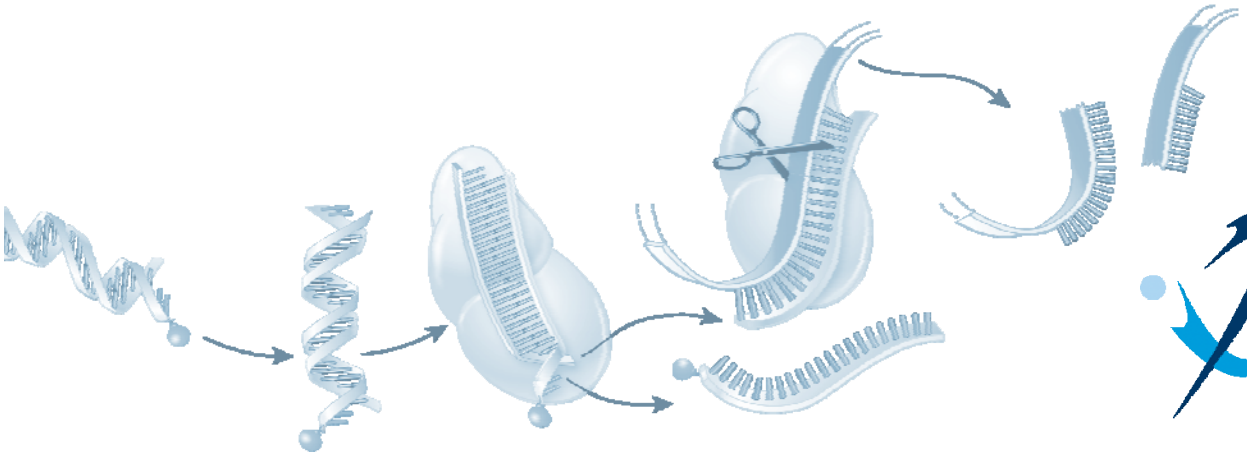

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Development of Antiviral RNAi Therapeutics

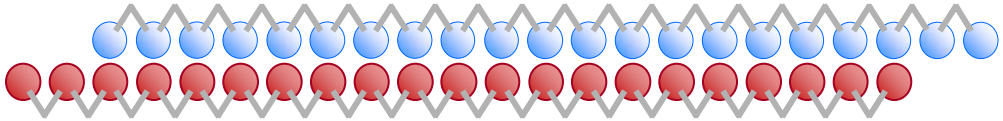
February 7, 2007

Agenda

- Technology Platform
- Respiratory disease programs (RSV, Flu)
- Ebola program

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



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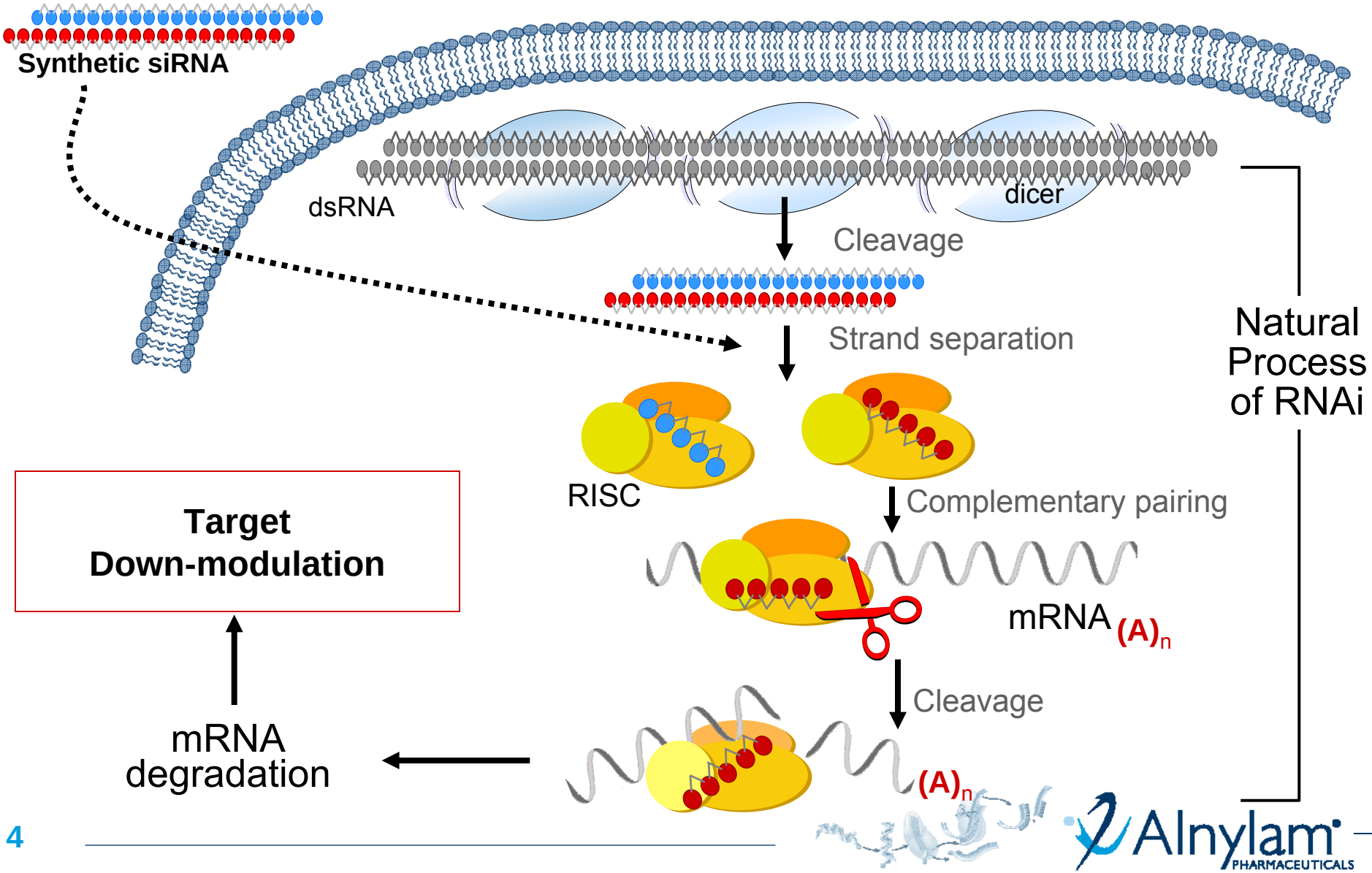
RNA Interference



