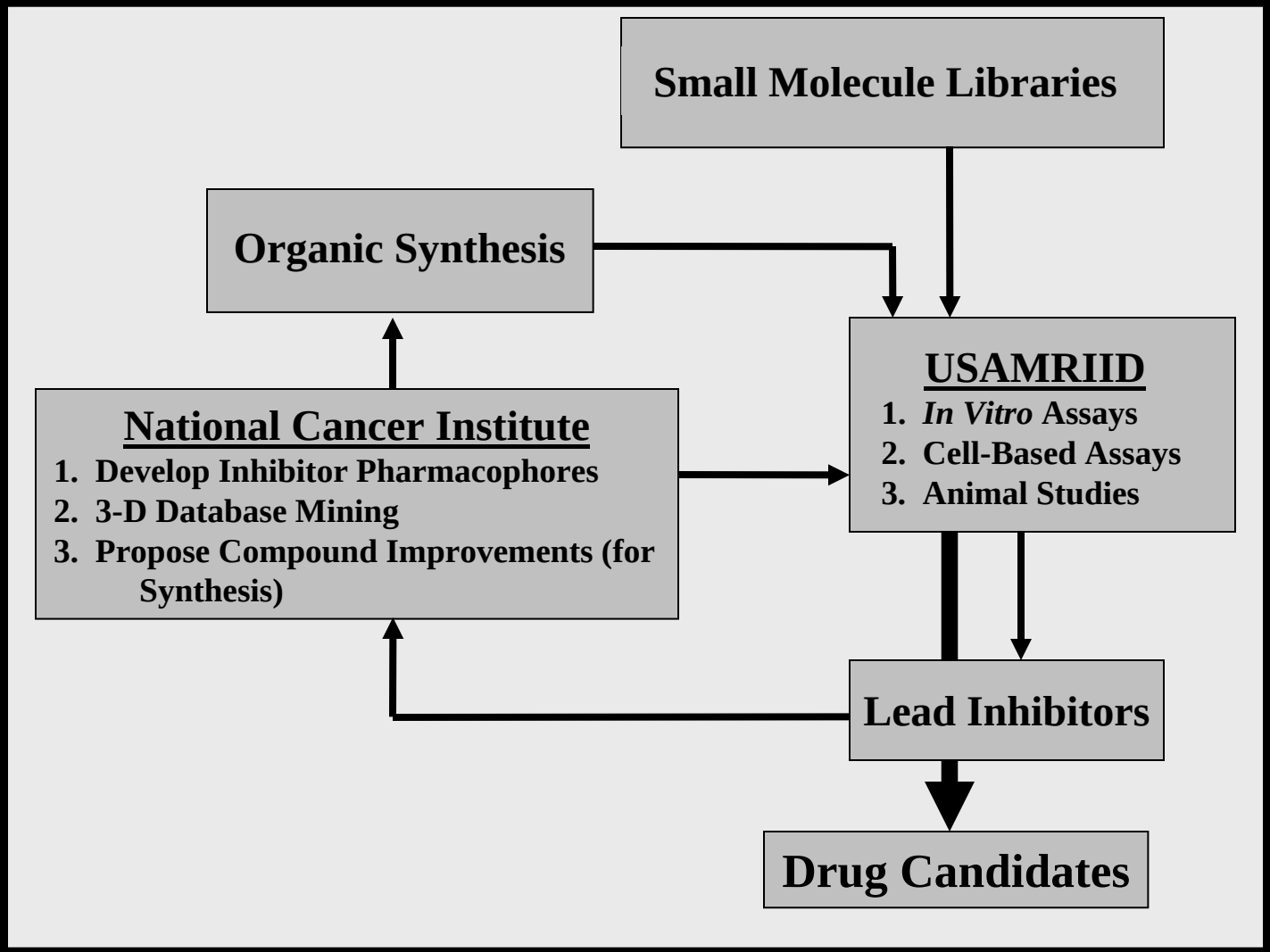
•**Approach:**

-Set up HTS assay: Use recombinant GFP-EBOV and a high-content imagers to identify small molecule inhibitors that inhibit viral replication

