Alnylam®
PHARMACEUTICALS

RNAi Therapeutics, the Alnylam Experience

Jared Gollob, MD

NIH/FDA Meeting

15 December 2011

Discussion Outline

Introduction and Background

Overview of Alnylam Lead Discovery Paradigm

Alnylam Pipeline

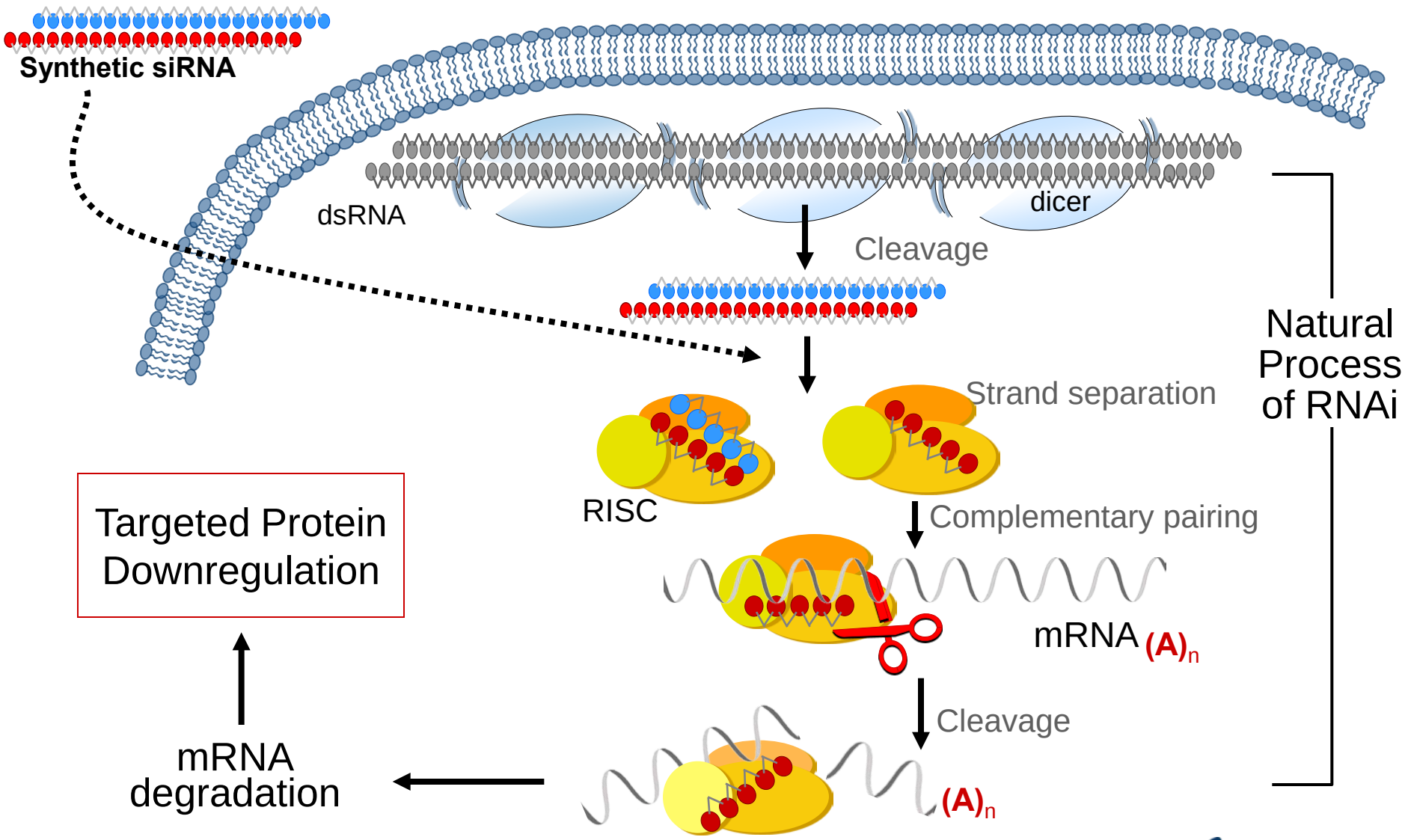
Program Review

- ALN-RSV01
- ALN-VSP02
- ALN-TTR01

Summary and Conclusions

Alnylam PHARMACEUTICALS

Therapeutic Approach to Harnessing RNAi



Alnylam PHARMACEUTICALS

RNAi Clinical Programs

Active Programs

		Phase I	Phase II	Phase III
Alnylam / CUBIST / KYOWA KIRIN	ALN-RSV	RSV	Phase IIb	
Quark	PF-04523655	Wet AMD	Phase II	
ZaBeCor	Excellair™	Asthma	Phase II	
santaris pharma a/s	miravirsen SPC3649	Hepatitis C (miR122)	Phase II	
NOVARTIS / Quark	QPI 1002	Delayed Graft Function	Phase I/II	
sylentis	SYL040012	Ocular Hypertension Glaucoma	Phase II Phase I	
Alnylam	ALN-VSP	Liver Cancer	Phase I	
TRANSDERM	TD101	Pachyonychia congenita	Phase I	
Alnylam	ALN-TTR	TTR-mediated Amyloidosis	Phase I	
SILENCE THERAPEUTICS	Atu027	Oncology – GI, Lung & Other	Phase I	
Tekmira	TKM-PLK1	Advanced solid tumor cancers	Phase I	
MARINA BIOTECH	CEQ508	Familial Adenomatous Polyposis	Phase I	
Alnylam	ALN-PCS	Hypercholesterolemia	Phase I	
Calando PHARMACEUTICALS	CALAA-01	Oncology – solid tumors	Phase I	
Quark	QPI 1007	Ocular neuroprotection	Phase I	
Silenseed	siG12D	Pancreatic Cancer	Phase I	

Based on public domain information on various programs, it is estimated that approximately 1000 humans have been exposed to siRNAs to date

Alnylam Programs | Licensed | Unlicensed

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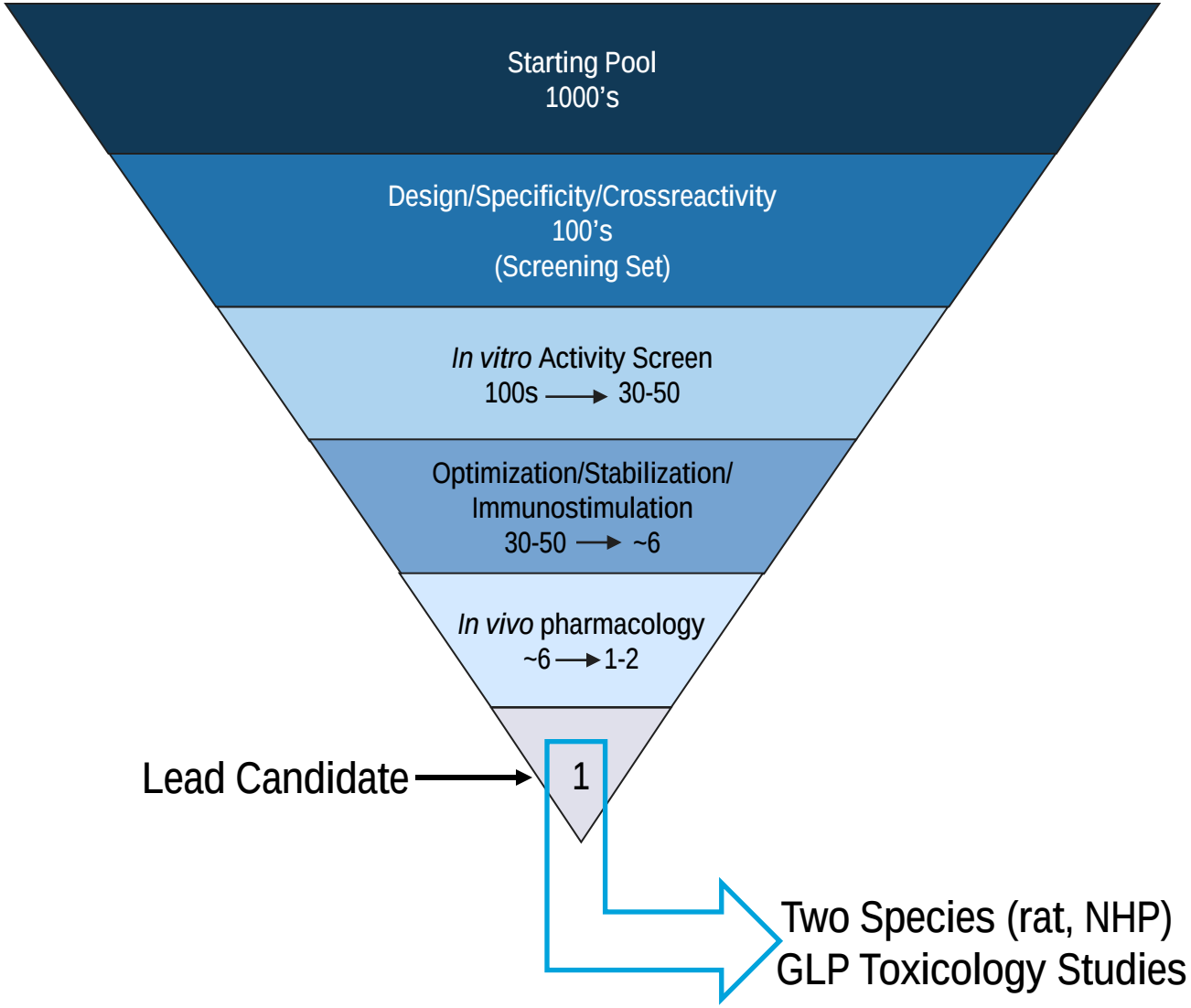
RNAi Lead Discovery



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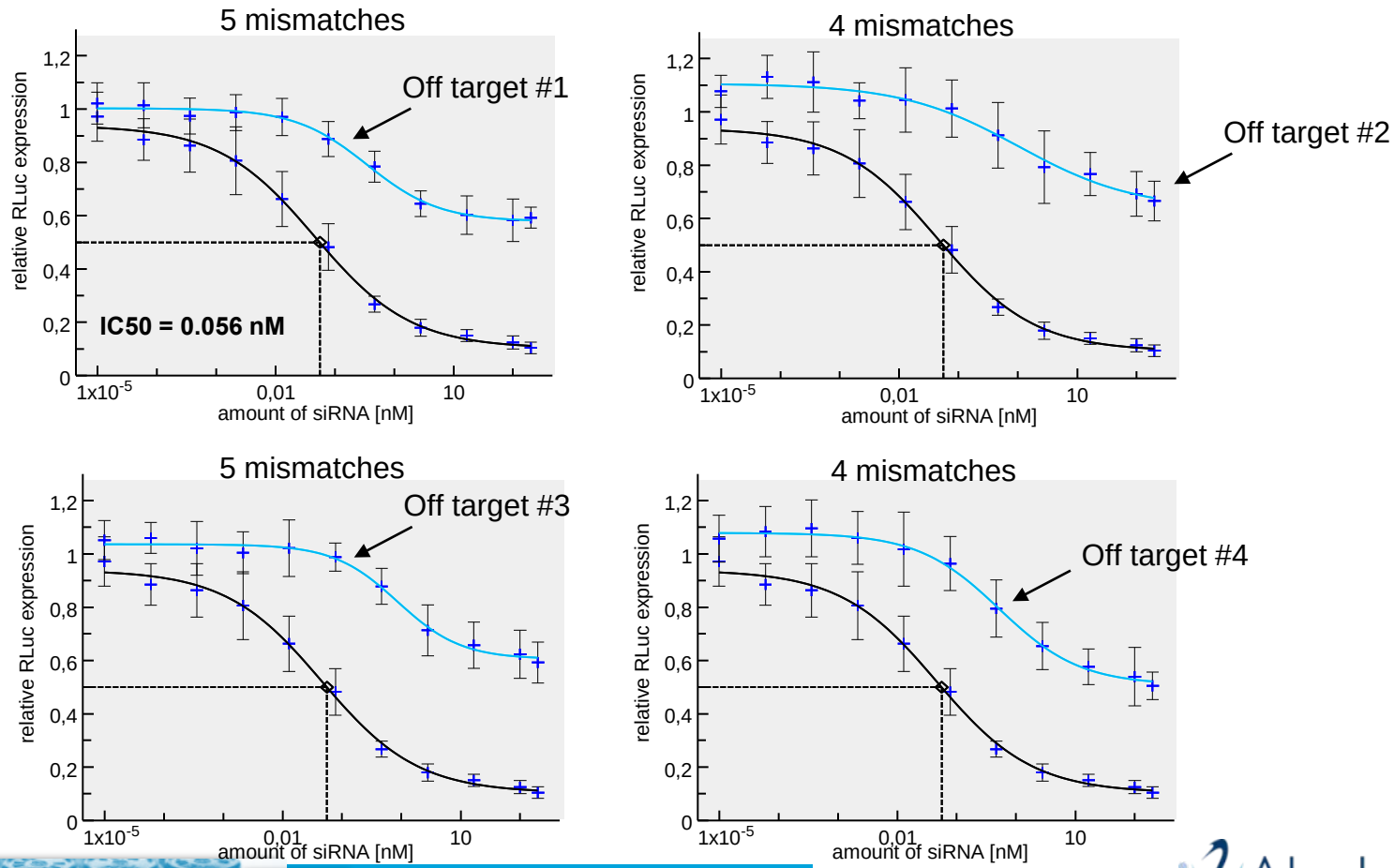
Sequence-mediated Off-targets