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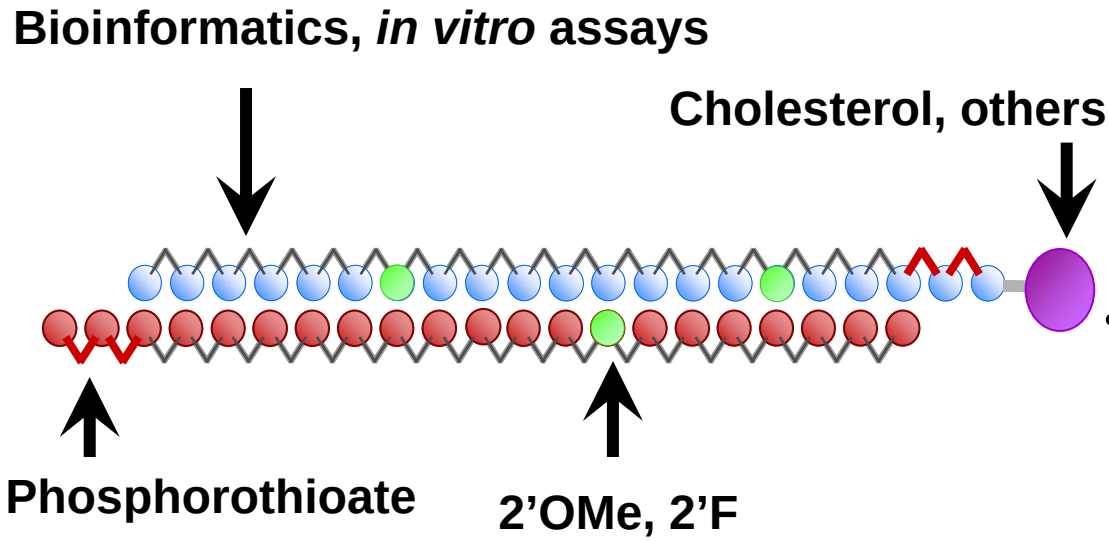
RNAi Product Platform

Turning siRNAs into Drugs



Incorporate minimal number of modifications required for appropriate pharmaceutical properties

- Select
  - »*In silico* design
  - »*In vitro* assays
- Stabilize
  - »Chemistry
- Deliver
  - »Conjugate

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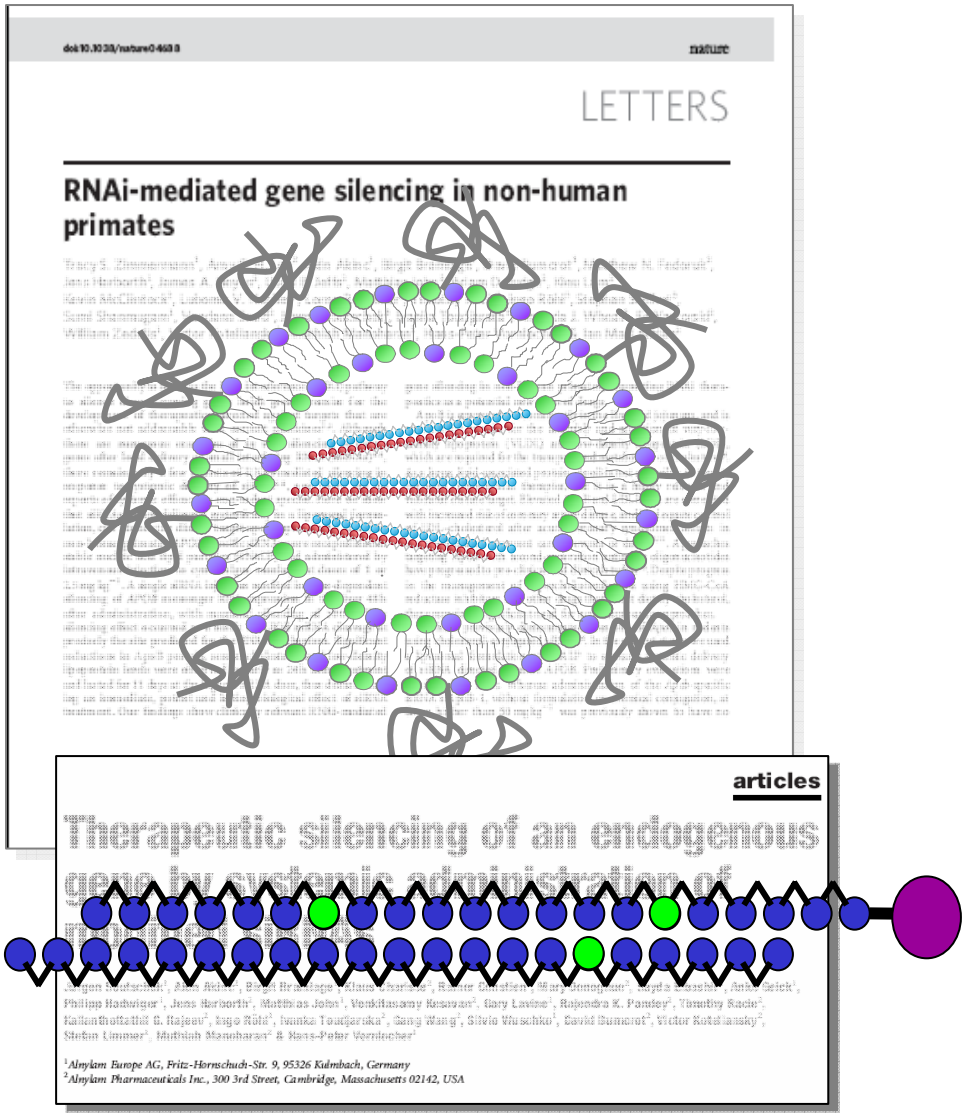
Delivery Technologies for RNAi

- Simple saline solutions are effective in local administration paradigms
  - »Ocular
  - »Respiratory
- Major advances made in systemic/parenteral delivery
  - » Conjugation
  - » Liposomes
- Ongoing work at Alnylam seeks to extend capabilities for a variety of applications
  - » Polymers
  - » Peptides
  - » Antibodies



