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Familial Support Group Meeting

Overview of ALN-TTR, a Novel RNAi Therapeutic for TTR Amyloidosis

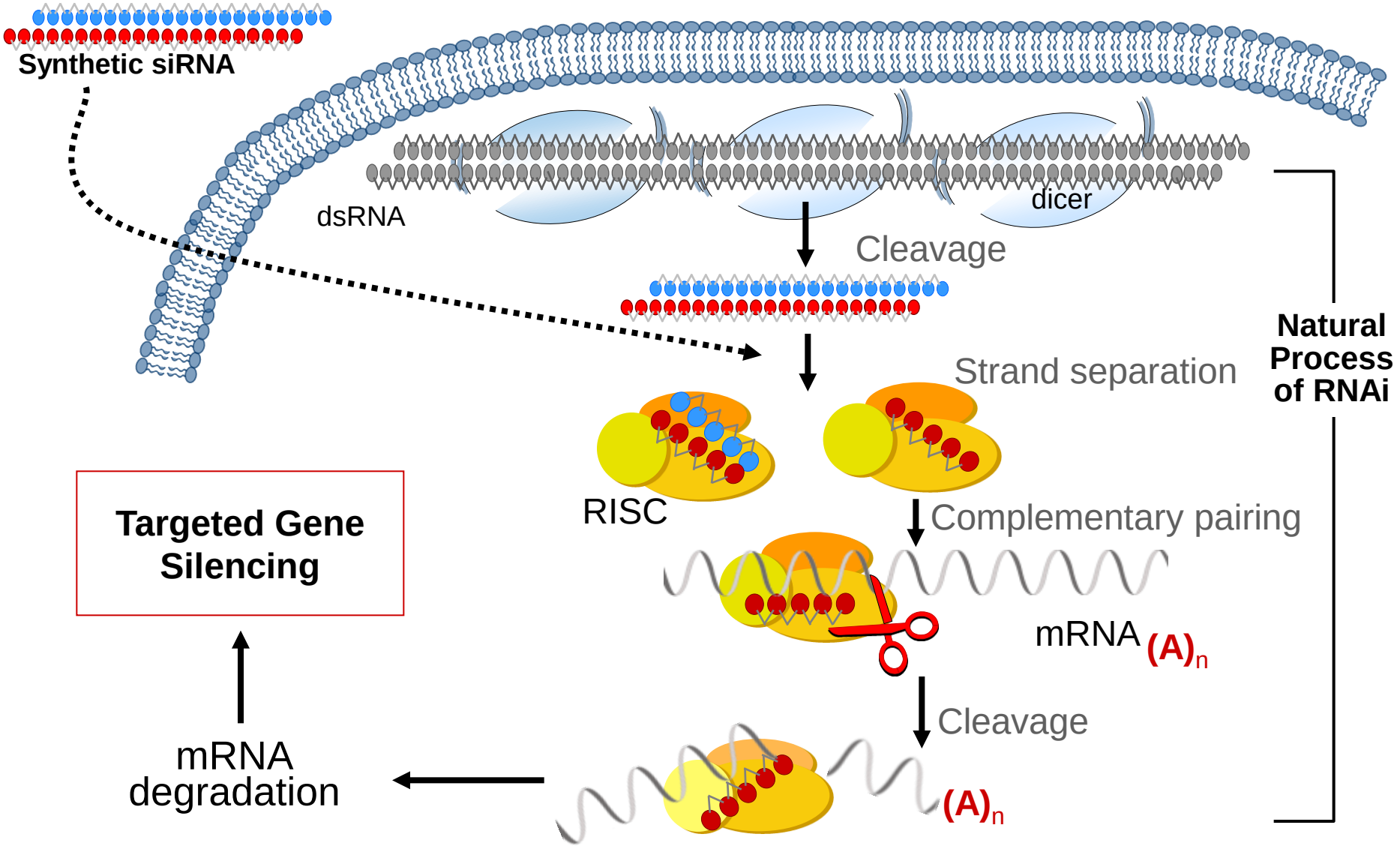
October 29, 2011

Jared Gollob, M.D.