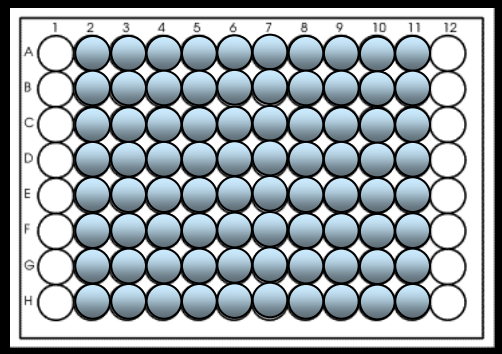
-Screen chemical libraries to identify hits (decrease in GFP)
-Validate hits: Use QRTPCR, and pfu
-Test in animal models: Start with rodents to NHPs.
-Identify the targets of the chemical (viral or host target)

Partners: Functional Genetics and Integrated Biotherapeutics

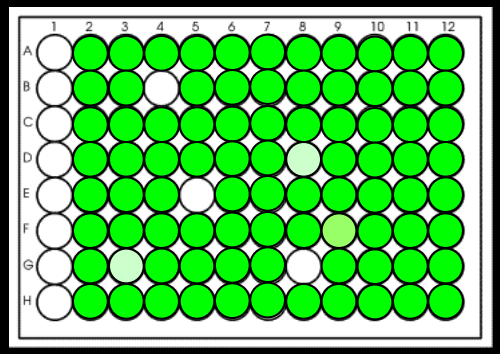
Initiated a major non-petidic small molecule drug discovery program