In Vitro Assays to Discriminate On vs. Off-Target Activity

- *In vitro* IC_{50} evaluation of highest probability off-targets can be conducted to evaluate whether closely related targets identified by genomics screens are silenced

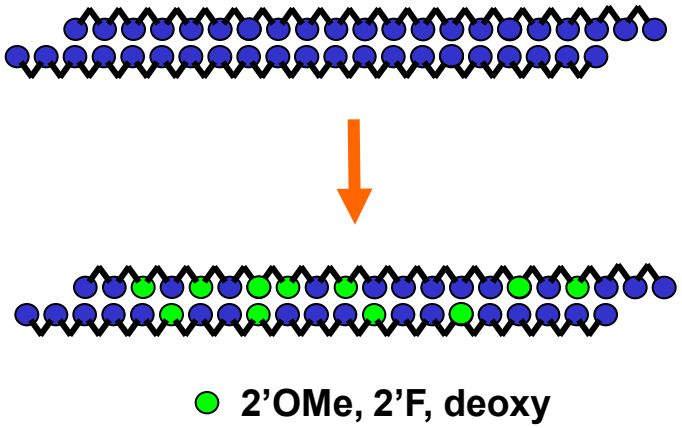
siRNA targeting On-Target gene vs. Four Potential Off Targets

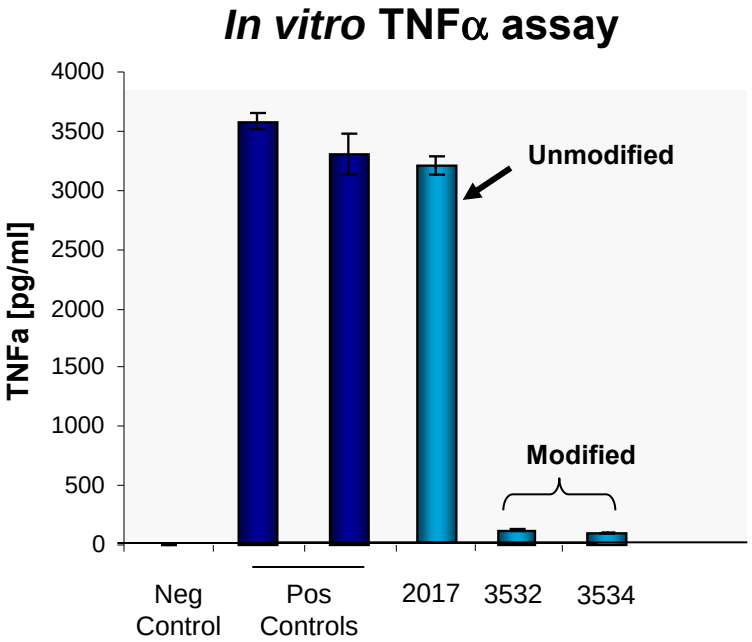


Chemical Modification to Mitigate Immunostimulatory Profile of siRNAs

Immunostimulatory effects are sequence, concentration (and structure) dependent

- Modifications at 2' position of ribose eliminate TLR-mediated innate immune stimulation by siRNAs *in vitro* (1, 2, 3, 4, 5)
- Modifications at the 2' position eliminate TLR-mediated innate immune stimulation by siRNAs *in vivo* (2, 5)
- Innate immune stimulation through RIG-I pathway can also be eliminated by 2' modifications (6)





(1) Sioud Eur J Immunol 36:1222 (2006)
(2) Judge Mol Ther 13:494 (2006)
(3) Cekaite JMB 365:90 (2007)
(4) Sioud BBRC 361:122 (2007)
(5) Robbins Mol Ther 15:1663 (2007)
(6) Hornung et al., Science 314:994 (2006)

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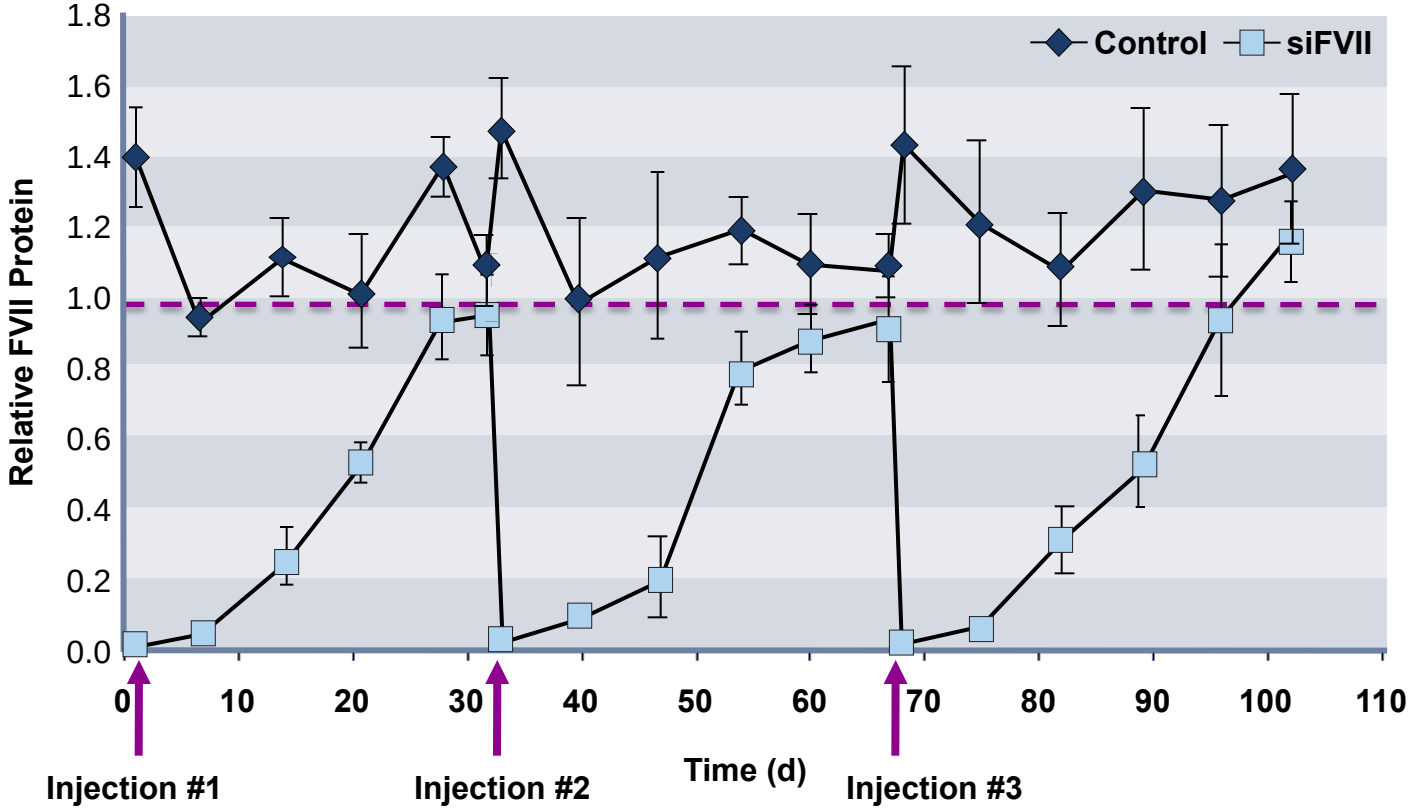
Systemic Delivery in Rodents

Example: *In Vivo* Silencing of Factor VII

Effective gene silencing with repeat dose administration over months

- Durable silencing that lasts ~1 month following each injection
- Demonstrates antibody-like pharmacology and feasibility for chronic indications
- No evidence of tachyphylaxis or neutralizing antibody formation



Akinc et al., *Mol Therapy*, 2009

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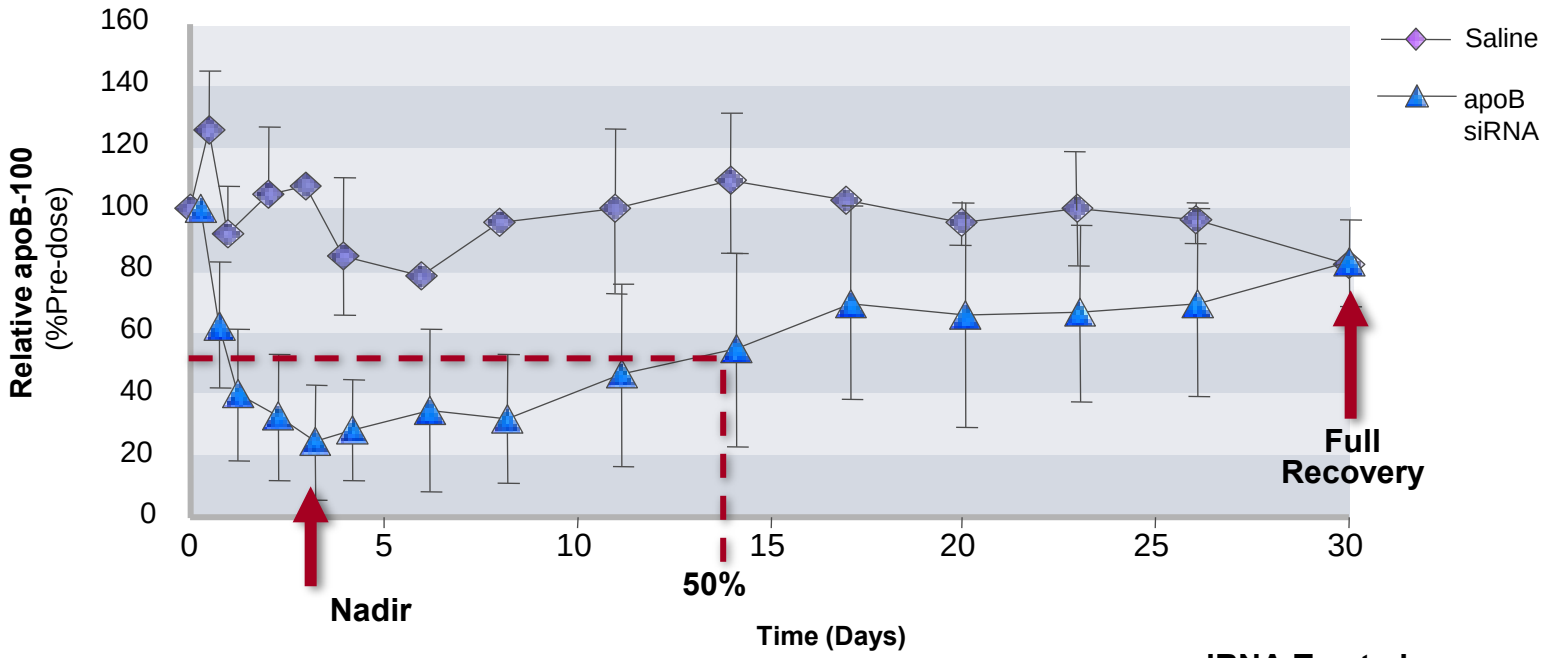
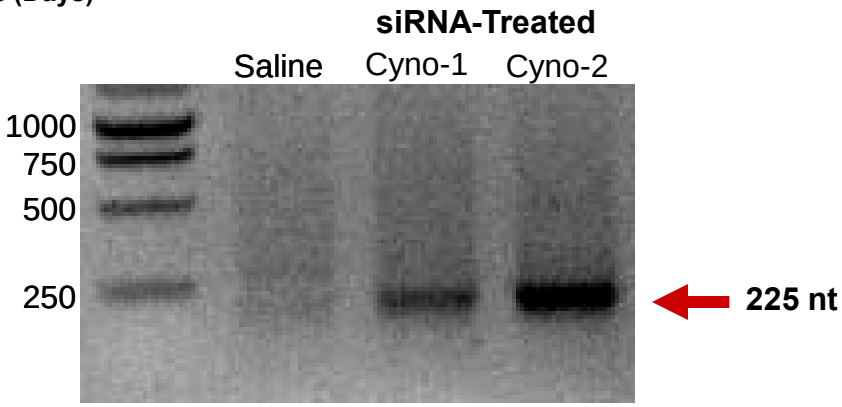
Silencing of apoB is Durable and RNAi-Mediated

Non-Human Primates



Determination by 5'-RACE PCR

- Amplification of product of specific molecular weight only occurs if cleavage took place at predicted site
- Verification by cloning and sequencing of amplified products