Agenda

- Background on RNAi and TTR Amyloidosis
- ALN-TTR Program: Preclinical Data
- Natural History Study of Serum TTR Levels
- ALN-TTR01 Phase 1 Study Design and Status
- Summary and Next Steps

RNAi Cellular Mechanism



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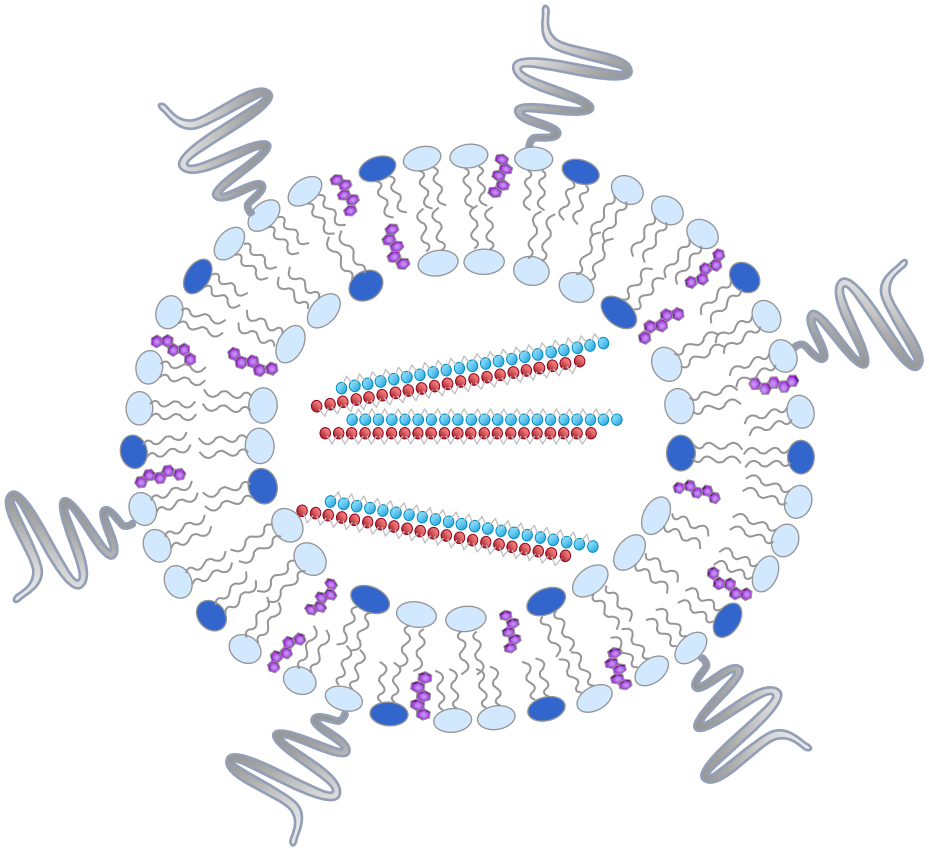
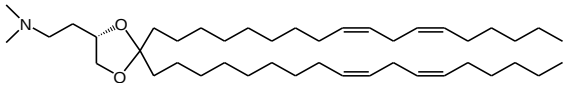
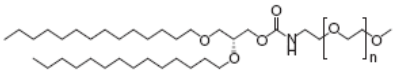
Lipid Nanoparticles (LNPs) for Systemic RNAi



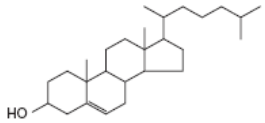
- Multi-component lipid formulation
  - » Amino lipid
  - » Structural lipid
  - » PEG lipid
  - » Cholesterol
- Highly efficient for liver delivery
  - » Hepatocyte-specific gene silencing achieved