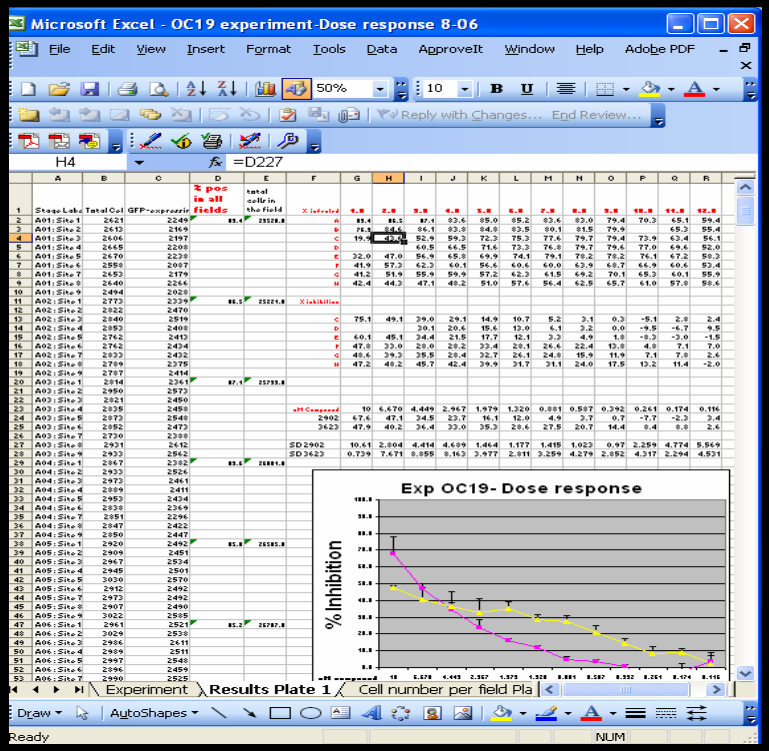
Ebola Virus Drug Screening



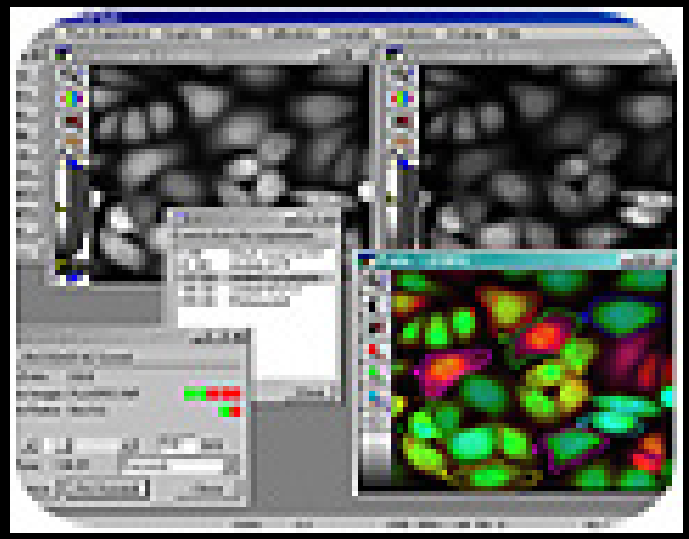
Infect
MOI:1
EBOV-GFP



48h, 37°C
Fix