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Agenda

- Technology Platform
- Respiratory disease programs (RSV, Flu)
- Ebola program

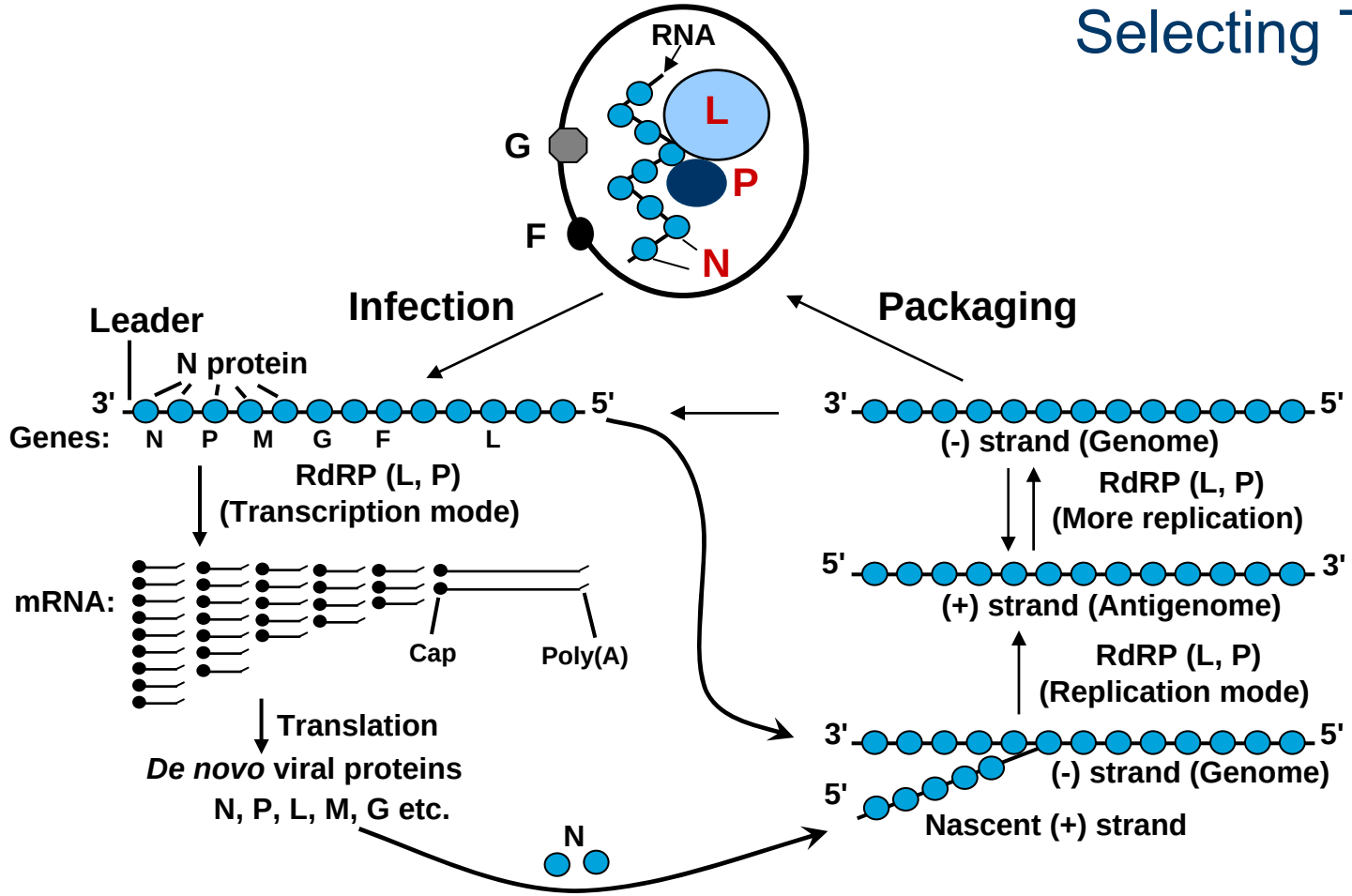
Respiratory Syncytial Virus (RSV) Program

Harness RNAi to treat a major infectious disease

- Significant unmet medical need
  - » >100,000 pediatric hospitalizations/yr in US
  - » >170,000 adult hospitalizations/yr in US
  - » Significant morbidity including asthma
- No effective therapies to treat RSV infection
  - » Synagis used for prevention
- ALN-RSV01 in clinic
  - » Target: RSV nucleocapsid "N" gene
    - — Highly conserved gene; essential for viral replication

Replication Cycle of RSV

Selecting Targets



Hypothesis

Silencing proteins present in the nucleocapsid, (**N, P, L**), required for RSV replication, can result in rapid viral elimination

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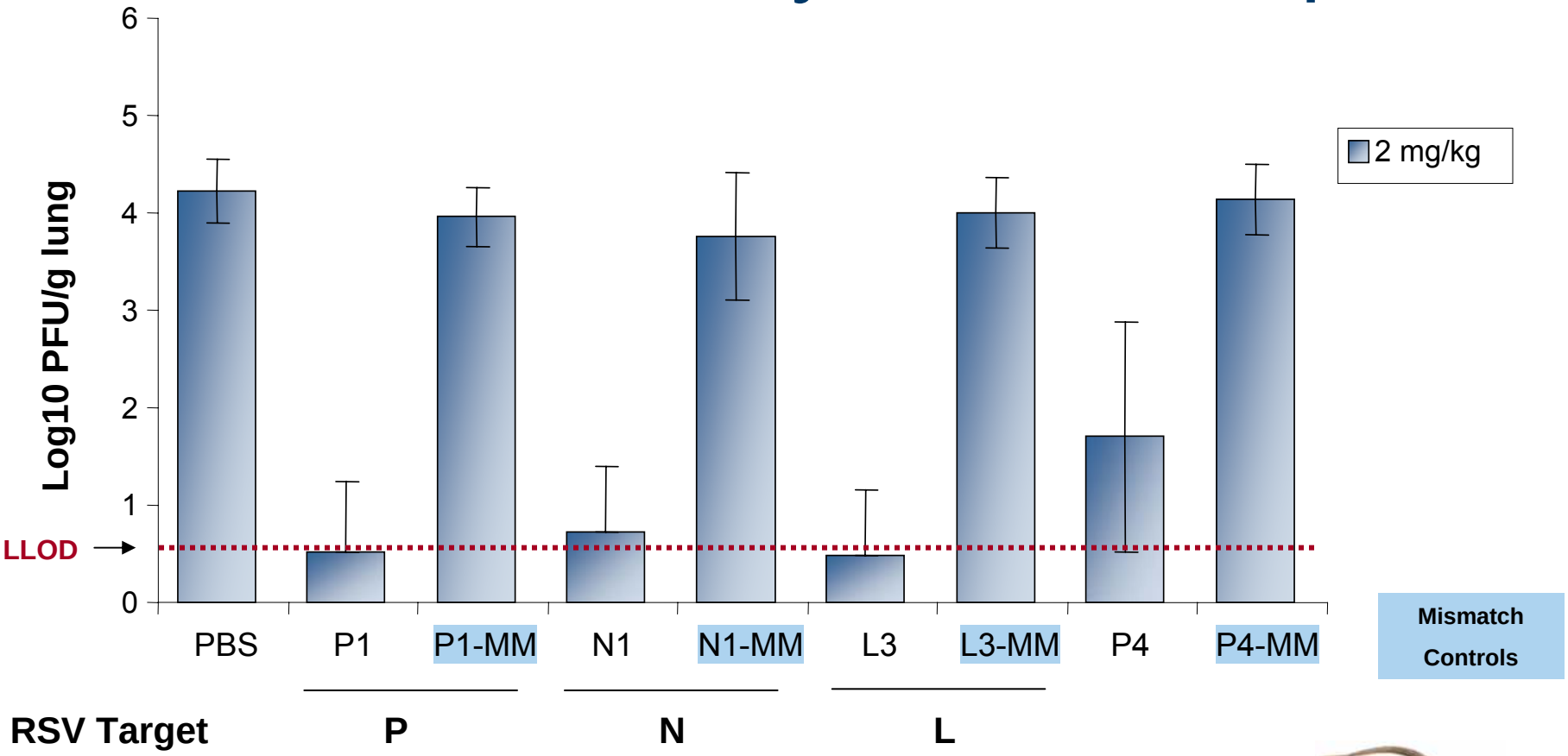
Inhibition of RSV by siRNA is Specific



siRNA i.n. (50ug) → **4 hrs** → **Virus (RSV/A_2) i.n. (10^6 pfu)** → **4 days** → **Viral Titer in Lung**