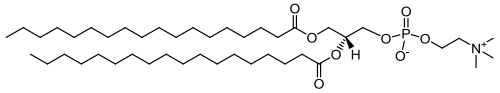
DLin-KC2-DMA



$mPEG_{2000}$-C14 glyceride



Cholesterol



DSPC

- Low surface charge
- Small uniform size particle <100 nm

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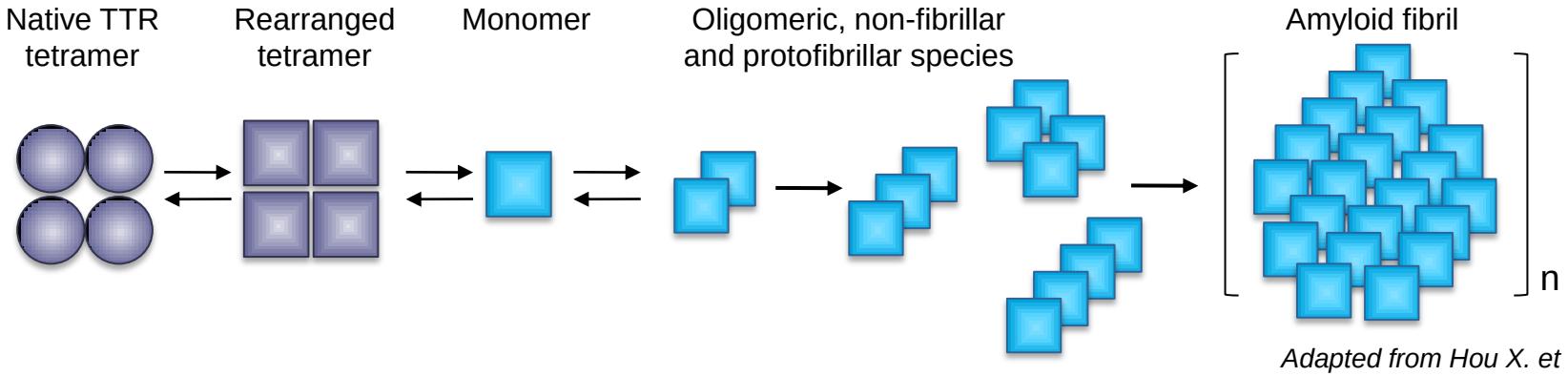
TTR Biology



Adapted from Hou X. et al., 2007

- TTR synthesized predominantly in liver, circulates as ~55 KDa tetramer
- Binds and transports serum retinol binding protein (RBP)/vitamin A and minor fraction of serum thyroxine (T4)
- Knock-out mice have mild peripheral phenotype
  - » Reduced levels of serum retinol, RBP, thyroid hormone without significant physiological effects
    - – Normal vitamin A metabolism, total liver retinol unchanged (Wei *et al*., 1995)
    - – Thyroid function normal (Palha *et al.,* 1994)
  - » Modest sensorimotor changes may represent developmental effect (Fleming *et al.,* 2007)

Transthyretin (TTR) Amyloidosis

- Autosomal dominant disease
- Deposition of mutant and wild-type TTR in tissues outside of liver
  - » Nerves and/or heart are main target organs depending on mutation
- Familial amyloidotic polyneuropathy (FAP)
  - » Prevalence of ~10,000 cases worldwide
  - » V30M most common mutation
- Familial amyloidotic cardiomyopathy (FAC)
  - » Prevalence of ~40,000 cases worldwide
  - » V122I most common mutation
- Liver transplant current standard of care for early stage V30M FAP
- Drugs in development
  - » TTR tetramer stabilizers (tafamidis, diflunisal)
  - » Agents targeting hepatic TTR synthesis (siRNA, ASO)

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ALN-TTR Summary

Molecule	Anti-TTR siRNA, modified duplex
Formulation and Dosing	Formulated in lipid nanoparticle
Molecular Hypothesis	An RNAi therapeutic targeting TTR gene will inhibit mutant and wild type TTR production in the liver
Therapeutic Hypothesis	Decreasing hepatic production of mutant and wild type TTR will decrease amyloid deposition in tissues and facilitate amyloid clearance from tissues, thereby halting progression or improving end-organ dysfunction
Potential Indications	Familial amyloidotic polyneuropathy (FAP) Familial cardiac amyloidosis (FAC)
Stage Status	ALN-TTR01 Phase I trial initiated in July 2010; Advancing ALN-TTR02 with 2nd generation LNP