Zimmermann et al., *Nature*, 2006

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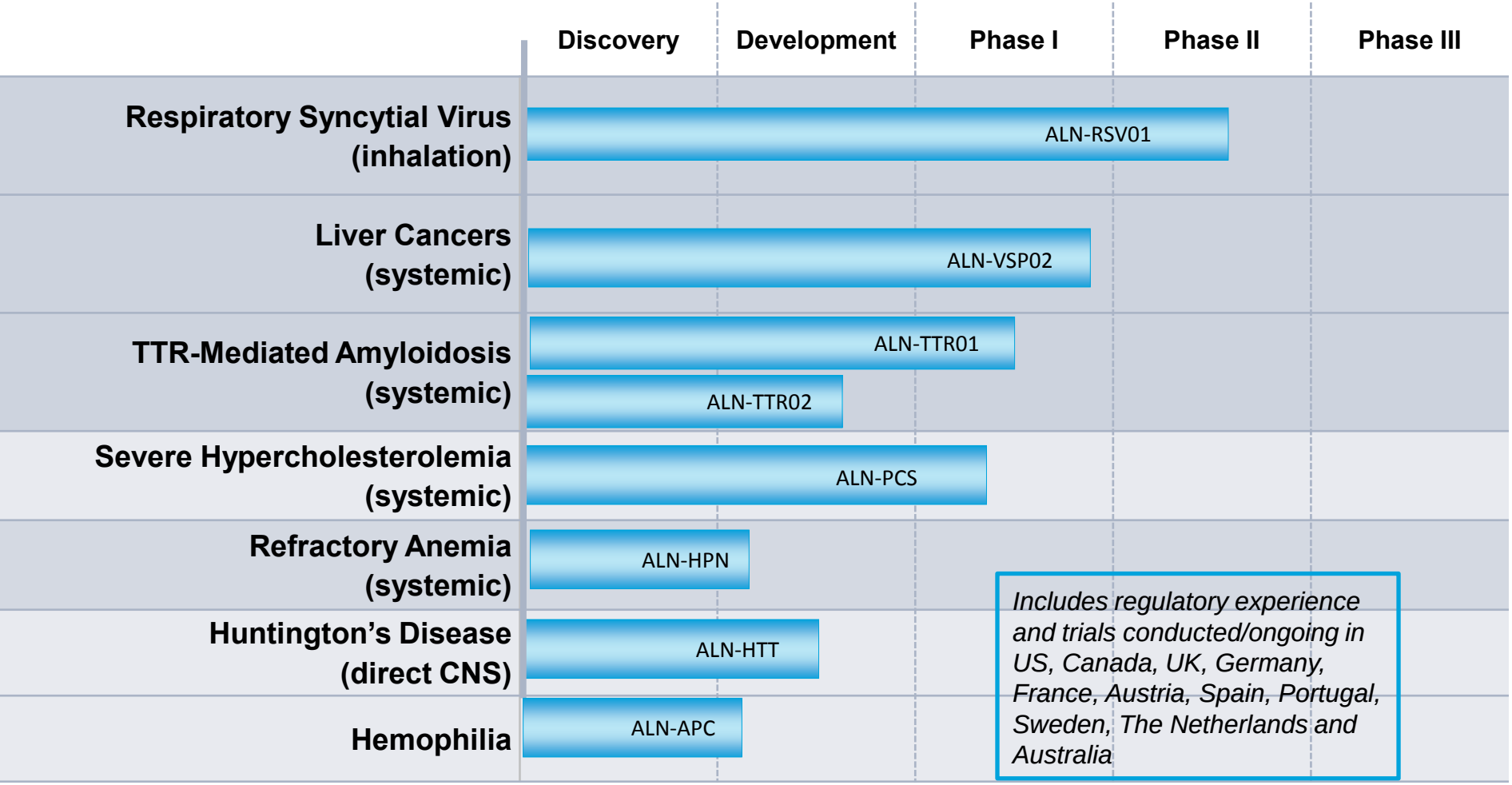
Alnylam Development Pipeline



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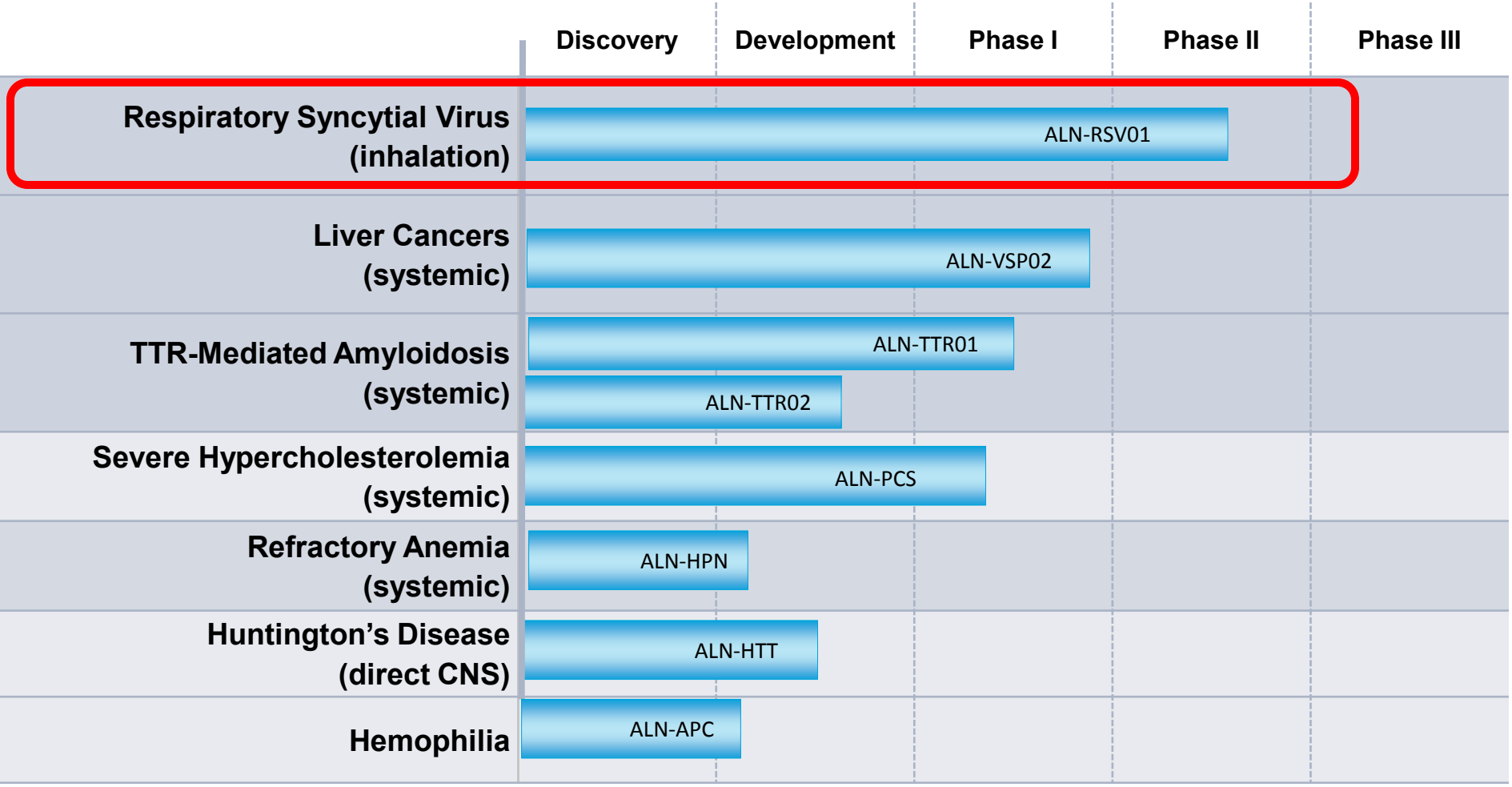

Alnylam PHARMACEUTICALS

ALN-RSV01 Clinical Development

Phase 1

Intranasal

Single and multi-dose safety and tolerability

101

102

COMPLETED

Inhalation

Single- and multi-ascending-dose safety and tolerability

104

COMPLETED

'Dose-fractionation' study

107

COMPLETED

Phase 2

Intranasal

Intranasal Experimental Infection Study

Model

GEMINI Pilot

Safety and Efficacy Trial

GEMINI

COMPLETED

Inhalation

Multi-dose study In RSV-infected lung transplant pts

106

COMPLETED

Phase 2b study in RSV-infected LT patients

109

ONGOING

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GEMINI Study Design and Outcomes

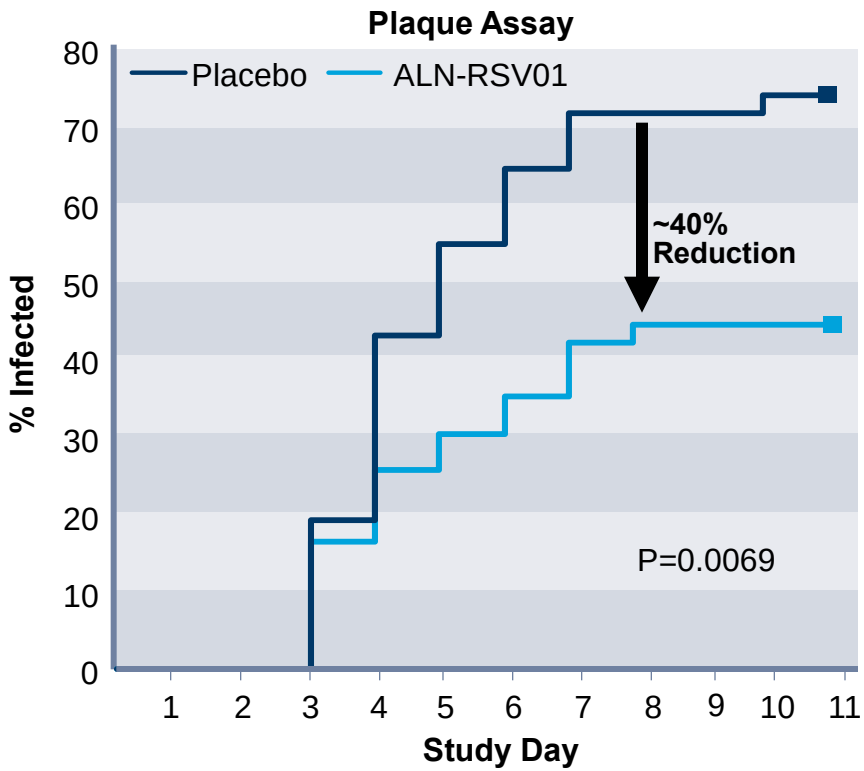
Human POC for RNAi Therapeutics

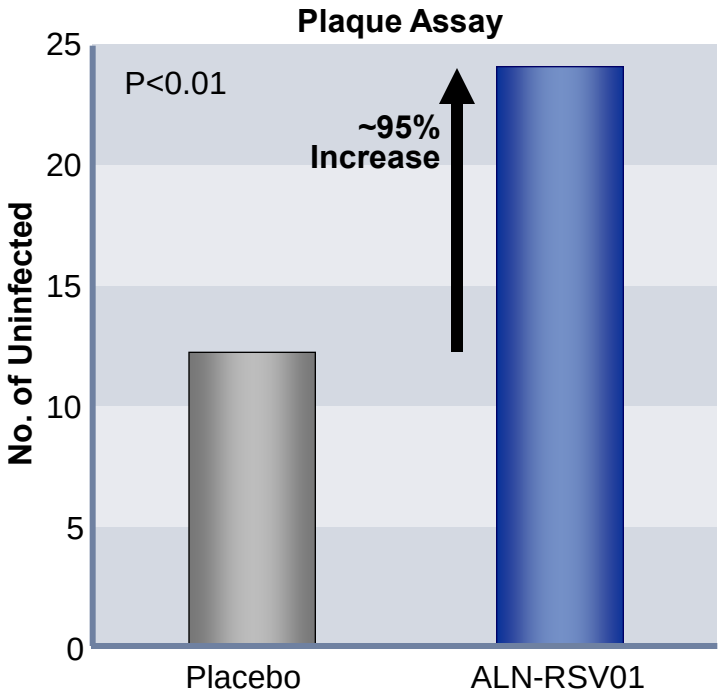
Design of GEMINI Study

Randomized, double-blind, placebo-controlled study (n=88, active:placebo 1:1)

All subjects were dosed with intranasal saline, or ALN-RSV01 (75-150 mg) once daily for 5 days

» RSV (4.7 log PFU) was instilled intranasally between doses 2 and 3





DeVincenzo et al., *PNAS*, 2010

Study 104 Design and Adverse Event Profile

Single Dose

Study Design

Randomized, double-blind, placebo-controlled study (n=109)