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RSV Summary

- RNAi is a natural and specific process applicable to the development of antivirals
- Rapid screening and selection of the siRNA sequence is possible
  - » Accelerated pre-clinical selection
  - » Consideration of therapeutics for orphan diseases
- ALN-RSV01 is a potent RSV antiviral *in vitro* and *in vivo*
  - » ≈4 log reductions with a single dose delivered topically to the resp tract
  - » Target site conservation across RSV clinical strains
- Topical and systemic ALN-RSV01 produce no significant adverse effects during GLP toxicology studies
- ALN-RSV01 well tolerated in Phase 1 intranasal trials
- Aerosolized ALN-RSV01 experimental infection studies on-going

Pandemic Flu Program

RNAi to address a possible influenza pandemic

- Effective preparedness requires multiple approaches
  - » Vaccines may not be optimally effective
  - » Existing treatment options limited
  - » RNAi therapeutics an attractive option
- Significant private-public sector partnership
  - » Novartis partnership
  - » Significant public sector support
    - — DARPA funding
    - — NIAID/NIH funding
  - » Leading academic group collaborations
    - — University of Georgia
    - — St. Jude Children's Research Hospital

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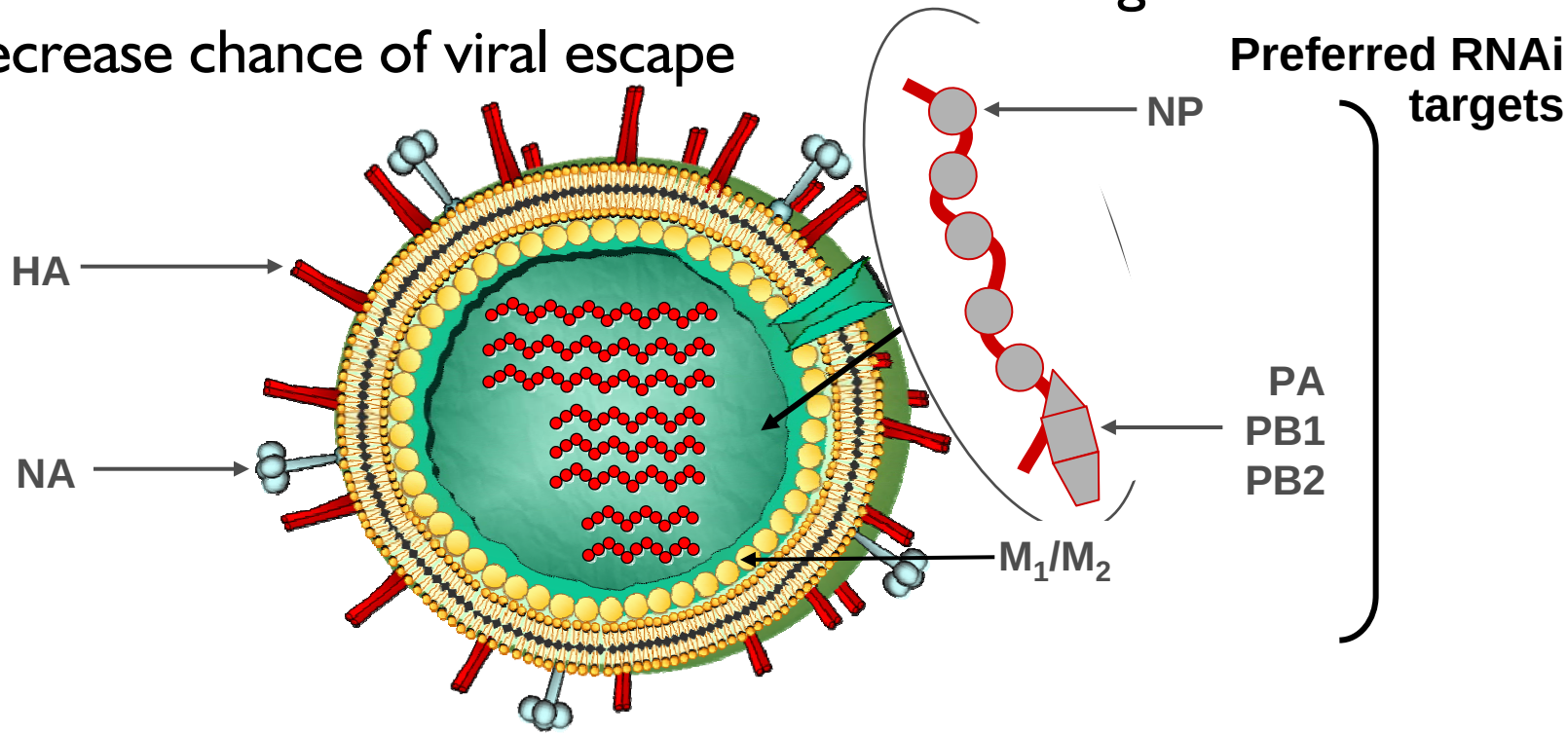
RNAi Therapeutics for Influenza

Target Selection Strategy

- Target RNAi therapeutic toward highly conserved genes required for viral replication
- Utilize combination of 2 siRNA to different flu genes to decrease chance of viral escape



Influenza Virus

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ALN-FLU01 Bioinformatics Shows 100% Strain Coverage

Target	Category*	Avian Flu (H5N1, H9N2, H7N3, H7N7)	Human Flu (H5N1, H9N2, H1N1, H2N2, H3N2)
Flu #1	% Identical Strains	99%	99%
	# Identical Strains	433	1162
Flu #2	% Identical Strains	95%	98%
	# Identical Strains	360	1131
ALN-FLU01	% Identical Strains	100%	100%
	# Identical Strains	439	1630