ALN-TTR siRNA Selection

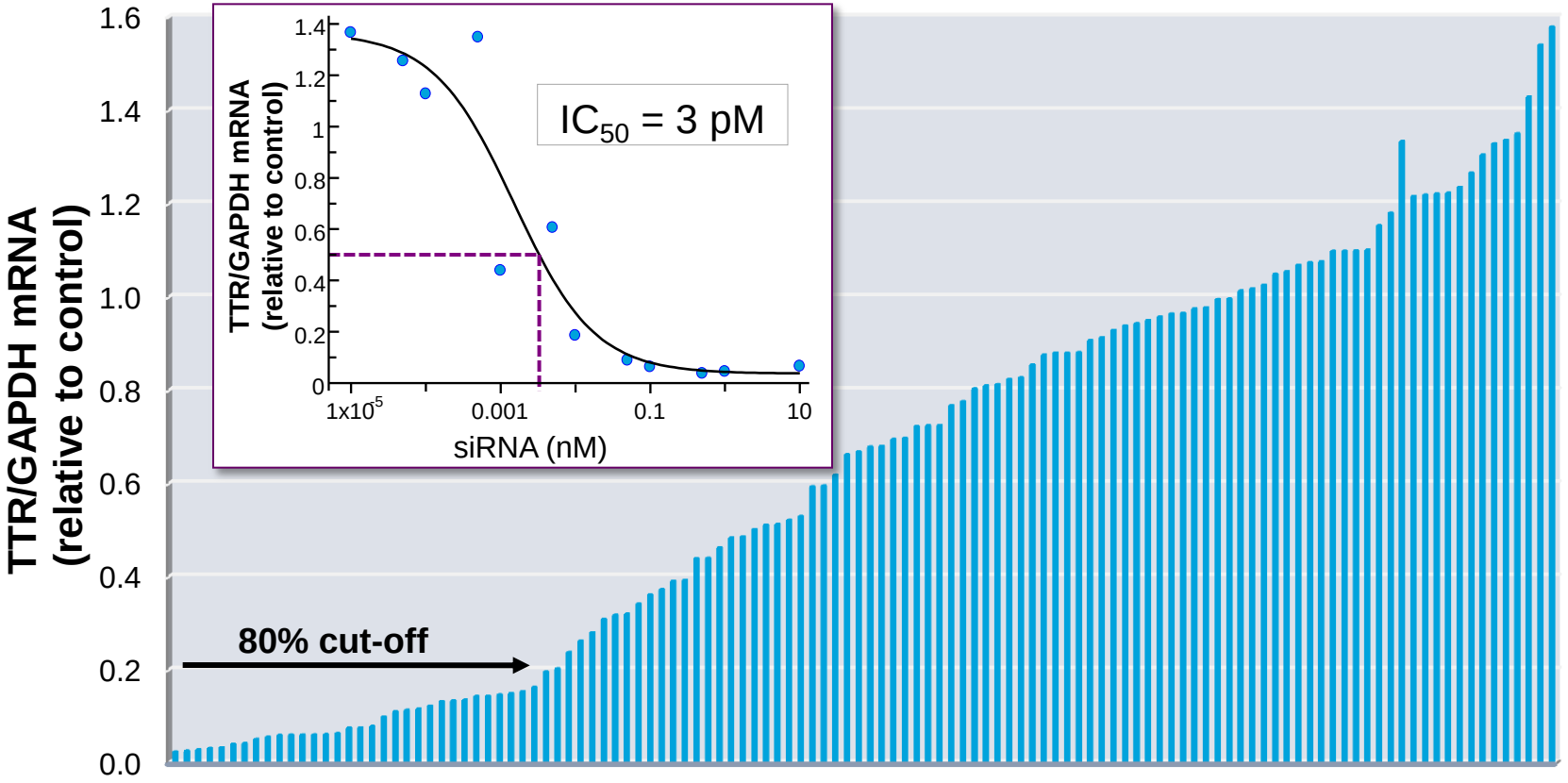
- >100 Mutations identified in TTR gene
- ALN-TTR targets region of TTR mRNA common to wild-type and all known mutant forms of TTR

ALN-TTR siRNA Lead Selection

In Vitro Screen

~140 siRNAs screened to identify lead candidate

- Assays performed in HepG2 cells
- TTR mRNA quantified by RT-PCR 24hr post-transfection