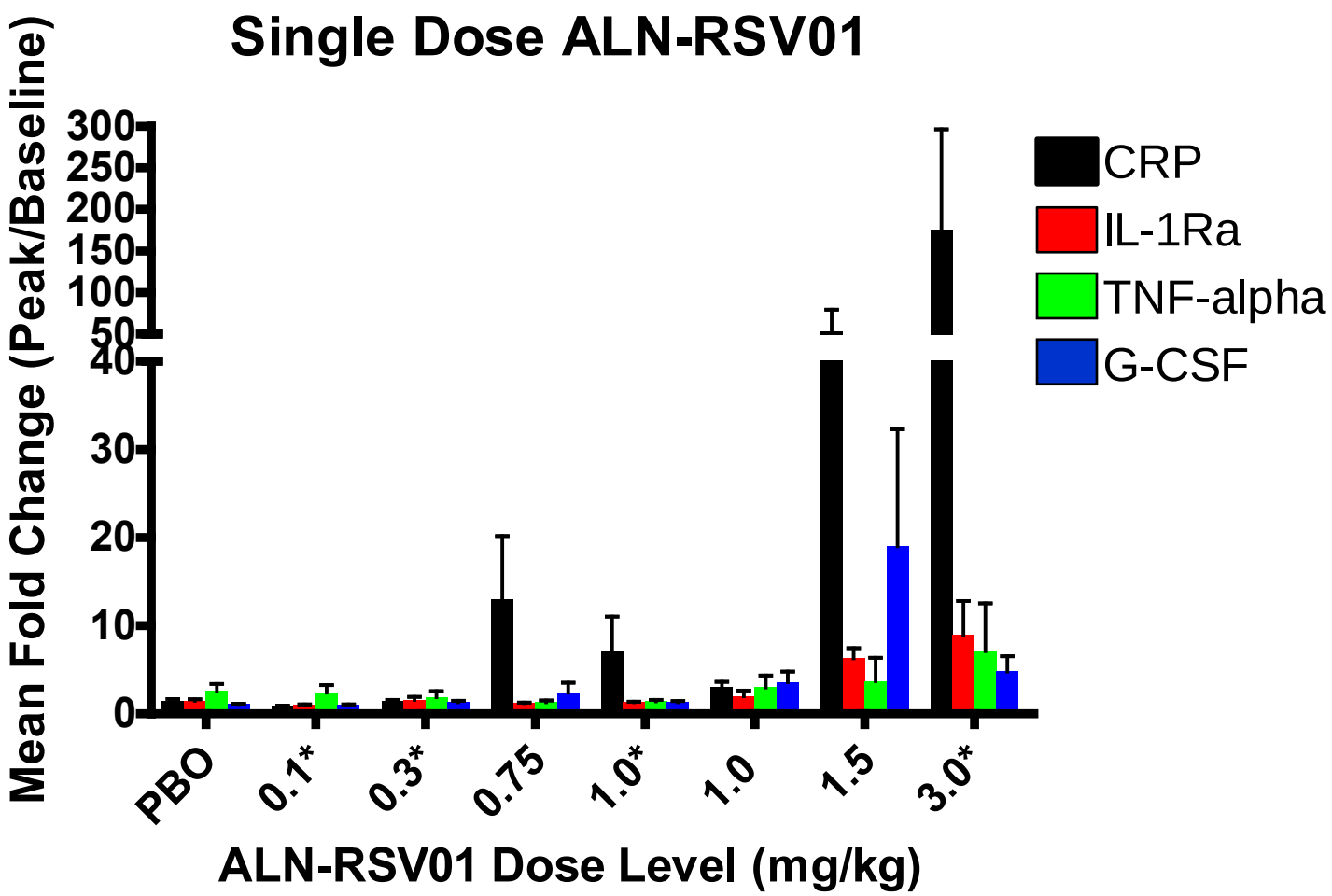
All subjects were dosed with inhalational saline, or ALN-RSV01 (0.1-3 mg/kg) once daily for up to 3 days

Dose (mg/kg)	0.1	0.3	0.75	1	1	1.5	3	All ALN-RSV01 (N=31)	Placebo (N=17)
N	5	5	5	5	5	3	3	31	17
Cough	2 (40)	1 (20)	3 (60)	3 (60)	2 (40)	2 (67)	3 (100)	17 (54.8)	1 (5.9)
Headache	1 (20)	1 (20)	3 (60)	1 (20)	1 (20)	2 (67)	2 (67)	11 (35.5)	2 (11.8)
Neutrophilia	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	3 (100)	3 (100)	7 (22.6)	0 (0)
Non-cardiac chest pain	0 (0)	1 (20)	0 (0)	0 (0)	1 (20)	1 (33)	1 (33)	4 (13)	0 (0)
Pharyngo-laryngeal pain	0 (0)	1 (20)	1 (20)	0 (0)	0 (0)	2 (67)	0 (0)	4 (13)	0 (0)
Chills	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	1 (33)	2 (67)	4 (12.9)	0 (0)

*numbers in parentheses indicate percentages

Alnylam PHARMACEUTICALS

Study 104: CRP and Cytokine Data Across ALN-RSV01 Dose Levels: Single Dose



* converted from mg to mg/kg dosing, based on 70 kg adult mass

Study 104 Design and Adverse Event Profile

Multi-Dose

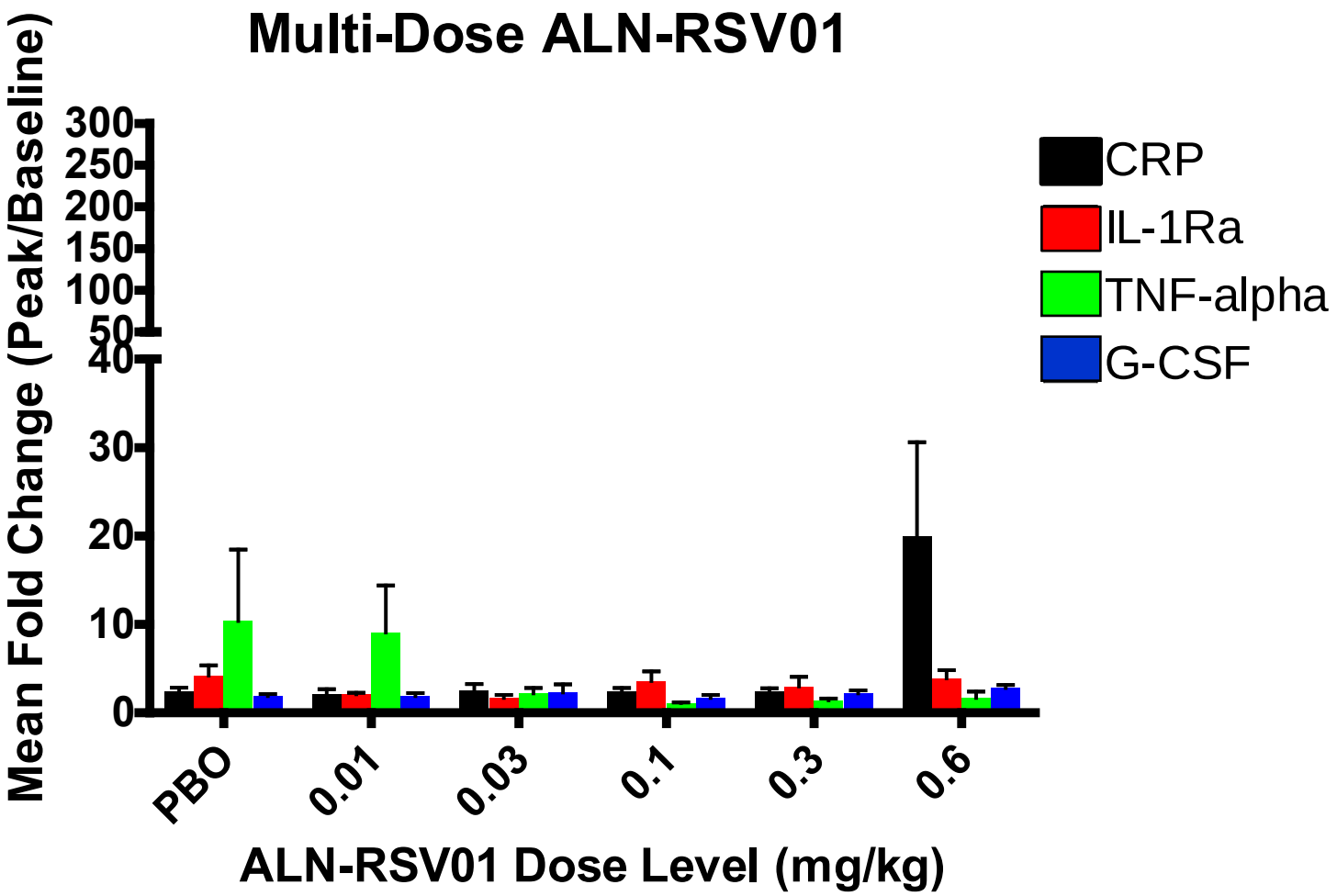
Dose (mg/kg)	0.01	0.03	0.1	0.3	0.6	All ALN-RSV01 (N=40)	Placebo (N=21)
N	8	8	8	8	8	40	21
Cough	2 (25)	2 (25)	4 (50)	1 (12.5)	7 (87.5)	16 (40.0)	4 (19.0)
Headache	0 (0)	2 (25)	2 (25)	0 (0)	1 (12.5)	5 (12.5)	2 (9.6)
Throat irritation	1 (12.5)	1 (12.5)	2 (25)	0 (0)	1 (12.5)	5 (12.5)	0 (0)
Pharyngo-laryngeal pain	2 (25)	0 (0)	1 (12.5)	0 (0)	1 (12.5)	4 (10)	1 (4.8)

*numbers in parentheses indicate percentages

- All AE mild except
  - » ALN-RSV01: one moderate severity episode headache
  - » Placebo: one moderate severity episode each of headache and cough
- AE short-lived, transient
- No serious adverse events

Alnylam PHARMACEUTICALS

Study 104: CRP and Cytokine Data Across ALN-RSV01 Dose Levels: Multi-Dose



ALN-RSV01 Lung Transplant Phase IIa Study

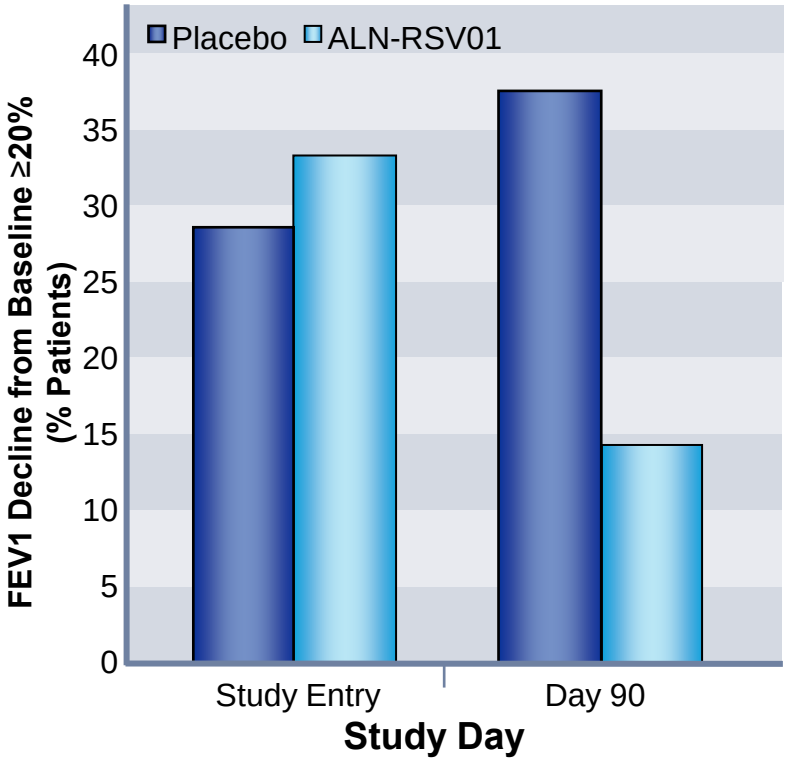
Study 109 Design

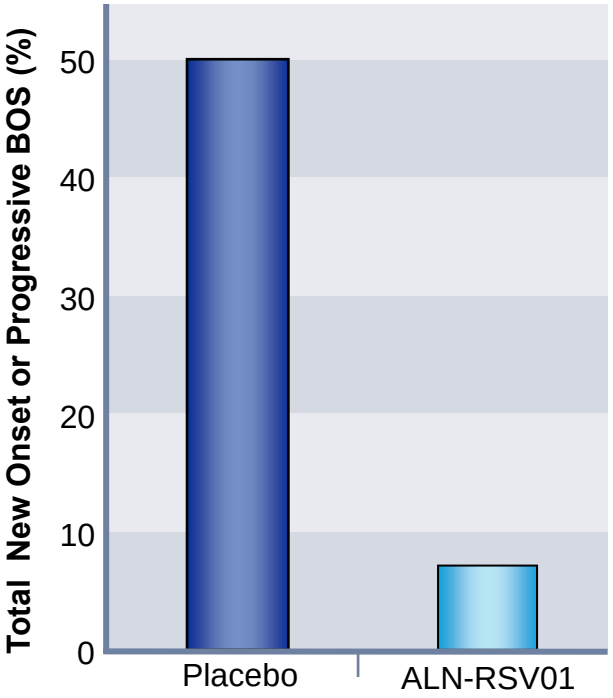
Randomized, double-blind, placebo-controlled study (n=24, active:placebo, 2:1) in RSV-infected lung transplant patients

Patients dosed with inhalational saline, or ALN-RSV01 (0.6 mg/kg) once daily for 3 days

Primary objective: safety, secondary: Day 90 incidence of bronchiolitis obliterans (BOS)

Day 90 Outcomes





**N=14 for ALN-RSV01 due to missed day 90 follow-up in one patient*

Zamora et al, *Am J Resp Crit Care Med*, 2011

Respiratory Treatment Emergent Adverse Events

Adverse Event (MedDRA Term)	ALN-RSV01 (N = 16) n (%)	Placebo (N = 8) n (%)
Nasal congestion	7 (43.8)	1 (12.5)
Cough	5 (31.3)	0
Dyspnoea exertional	4 (25.0)	1 (12.5)
Rhinorrhoea	4 (25.0)	3 (37.5)
Wheezing	4 (25.0)	5 (62.5)
Diarrhoea	4 (25.0)	0
Pharyngolaryngeal pain	3 (18.8)	1 (12.5)
Dyspnoea	2 (12.5)	0
Epistaxis	2 (12.5)	0
Hemoptysis	2 (12.5)	0
Productive cough	2 (12.5)	1 (12.5)
Sneezing	2 (12.5)	0