* Based on target sequences available on 3 May 2006

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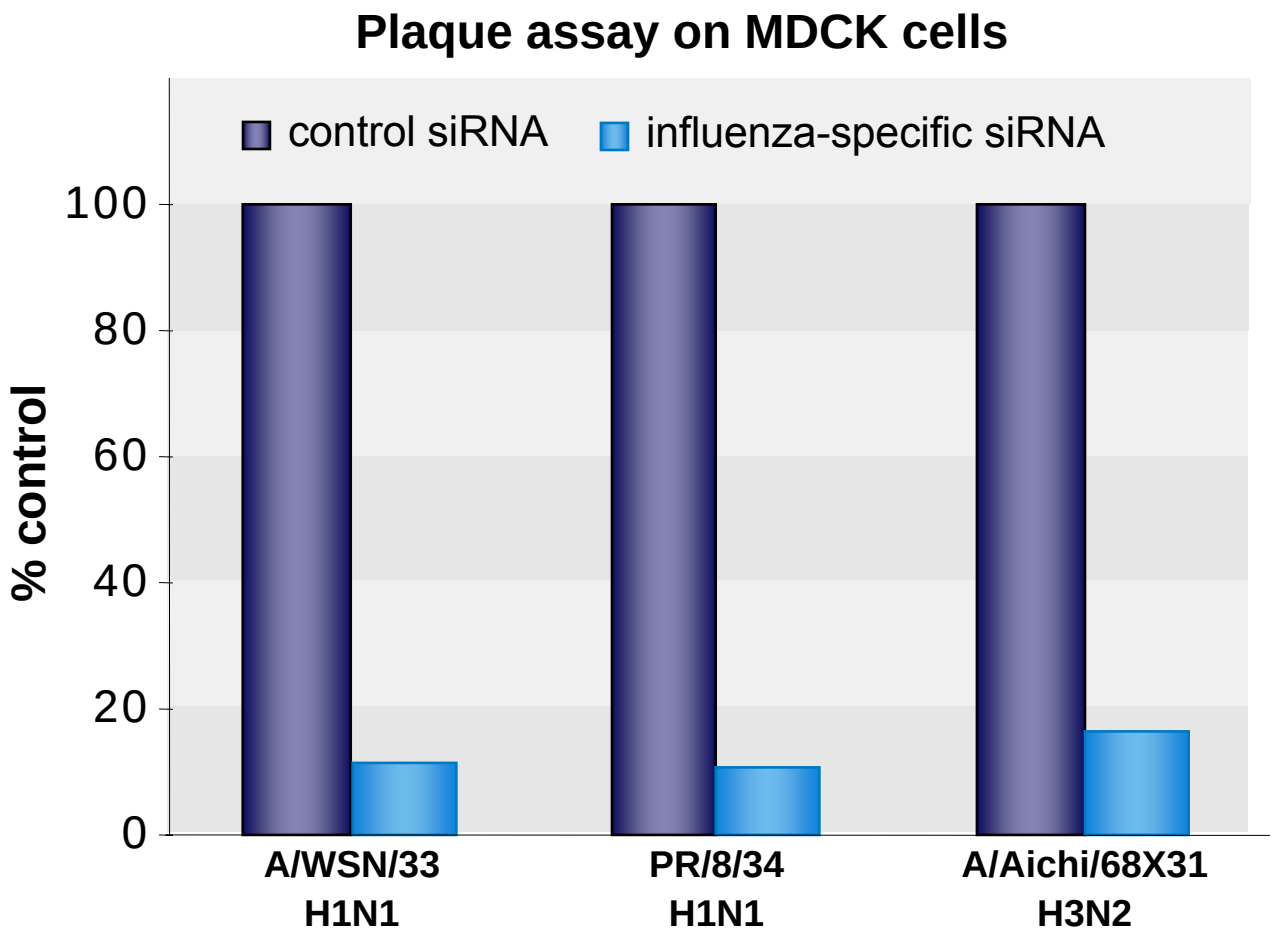
Anti-viral Activity Toward Multiple Human Flu Strains



Flu specific siRNA can inhibit viral replication of different flu strains

ATS, May 2006. Dr. M. Tompkins (UGA)

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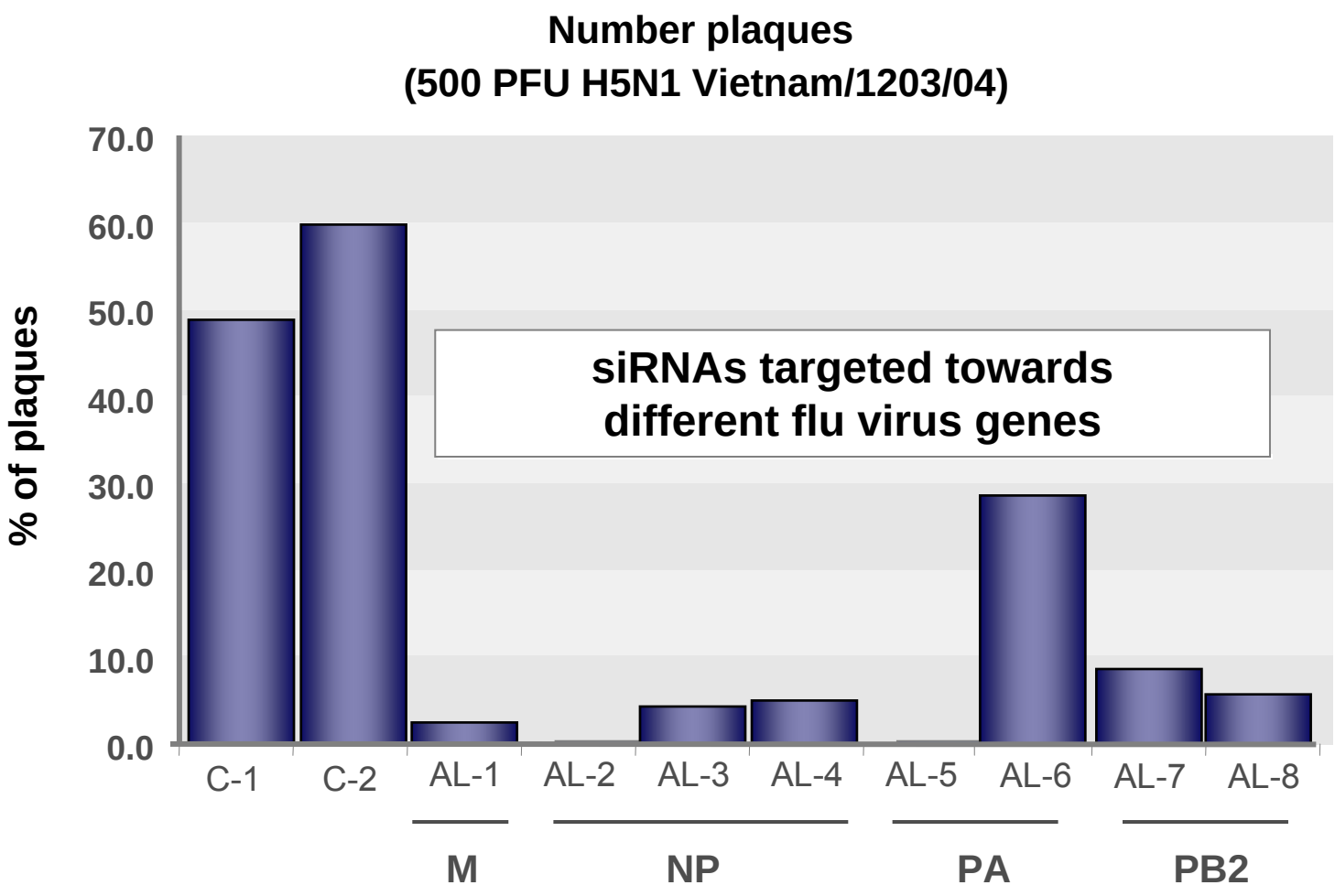
RNAi Against Human H5N1 Flu