AALSD: Liver Meeting, Nov 2009

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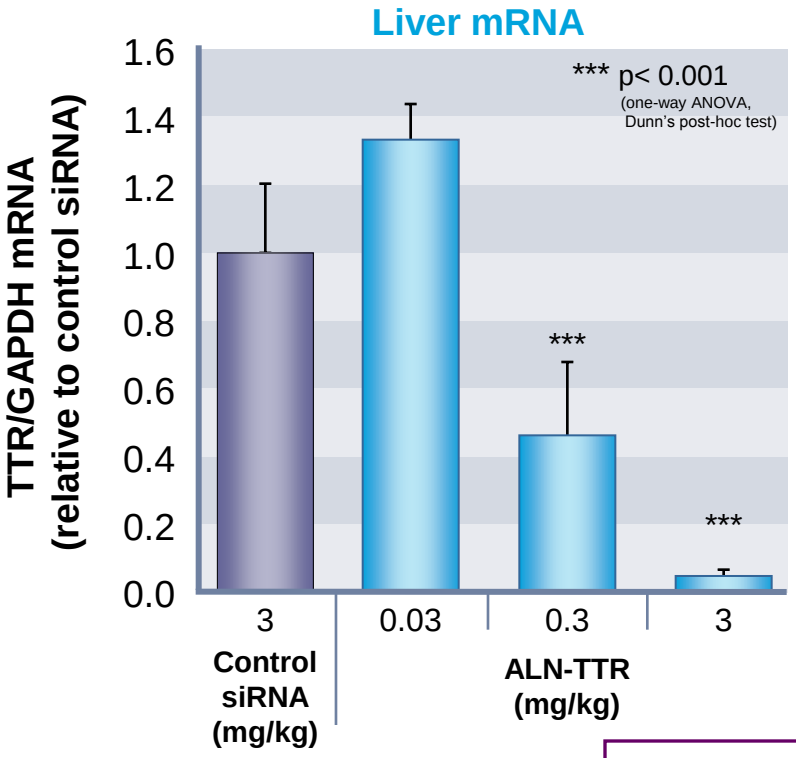
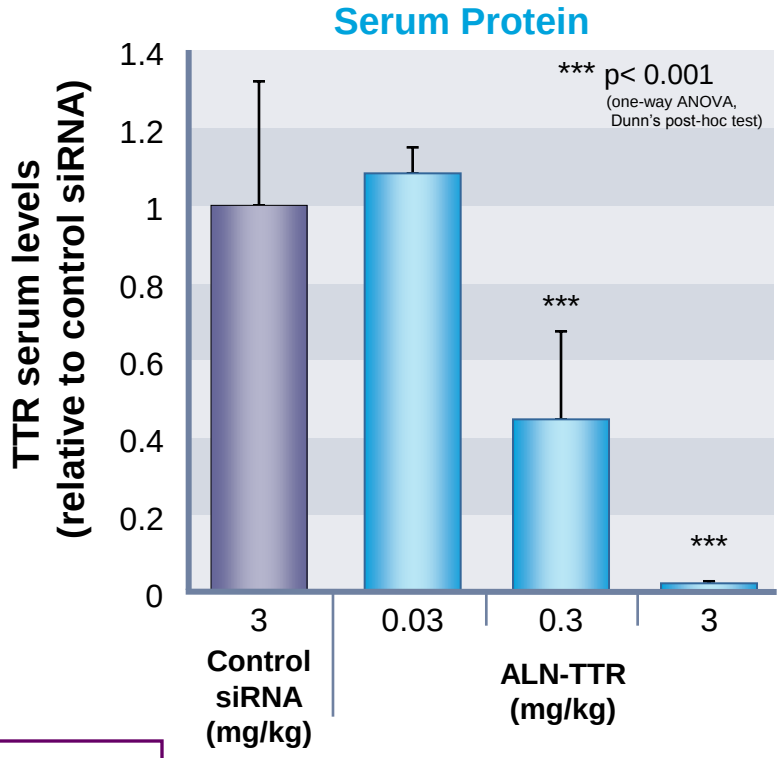
ALN-TTR Silences Mutant Human TTR

V30M TTR Transgenic Mouse Model

ALN-TTR silences human V30M TTR mRNA and suppresses mutant protein levels

- Single i.v. dose of ALN-TTR or control siRNA
- Liver mRNA and serum TTR levels measured 48hr post-dose