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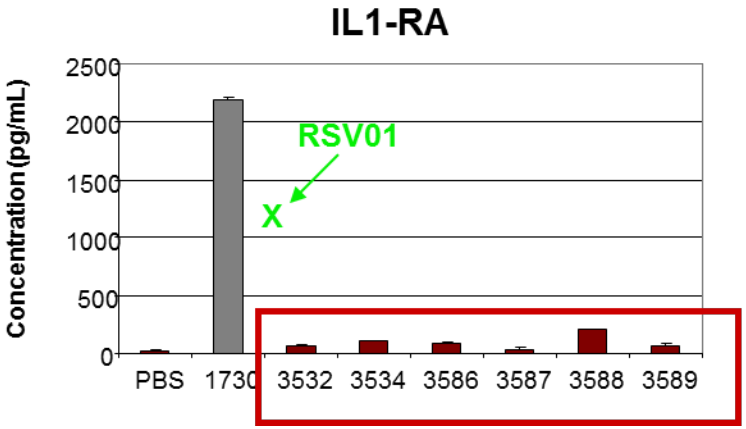
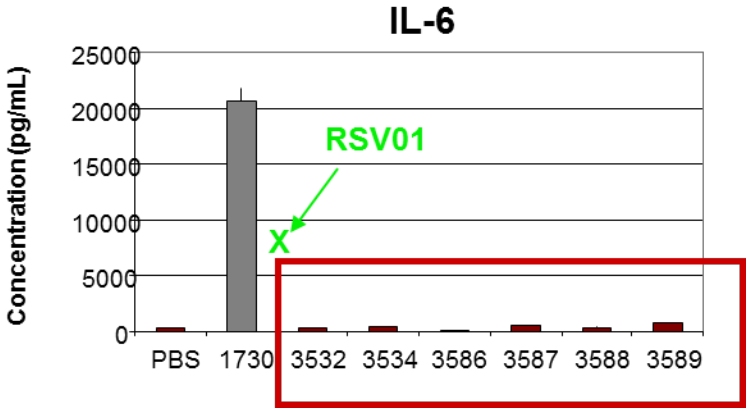
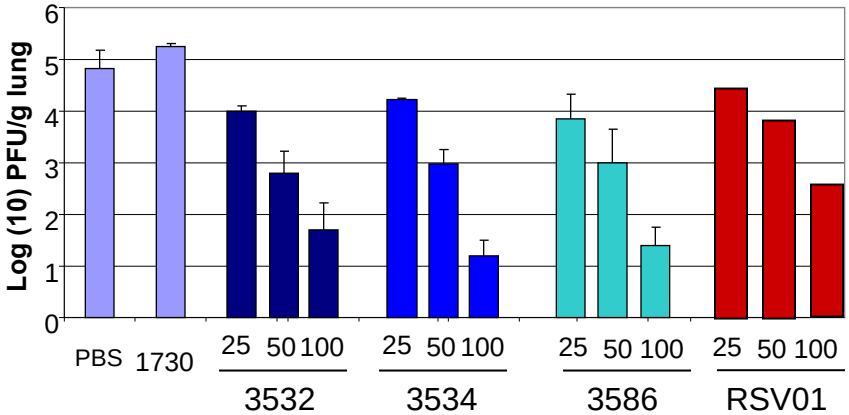
Chemical Modifications Attenuate Cytokine Profile

Cytokine Profiles of Various Chemically Modified siRNA Candidates in NHP BAL





In vivo Activity in Mouse RSV Model



1730 is a mismatch control siRNA which is highly immunostimulatory

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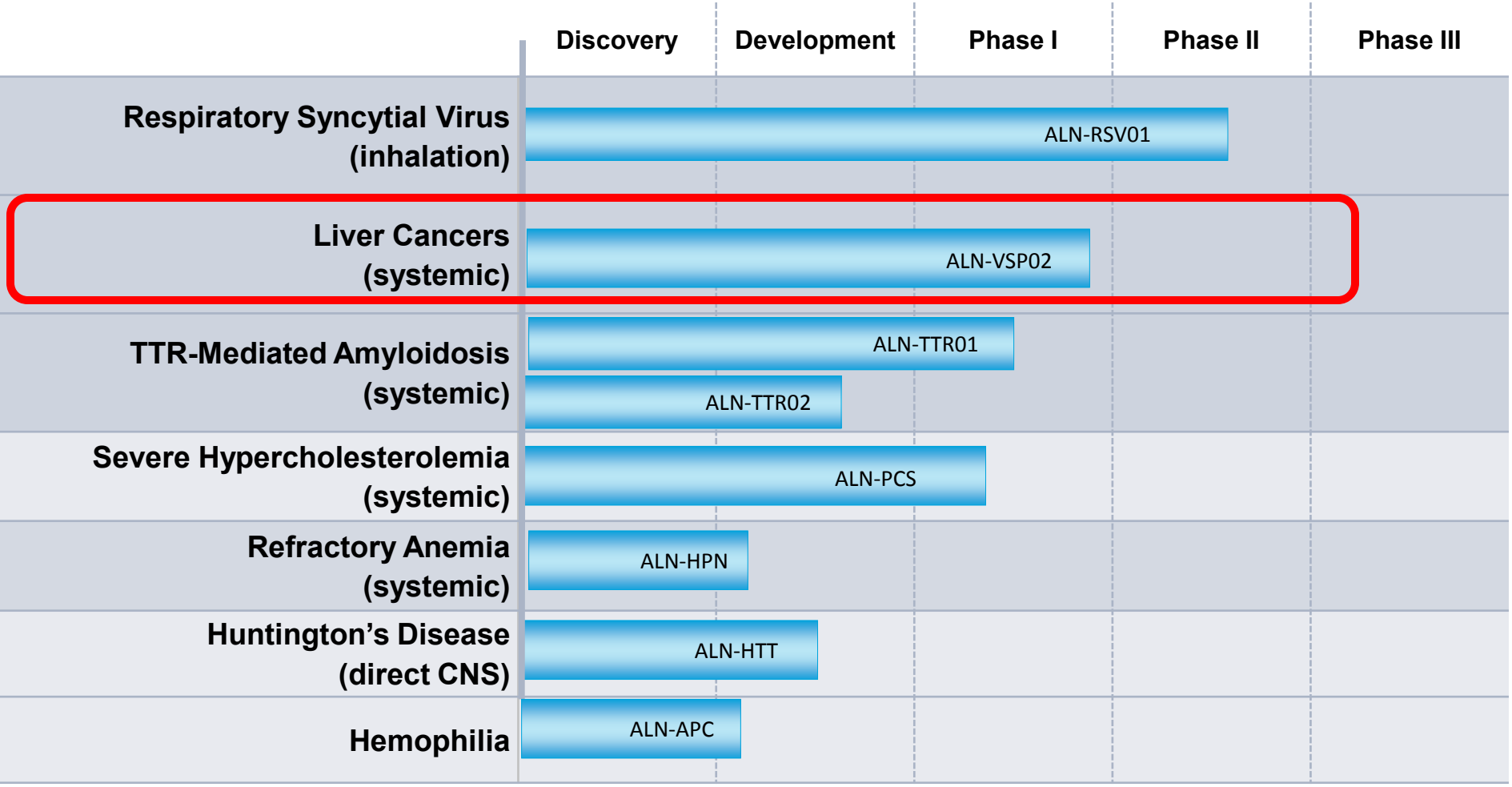
Alnylam Development Pipeline



Alnylam PHARMACEUTICALS

Major GLP Toxicology Studies Supporting ALN-VSP02

GLP Studies

8-Week Repeat-Dose Rat

- NOAEL = 1 mg/kg, based on increases in ALT, AST and histopathology in liver (3 mg/kg - vacuolation: 6/5 mg/kg - hemorrhage, bridging necrosis, fibrosis, mixed cell infiltration)

8-Week Repeat-Dose NHP

- NOAEL = 1 mg/kg, based on histopathology in spleen at 3 mg/kg

Additional studies (non-contributory)

- Rat and NHP safety pharmacology studies
- Genotoxicity

Alnylam PHARMACEUTICALS

ALN-VSP02 Phase 1 Study Design

Open label, multi-center, dose escalation

Up to ~68 patients with primary or secondary liver cancer

Primary objective

- Safety and tolerability in patients with advanced solid tumors with liver involvement

Secondary objectives

- Characterize PK
- Assess preliminary evidence of antitumor/antiangiogenic activity
  - Liver (tumor) biopsies
  - DCE-MRI scans

Treatment regimen

- ALN-VSP02 by 15 minute IV infusion every 2 weeks
- Dose levels: 0.1, 0.2, 0.4, 0.7, 1.0, 1.25, 1.5, 1.7 mg/kg

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Tumor Response Summary

Dose Level (mg/kg)	N (evaluable for response)	Avg # of Doses Received (range)	# Pts with Stable Disease or Better for ≥ 2 mos	# Pts Who Went on to Extension Study (>8 doses) to Date
0.10	3	3 (2-4)	0	0
0.20	3	4 (4-4)	0	0
0.40	7*	4.6 (2-11)	1	1
0.70	5	9.6 (3-23)	3 (includes 1 PR with ~70% tumor reduction)	2
1.00	11†	6.7 (2-12)	7	4
1.25	7	2.4 (1-6)	2	0
1.50	1	4	0	0

PR: partial response
*Includes 1 patient whose first dose was given at 0.7 mg/kg
†Includes 2 patients whose first dose was given at 1.25 mg/kg

Stable disease or better in:

1/13 pts (8%) at ≤ 0.4 mg/kg
12/24 pts (50%) at ≥ 0.7 mg/kg

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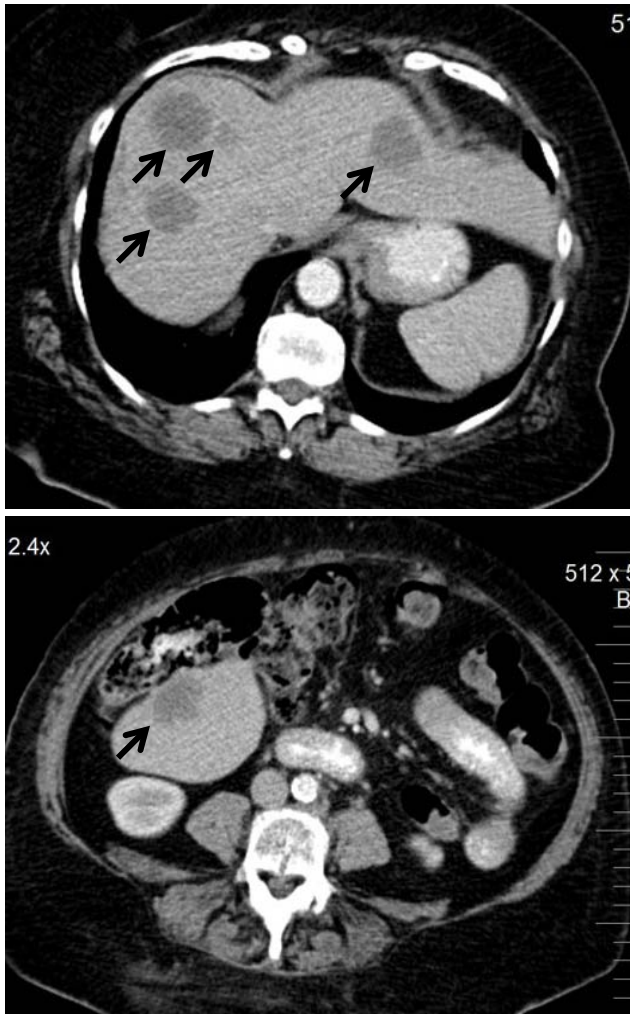
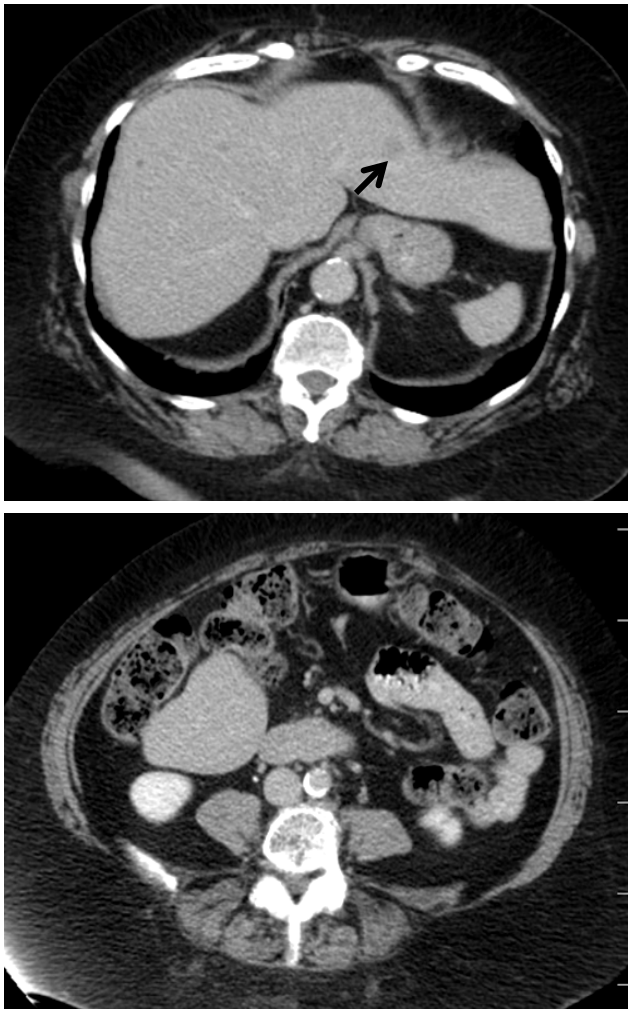
Major Response in Endometrial Cancer: Patient 021