ATS, May 2006. Dr. R. Webby (St. Jude)

Agenda

- Technology Platform
- Respiratory disease programs (RSV, Flu)
- Ebola program

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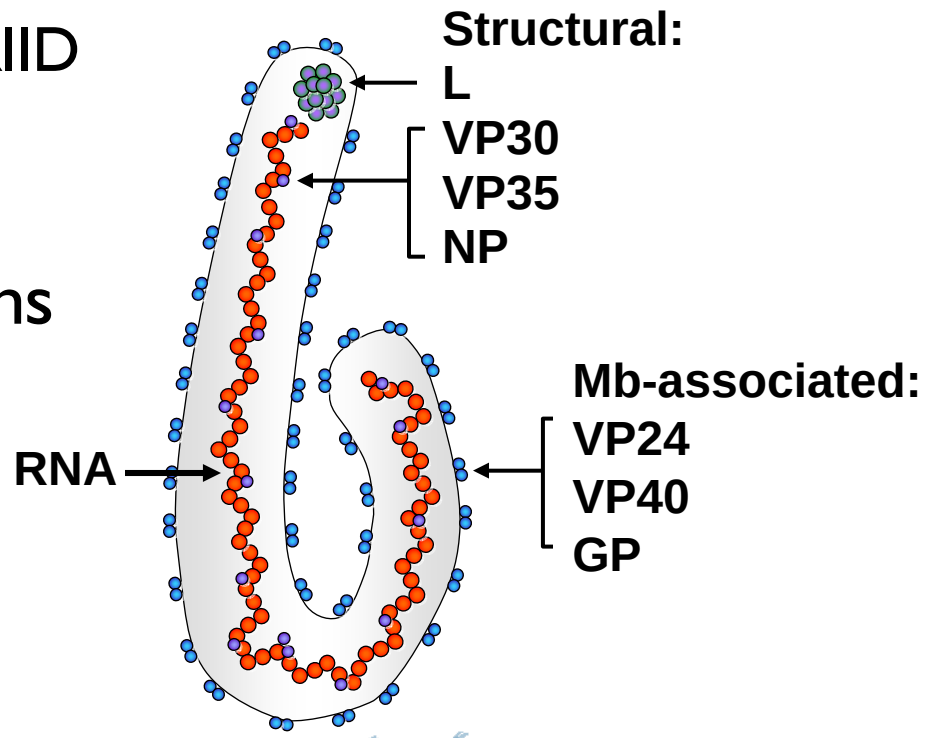
Ebola Program

RNAi targeting threats of bioterrorism

- $23M in federal funding to develop RNAi anti-viral targeting Ebola (NIH contract HHSN266200600012C)
  - » Severe, often fatal infection
  - » Collaboration with USAMRIID (Drs. Bavari and Warfield)
- Target all pathogenic strains
  - » Zaire and Sudan
- Non-biased approach to selecting viral gene targets



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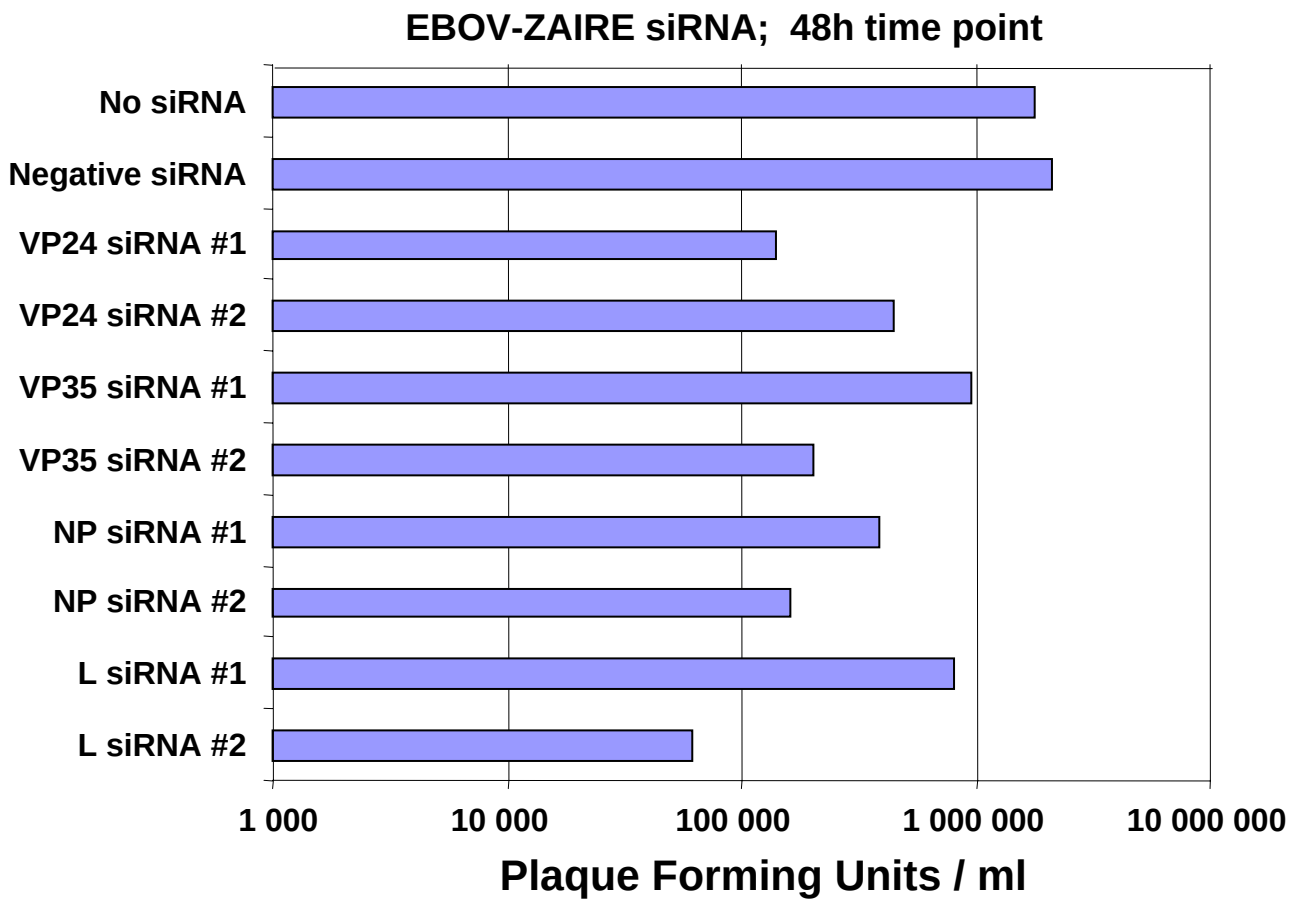
USAMRIID *In vitro* Proof of Concept

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.