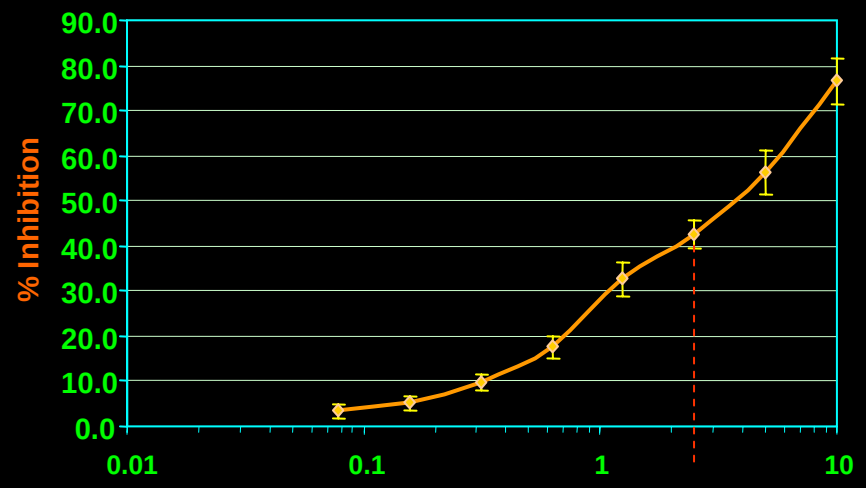
Data
Analysis



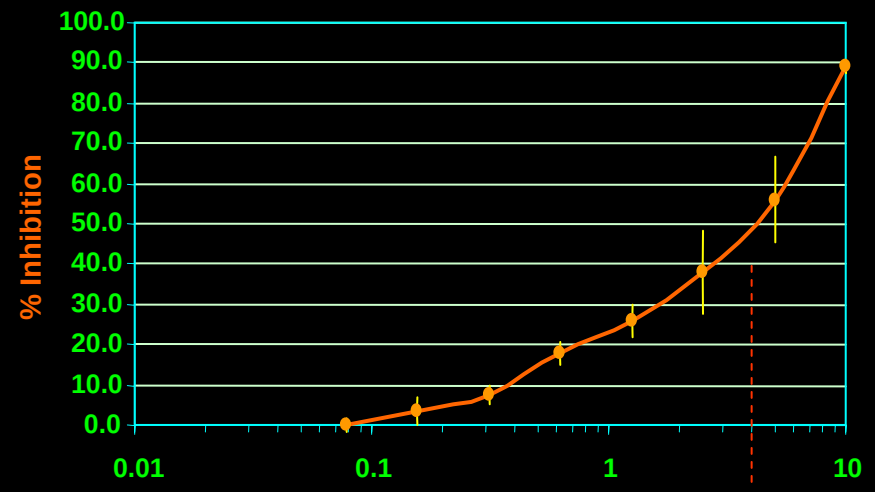
Discovery 1 High Content Imaging System

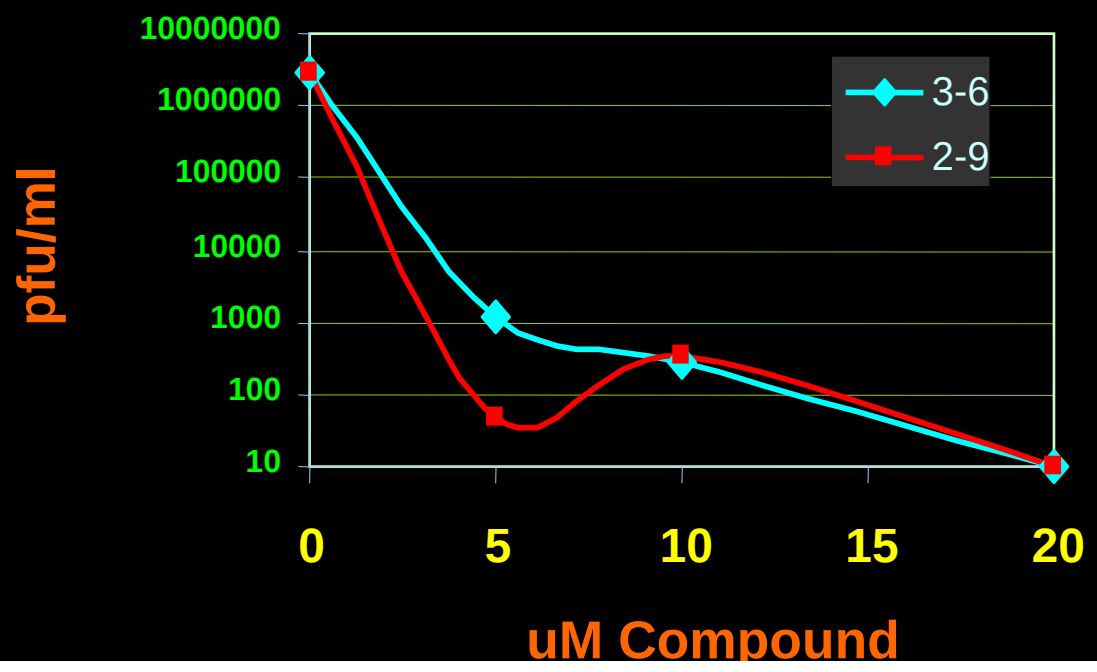