70% Regression of Liver Metastases

Pre-Treatment



After 12 Doses ALN-VSP02*



*Response ongoing after 24 doses

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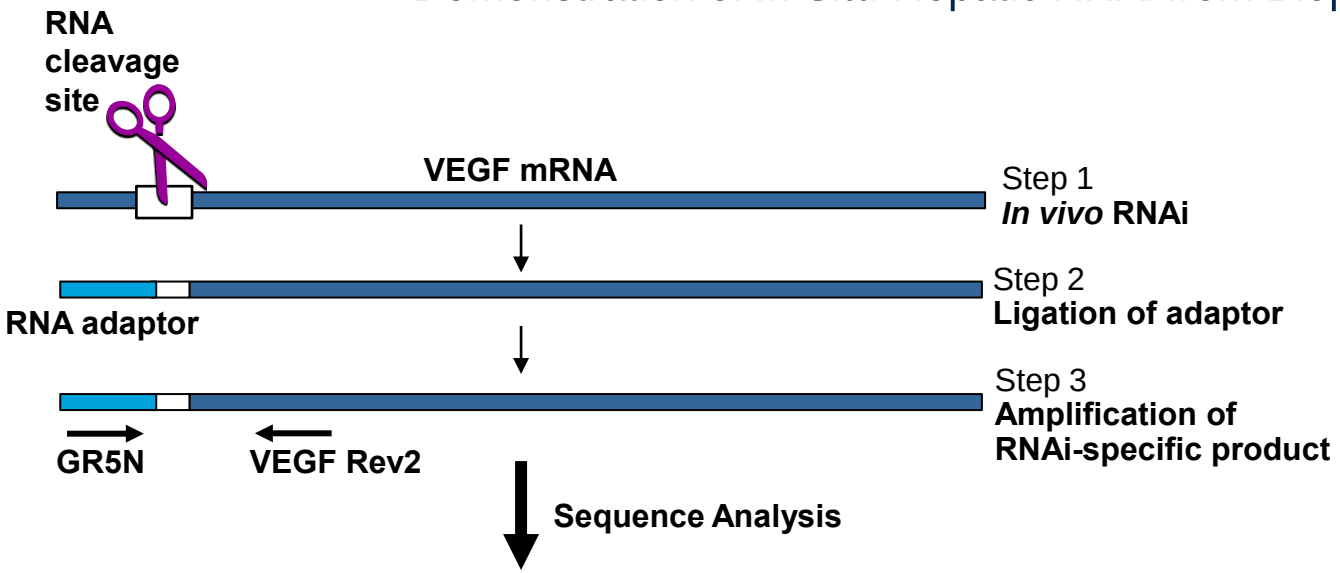
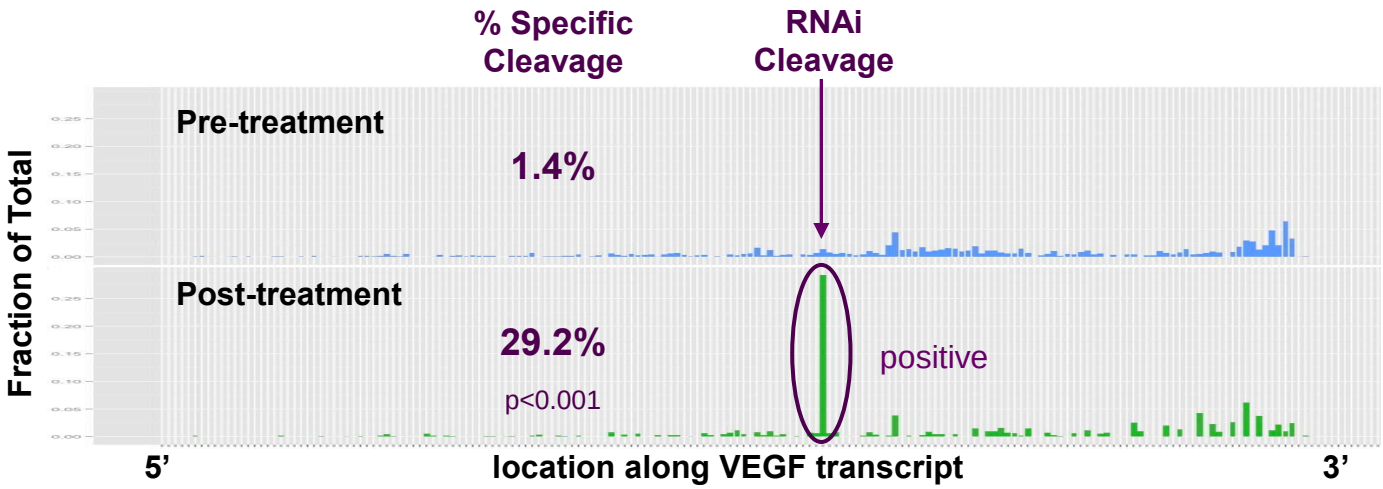
VEGF 5' RACE Assay

Demonstration of *In Situ* Hepatic RNAi from Biopsy Samples





Illustrative Example: Patient 016

Alnylam PHARMACEUTICALS

ALN-VSP02 Phase 1 Study

Adverse Events

Toxicity**	% of Pts (n=41)	ALN-VSP02 Dose Level (mg/kg)						
		0.1 (n=3)	0.2 (n=3)	0.4 (n=6)	0.7 (n=7†)	1.0 (n=9)	1.25 (n=11‡)	1.5 (n=2)
Fatigue	24%	1, 0, 0, 0	0, 0, 0, 0	0, 2, 0, 0	2, 1, 0, 0	1, 0, 0, 0	1, 1, 0, 0	1, 0, 0, 0
Nausea	17%	1, 0, 0, 0	0, 1, 0, 0	1, 1, 0, 0	1, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0
Fever	15%	0, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0	2, 0, 0, 0	2, 0, 0, 0	0, 0, 0, 0
Infusion-Related Reaction	15%	0, 0, 0, 0	0, 0, 0, 0	0, 1, 0, 0	0, 1, 0, 0	0, 3, 0, 0	0, 1, 0, 0	0, 0, 0, 0
Vomiting	12%	2, 0, 0, 0	1, 1, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0
AST Elevation	12%	0, 1, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 1*, 0	2, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0
Rash/Flushing	12%	1, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0
Thrombocytopenia	12%	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 1*, 0	2, 0, 0, 0	0, 0, 2, 0	0, 0, 0, 0
Chills/Rigors	10%	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	1, 2, 0, 0	0, 0, 0, 0
Diarrhea	7%	0, 0, 0, 0	0, 1, 0, 0	1, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
Abdominal Pain	7%	1, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0	0, 1, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
Headache	7%	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0
Hypertension	5%	0, 0, 0, 0	0, 0, 0, 0	0, 1, 0, 0	0, 1, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
ALT Elevation	5%	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 1*, 0, 0	1, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
Dizziness	5%	1, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0
Anorexia	5%	1, 0, 0, 0	0, 0, 0, 0	1, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0

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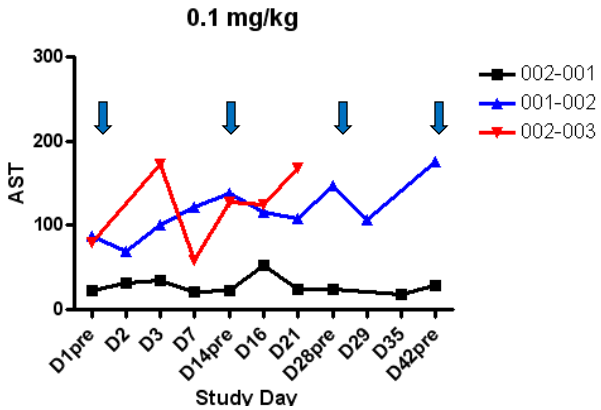
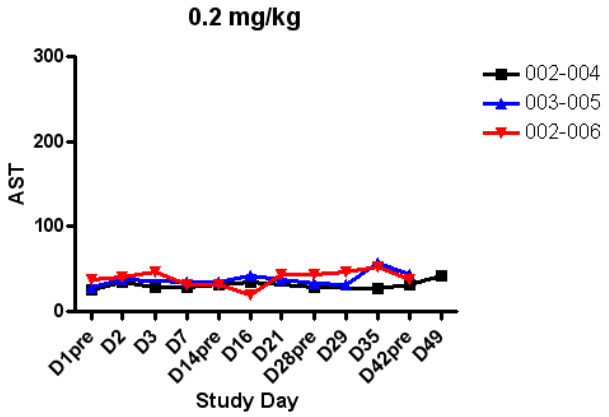
ALN-VSP02 Phase 1 Study