ED_{50} ~ 0.15 mg/kg

RNAi, MicroRNAs, 2009

Collaboration with M. Saraiva

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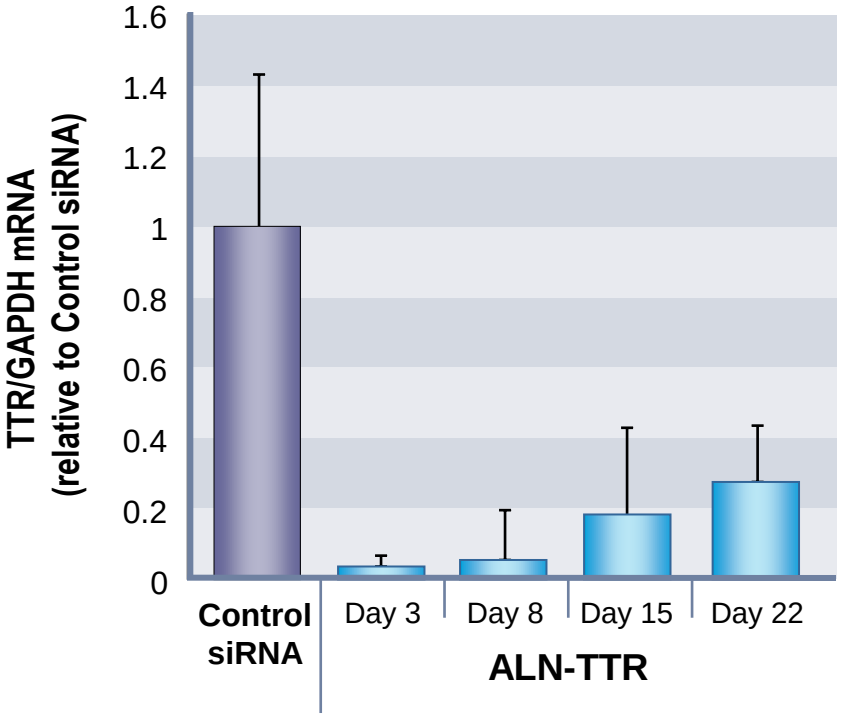
ALN-TTR Silencing is Durable

V30M TTR Transgenic Mouse Model

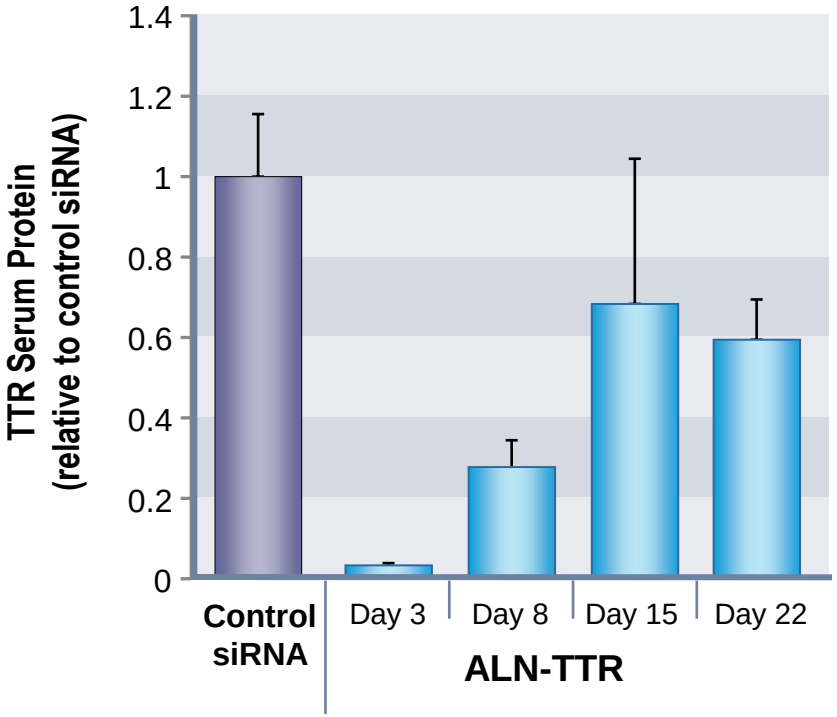
ALN-TTR efficacy is both rapid and durable

- Single i.v. dose of ALN-TTR or control siRNA; 1 mg/kg
- Liver mRNA and serum protein levels measured on Days 3, 8, 15 and 22 post-dose