Examples of EBOV inhibitors

Cpd3-6

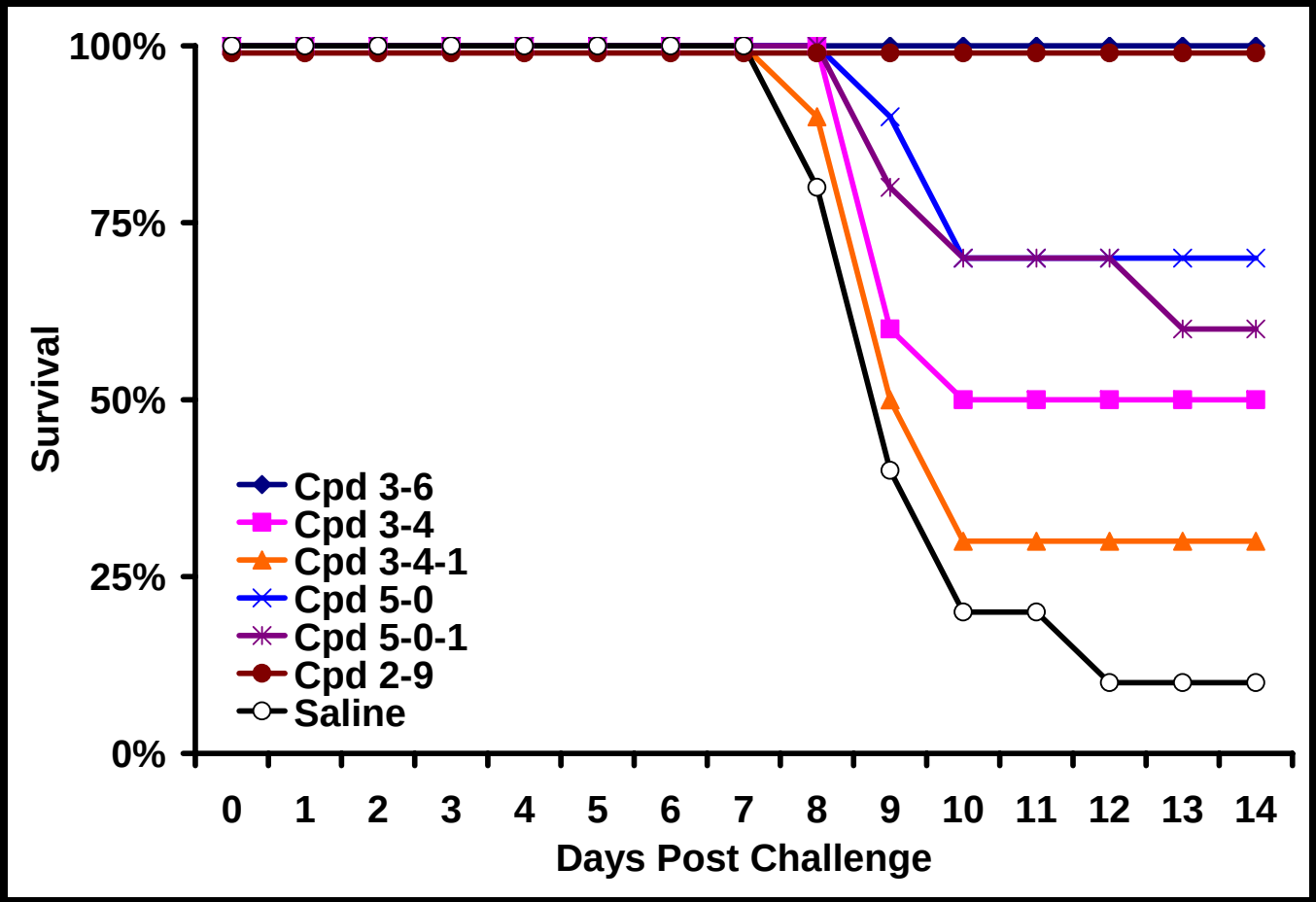


Cpd2-9



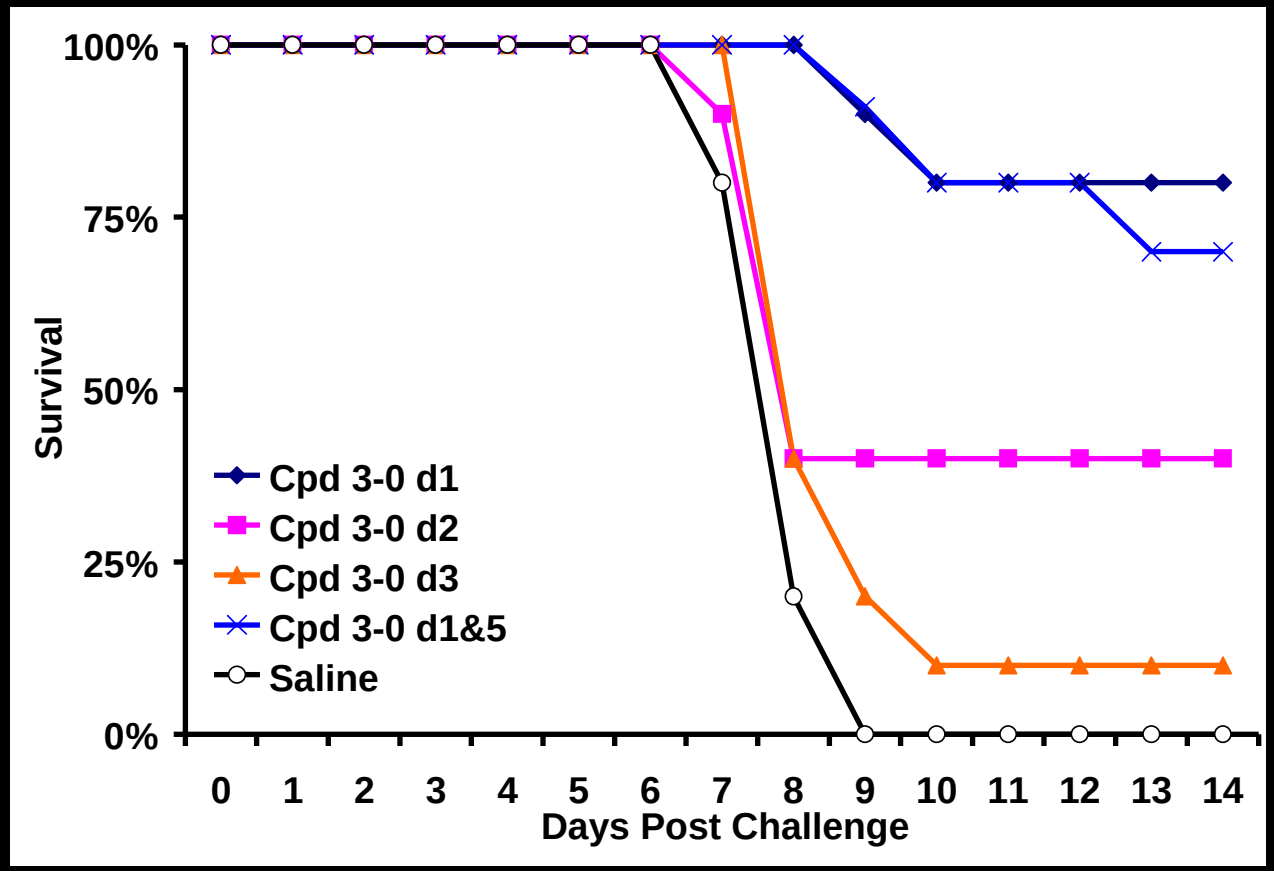


Protection of Mice against lethal EBOV challenge