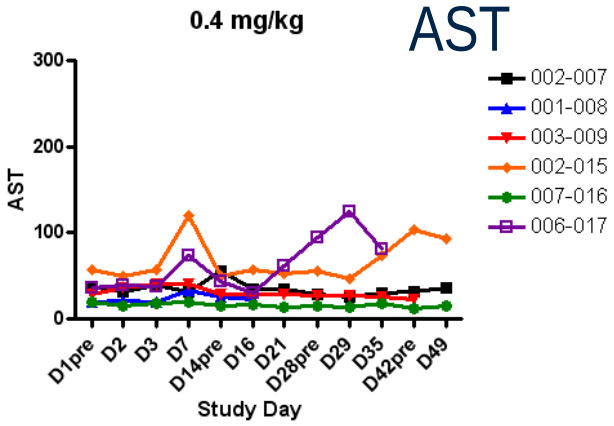
AST

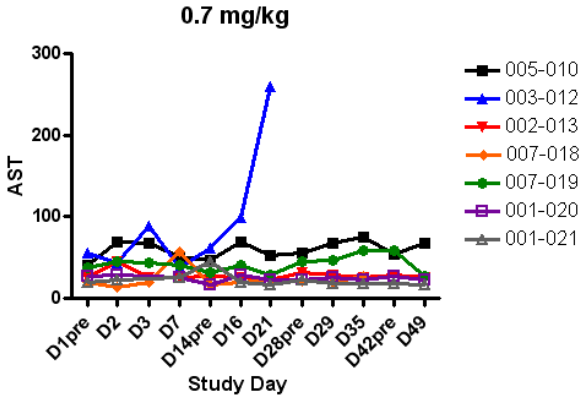
ALN-VSP02 dosing

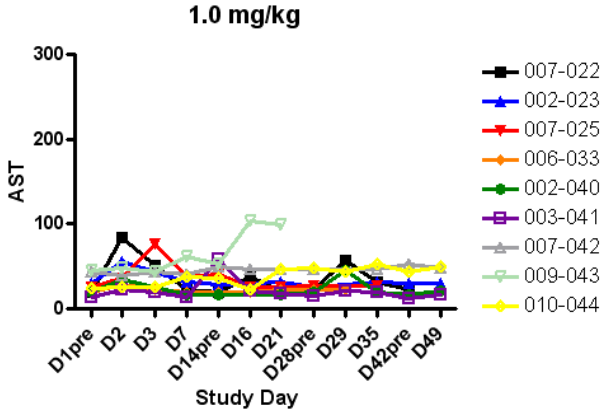
Pre: Prior to dosing

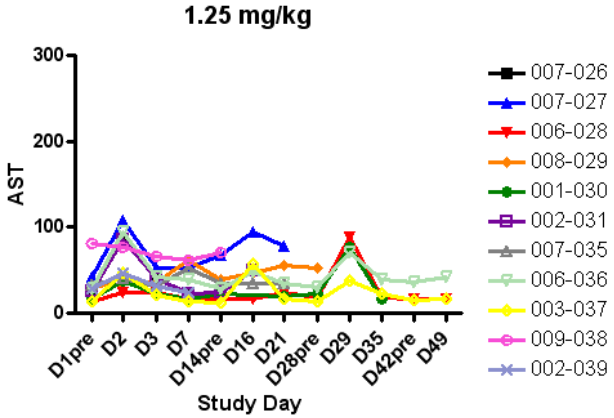


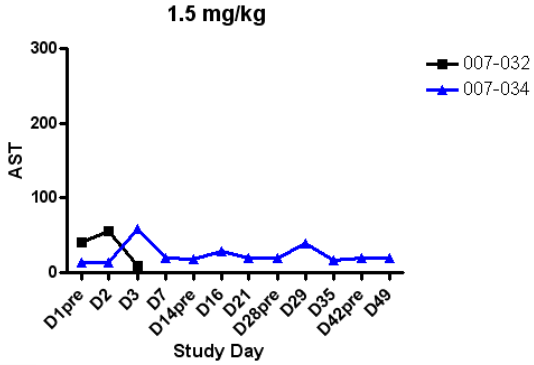












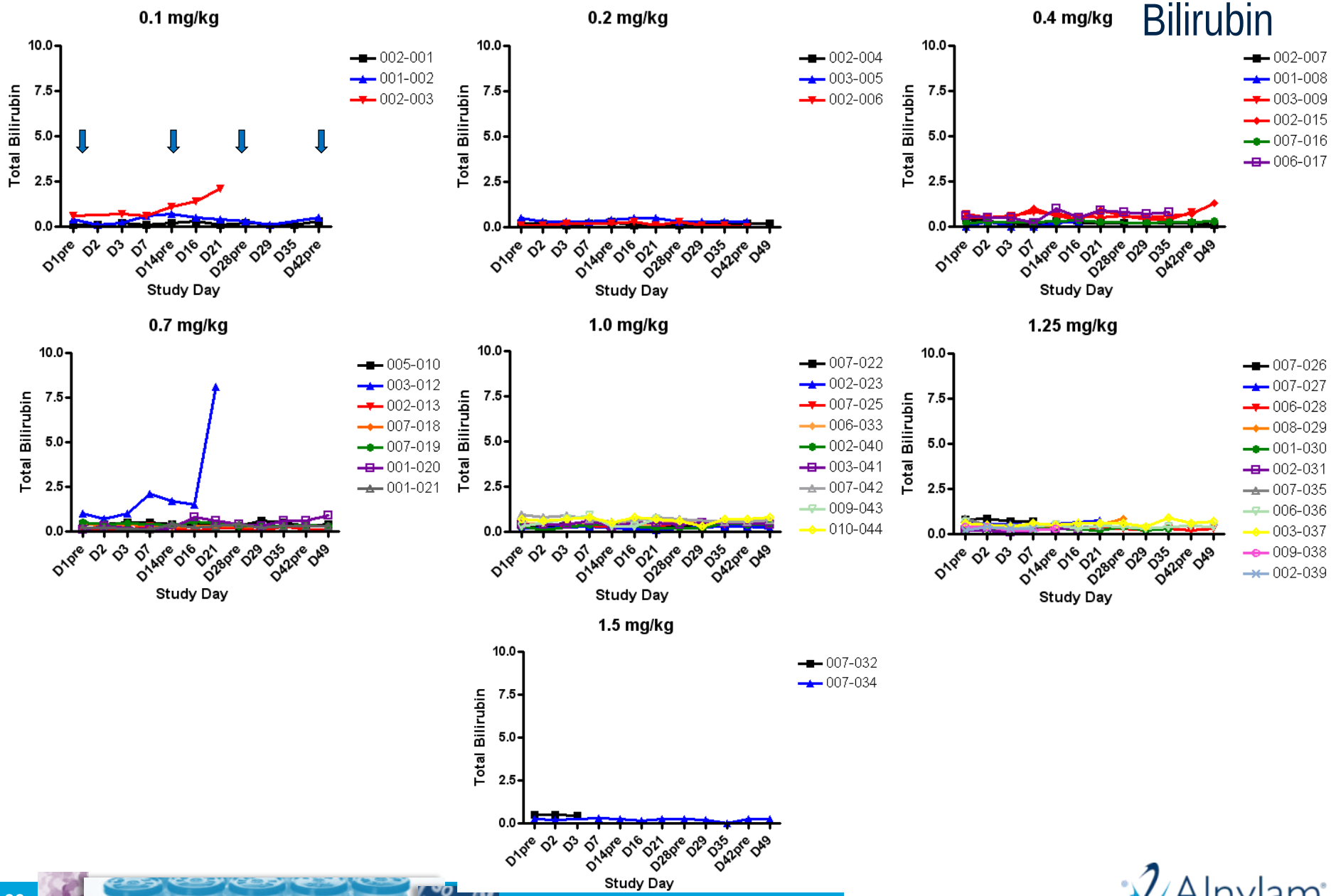
Alnylam PHARMACEUTICALS

ALN-VSP02 dosing

Pre: Prior to dosing

ALN-VSP02 Phase 1 Study

Bilirubin



Alnylam PHARMACEUTICALS

ALN-VSP02 Phase 1 Study

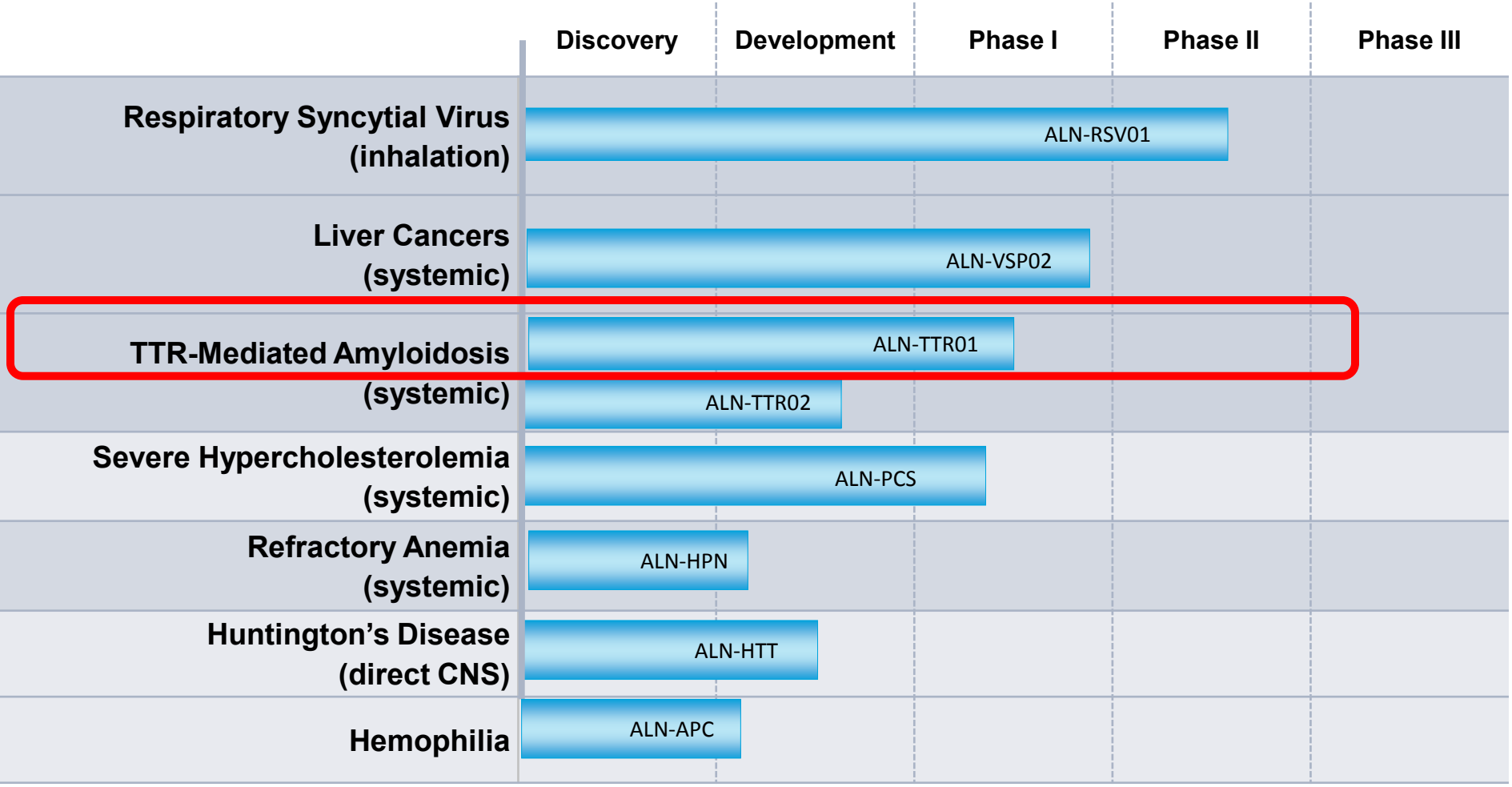
Serious Adverse Events

Patient #	Tumor Type	Dose Level (mg/kg)	Event(s)	Relatedness
003-012	Pancreatic neuroendocrine	0.7	1. Liver failure 2. Hepatic encephalopathy Both arising 5 days post-dose 2, resulting in death	Possibly related
001-021	Endometrial	0.7	1. Elevated WBC 2. Fever Both occurred post-dose 23. Hospitalized to rule out infection and then resumed dosing on study.	Possibly related
001-030	Endometrial	1.25	Elevated WBC post-dose 3. Hospitalized to rule out infection and then resumed dosing on study.	Possibly related

5 possibly related SAEs in 3 patients
23 unrelated SAEs in 10 patients
No ALN-VSP02 related discontinuations

Alnylam PHARMACEUTICALS

Alnylam Development Pipeline



Alnylam PHARMACEUTICALS

Transthyretin (TTR)-Mediated Amyloidosis (ATTR)

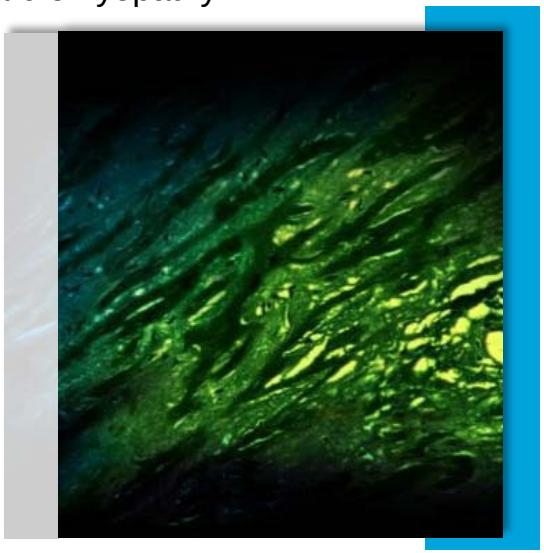
ALN-TTR01 Program

RNAi to treat genetic disease

ATTR is significant orphan disease
- » ~50,000 patients worldwide

Clinical pathology
- » Onset ~40 to >60 yr
- » Two predominant forms
  - – Familial amyloidotic polyneuropathy (FAP)
  - – Familial amyloidotic cardiomyopathy (FAC)
- » Peripheral sensorimotor neuropathy, autonomic neuropathy, and/or cardiomyopathy
- » Fatal within 5-15 years

Liver transplant current standard of care
- » <3,000 Patients eligible



First RNAi drug for ATTR

Targets wild-type and all mutant forms of TTR

Lead siRNA candidate selected (formulated in SNALP)

Potent, in vitro IC50 = 3 pM

Highly specific for TTR
- »Both wild-type and mutant TTR

Non-immunostimulatory

IND-enabling safety package similar to ALN-VSP02 (single dose studies, similar findings)

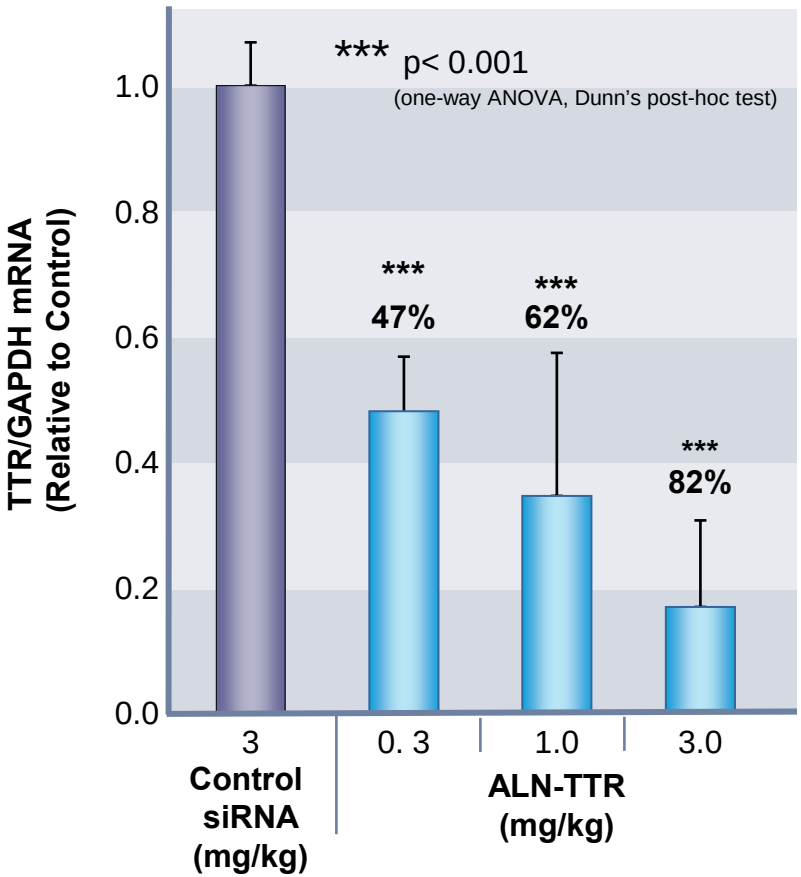
Alnylam PHARMACEUTICALS

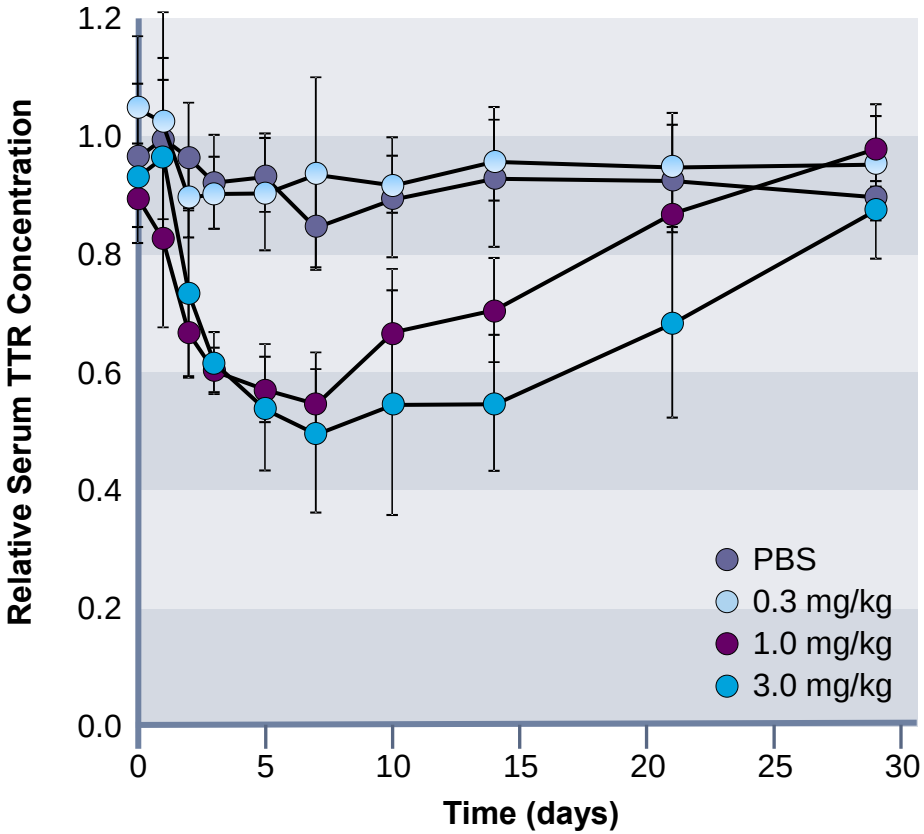
ALN-TTR01 Activity
Non-Human Primates

ALN-TTR01 shows dose-dependent silencing of TTR mRNA and protein

Single i.v. infusion of ALN-TTR01 or control siRNA

Liver mRNA levels measured 48hr post-dose, serum protein levels measured over 28d post-dose