Data: Kelly Warfield and Sina Bavari, USAMRIID

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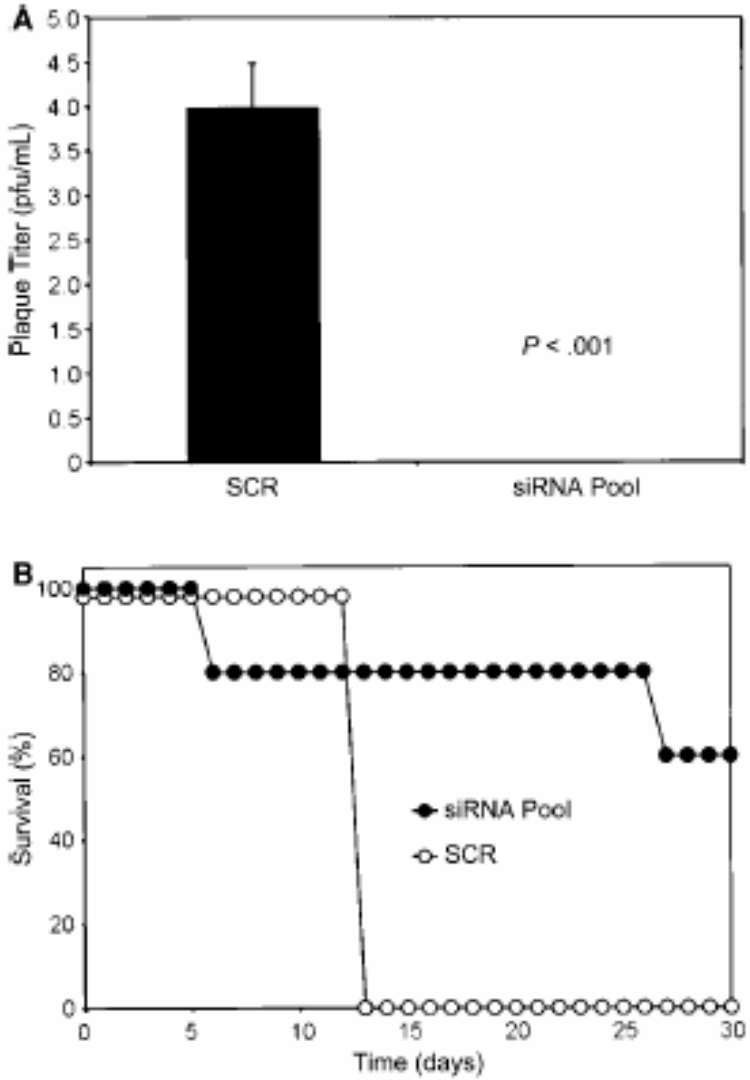
In vivo Proof of Concept

Liposomally delivered Ebola siRNA protects from lethal infection



- Guinea pig Zaire EBOV infection model
- Pooled Ebola or scrambled siRNA
- 1 mg/kg siRNA in SNALP2 liposomal formulation given i.p. 1 hr after EBOV infection and then daily on days 1-6.
- Sacrifice on day 7 for viremia (n=5 per group)

Reference: Geisbert et al., *JID* (2006) 193:1650-7.

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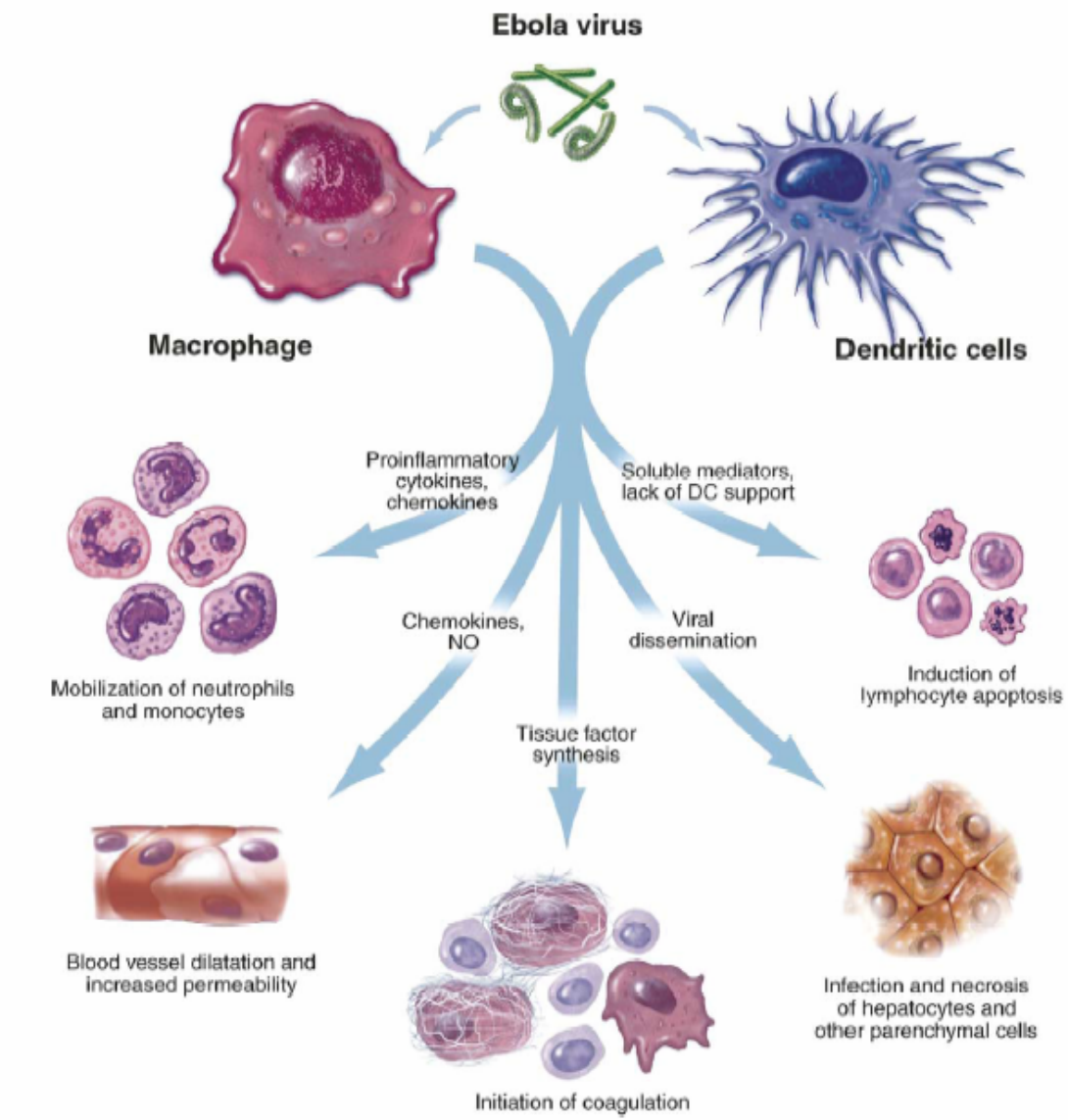
In Vivo Delivery to Appropriate Cells



- Macrophages and dendritic cells are primary cells involved in early infection
- Hepatocytes and other parenchymal cells involved in late infection

Bray and Geisbert, *Int. J. Biochem. Cell Biol.* (2005) 37:1560-6.