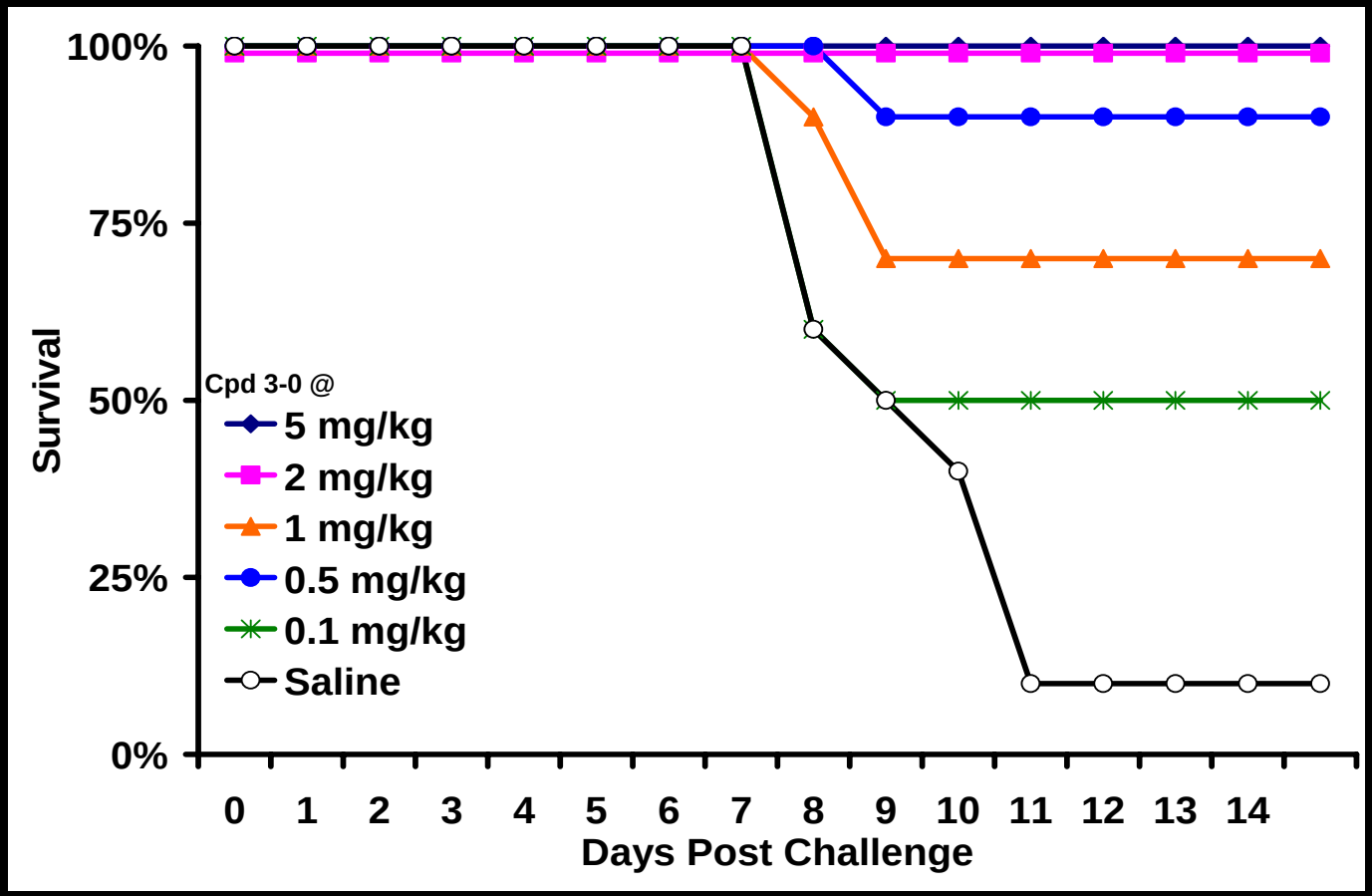
Compounds were given only 3 times at 5-25 mg/kg
Mice were challenged with 1,000 pfu mouse adapted ZEBOV