Keystone RNAi, Feb 2009

ALN-TTR01 Phase I Study Design

Study Design

Randomized, placebo-controlled, single-blind, single-dose escalation study

- »3:1 randomization
- »4 patients/cohort

Up to 36 patients with ATTR

- »Conducted in Portugal (T. Coelho, A. Silva), Sweden (O. Suhr), France (D. Adams, P. Lozeron) and UK (T. Mant, P. Hawkins)

Primary Objective

Evaluate safety and tolerability of ALN-TTR01

Secondary Objective

Characterize plasma PK

Assess preliminary pharmacodynamics and clinical activity

- »Serum TTR, RBP, Vitamin A levels

Study Status

Dose escalation completed; accruing additional patients at top dose

Alnylam PHARMACEUTICALS

ALN-TTR01 Phase I Study

Adverse Events

	ALN-TTR01 Dose Level (mg/kg)							All ALN-TTR01 (n=23)	Placebo** (n=8)
	0.01 (n=3)	0.03 (n=3)	0.10 (n=3)	0.20 (n=3)	0.40 (n=3)	0.70 (n=3)	1.00 (n=5)		
TEAE*	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Infusion-Related Reaction	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1(33.3)	1(33.3)	1 (20.0)	3 (13.0)	0 (0.0)
Fatigue	1 (33.3)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.7)	0 (0.0)
Headache	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)	1 (12.5)
Constipation	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)
Nausea	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)
1st Degree A-V Block	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)	0 (0.0)

No SAEs related to study drug administration

**Includes TEAEs probably or possibly related to study drug: all mild-moderate in severity. No significant increases in liver function tests (LFTs).*

*** One placebo patient underwent elective hospitalization for liver transplant, scored as SAE unrelated to study drug.*

Preliminary, Study Ongoing

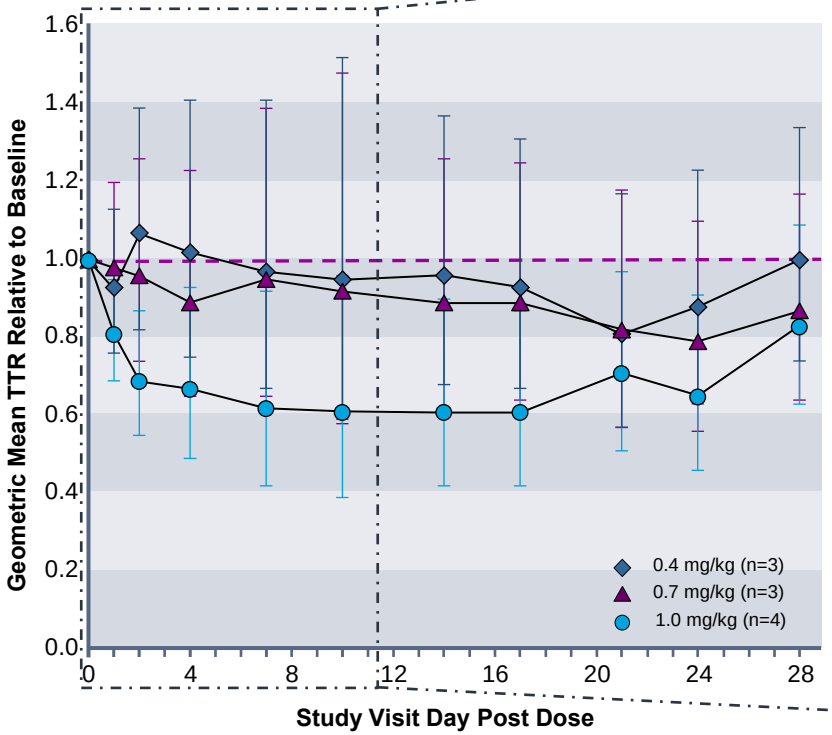
Alnylam PHARMACEUTICALS

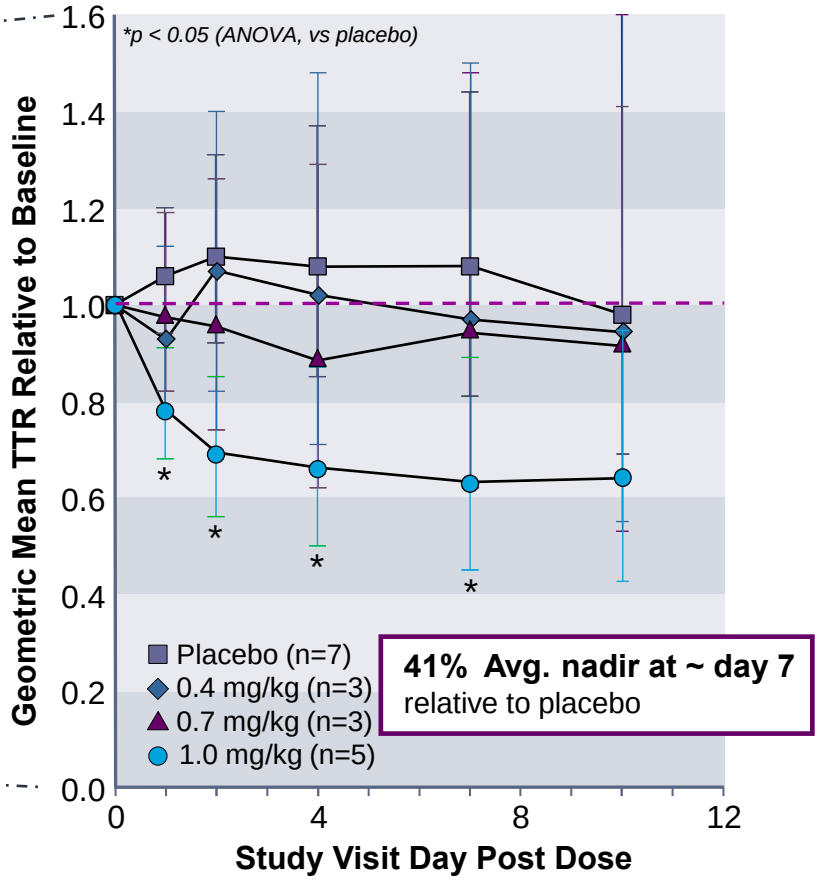
ALN-TTR01 Phase I Study

Dose-Dependent TTR Lowering at 0.4 to 1.0 mg/kg

ALN-TTR01 single dose results in rapid, dose-dependent, and durable lowering of serum TTR protein levels

- All patients show TTR lowering at 1.0 mg/kg, ranging 25-81%
- Average nadir at approximately Day 7 of 41% relative to placebo (geometric mean vs. placebo, p=0.02)



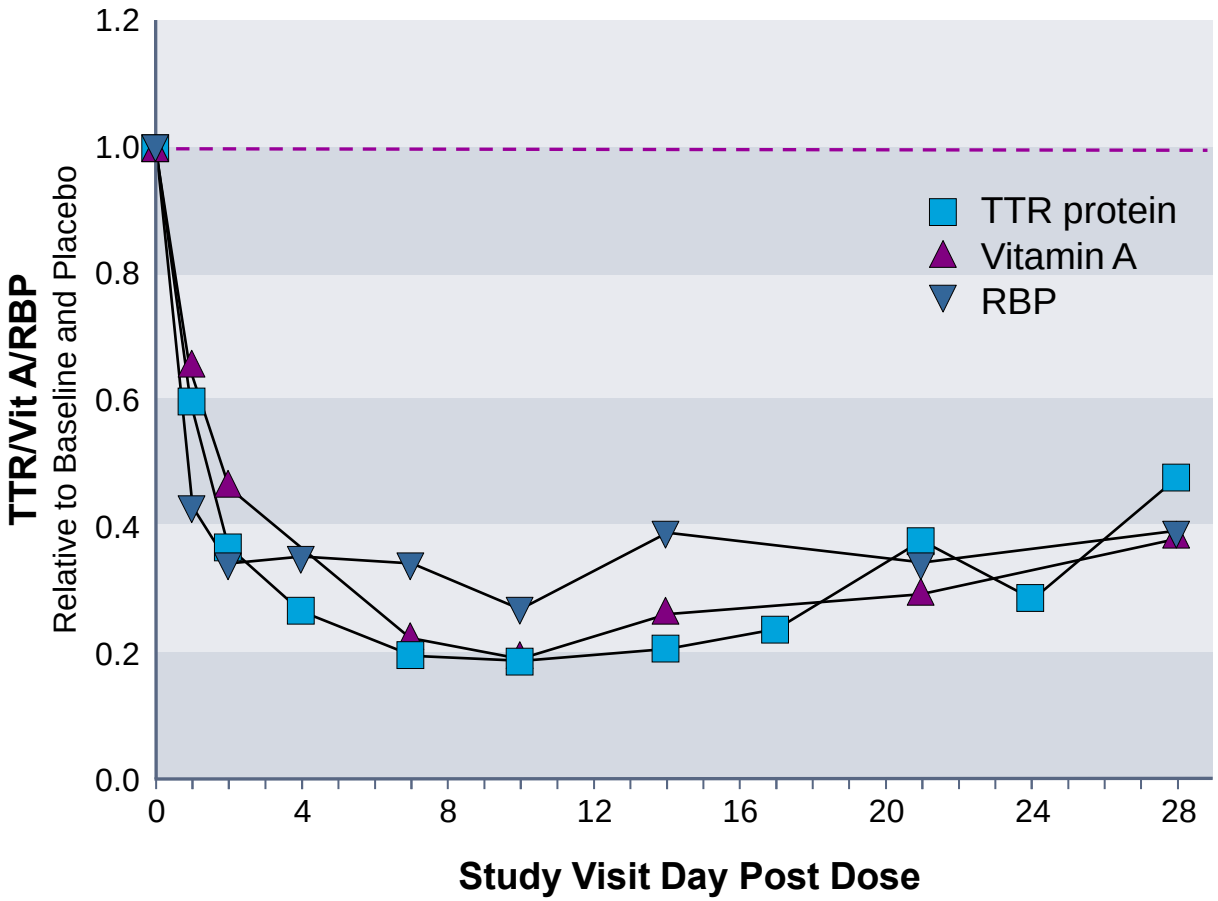


Preliminary, Study Ongoing

Alnylam PHARMACEUTICALS

ALN-TTR01 Phase I Study

Robust RNAi in Patient at 1 mg/kg ALN-TTR01



Single 1 mg/kg dose lowers serum TTR protein, RBP and vitamin A levels

TTR protein reduced by 81% at nadir

Rapid onset of effect with >50% lowering at day 2

Nadir at ~day 7

Durable effect with ~50% lowering at day 28

Preliminary, Study Ongoing

Alnylam PHARMACEUTICALS

Discussion Outline

Introduction and Background

Overview of Alnylam Lead Discovery Paradigm

Alnylam Pipeline

Program Review

- »ALN-RSV01
- »ALN-VSP02
- »ALN-TTR01

Summary and Conclusions

Alnylam PHARMACEUTICALS

Alnylam RNAi Experience
Summary and Conclusions

The global RNAi pipeline shows a significant and growing experience with siRNA-based therapeutics

Alnylam has a multi-product pipeline and a growing database working in consensus with US and ex-US regulatory authorities

ALN-RSV01

- >0.6 mg/kg, mild, transient flu-like syndrome associated with reversible cytokine effects
- Second generation compound shows cytokine attenuation based on chemical modification
- Human proof of concept established via intranasal, and inhalational route at doses up to 0.6 mg/kg

ALN-VSP02

- Safety encouraging with multi-dosing for up to 18 months
- Liver signal from rat ***NOT*** realized in human experience to date
- Data suggest anti-tumor response across multiple tumor types at doses ≥0.7 mg/kg
- Biopsies showed *in situ* RNAi

ALN-TTR01

- Excellent safety profile in ongoing Phase 1 study
- Clear evidence of dose-dependent, reversible TTR knockdown (25-81%, mean ~40%) without any evidence of hepatotoxicity

Alnylam PHARMACEUTICALS