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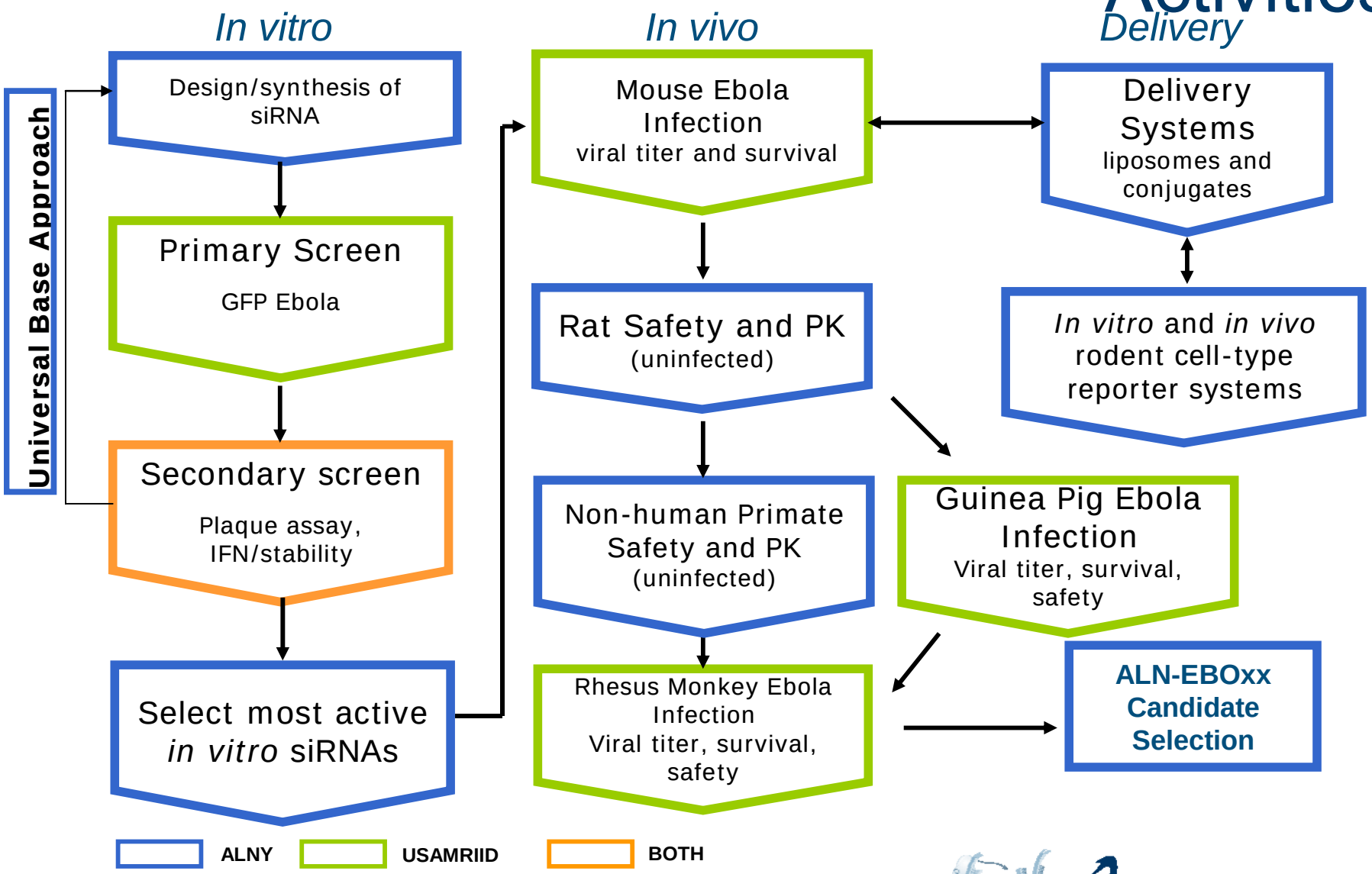
Summary of Product Development Activities



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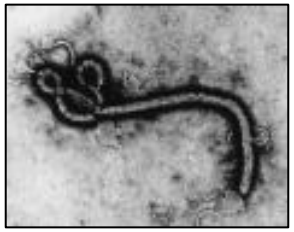
Rapid Response Medical Countermeasure Platform



Ebola

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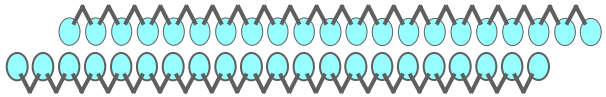
GAAG

ALN-EBOxx



Marburg

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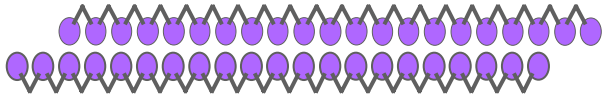
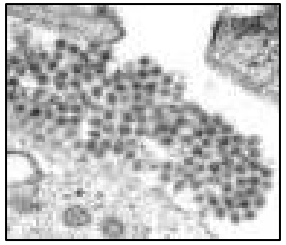
ALN-MARxx





Dengue

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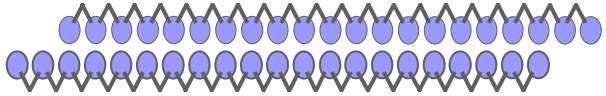
ALN-DENxx



SARS

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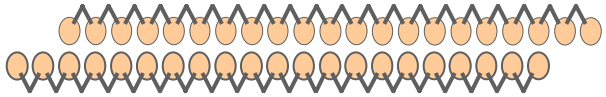
ALN-SARxx



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Summary

- RNAi offers the opportunity to rapidly develop broad-spectrum medical countermeasures
  - » Ability to therapeutically target any gene
  - » Identify active therapeutics in weeks to months
  - » Ideally suited as antiviral therapeutic for existing or newly emerging threats

- Validation of RNAi approach
  - » ALN-RSV01 drug in clinical stage for RSV infection
  - » Advanced development of ALN-FLU01
  - » Alnylam's systemic RNAi Therapeutic programs

- Alnylam's Ebola program progressing rapidly
  - » Identify siRNA that target all pathogenic Ebola strains
  - » Collaboration with USAMRIID
  - » NIAID-funded