Drug-like small molecules protect after a single injection



Compounds were given only once or twice at 1-10 mg/kg
Mice were challenged with 1,000 pfu mouse adapted ZEBOV

Drug-like small molecules protect in a dose-dependent manner



Compounds were given a single dose or three doses 1 day after infection
Mice were challenged with 1,000 pfu mouse adapted ZEBOV

Summary of Development: Small molecule inhibitors of EBOV replication

- Several libraries were screened >12,500 compounds
  - 15 validated hits were ID in the diversity set
  - Over 25 hits in a single focused sublibrary

- Secondary screen (plaque assay with wild-type virus)

- Tertiary Screen (Dose responses) → Selection of leads

- Mouse experiments : Found several novel Txs with excellent bioavailability.

- Ongoing:
  - Mechanistic characterization
  - Gas phase pharmacophore
  - Scale-up synthesis for guinea pig and NHP studies

Overall summary of our Filovirus TX efforts

- Innate immune modulation by VLP protects against both EBOV and MARV most likely through NK cell activation.
- Identified several host factors from proteomic analysis and KD of some of these host factors protect against EBOV-MARV is in process.
- Targeting common viral processing pathways such as VPS4 shows Tx promise.
- Systematic genome wide siRNA screen has led to several promising lead sequences *in vitro* and *in vivo*.
- Identified second generation PMOs which protects mice and guinea pigs against MARV and NHP in a post-exposure Tx against EBOV.
- Identified several scaffolds/druggable small molecule inhibitors of EBOV.

Acknowledgments

Nichole Posten
Meagan Cooper
Jay Wells
Sean VanTongeren
Daniel Reed
Doug Lane
Tara Kenny
Shirin Badie

Kelly Warfield
Dana Swenson
M. Javad Aman

Gordon Ruthel
Rekha Panchal

George Kallstrom
Gretchen Demmin
Lynn Silvestri

Derron A. Alves
G. Gene Olinger
Carlton Rice

Acknowledgements

USAMRIID
Kelly Warfield
Dana Swenson
Gordon Ruthel
Rekha Panchal
Steven Bradfute
Warren Kalina
Kylene Hall
Meagan Cooper
Xiaoli Chi
Jay Wells
Nichole Posten
Edna Torres-Melendez
Connie Schmaljohn
Alan Schmaljohn (previous)
Diane Negley
Mary Kate Hart (Previous)
William Pratt
Mike Parker
Pathology Division

NCI-DTP
Rick Gussio
Tam Nguyen
James Burnett

AVI BioPharma
Pat Iversen
Alan Timmins
Rob Blouch
Hong Moulton
Dave Stein

Functional Genetics
Michael Goldblatt
Michael Kinch

Integrated Biotherapeutics
M. Javad Aman
Sven Enterlein

Alnylam
Zak Zimmerman
Tony DeFougerolles
Anna Borodovsky
Tanya Novobrantseva
Andrew Sprague
Akin Akinc
Philip Hadwinger
Martin Maier
Pamela Tan

Funding
DoD: DTRA/TMTI
HHS: NIH