Liver mRNA



Serum Protein



AALSD: Liver Meeting, Nov 2009
Collaboration with M. Saraiva

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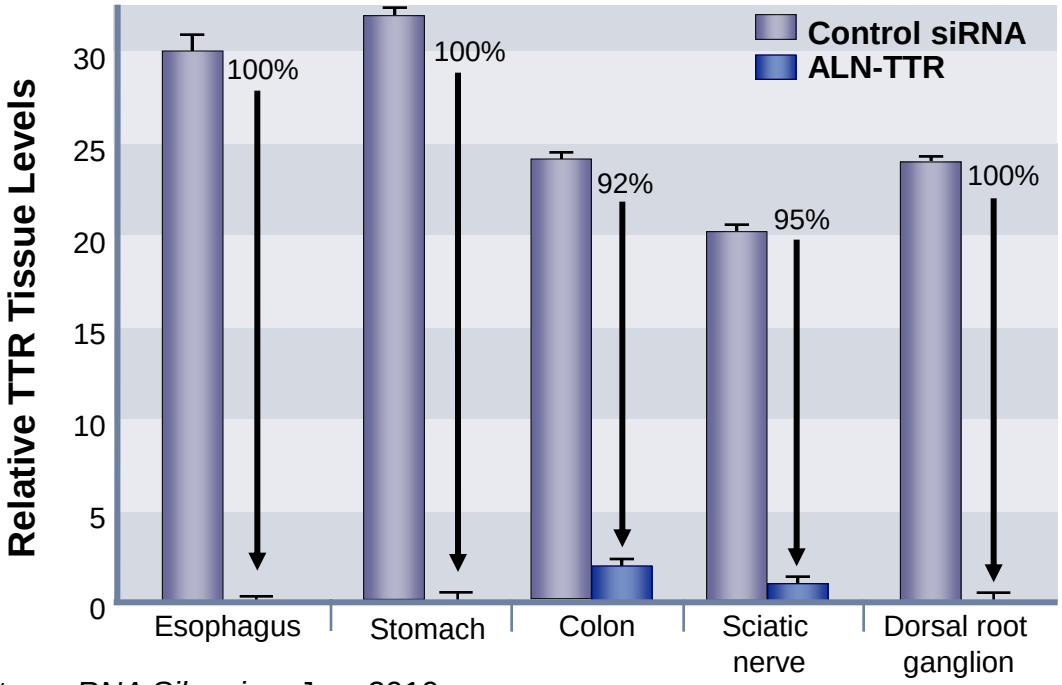
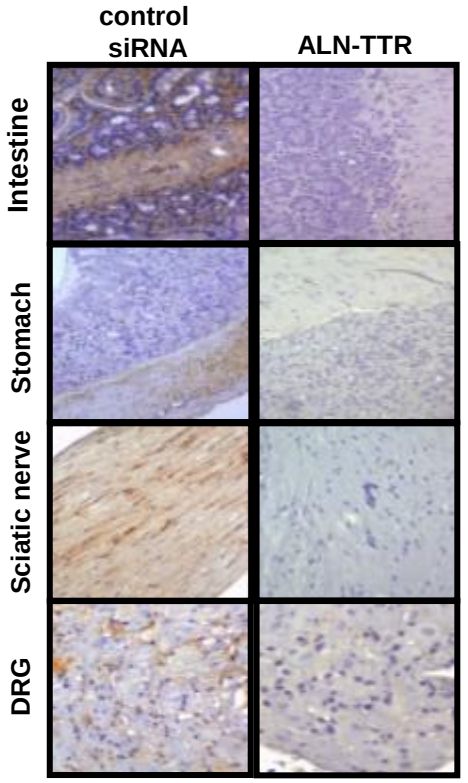
ALN-TTR Prophylactic Efficacy

V30M TTR Transgenic Mouse Model

ALN-TTR blocks pathogenic accumulation of mutant human TTR in peripheral tissues

- >95% Reduction of V30M hTTR deposition
- Multi-dose i.v. injections of ALN-TTR or control siRNA, 3 mg/kg (days 0, 14, 28)
- Quantitation of V30M hTTR deposition by immunohistochemistry (day 56)





Collaboration with M. Saraiva

Keystone: RNA Silencing, Jan. 2010
Collaboration with M. Saraiva

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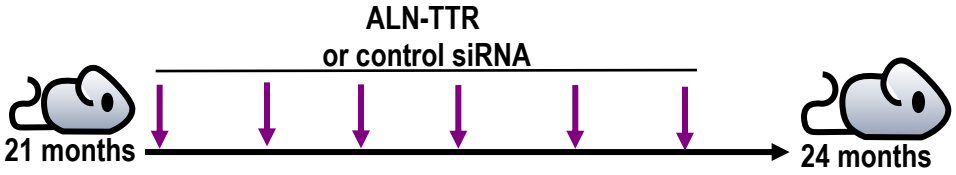
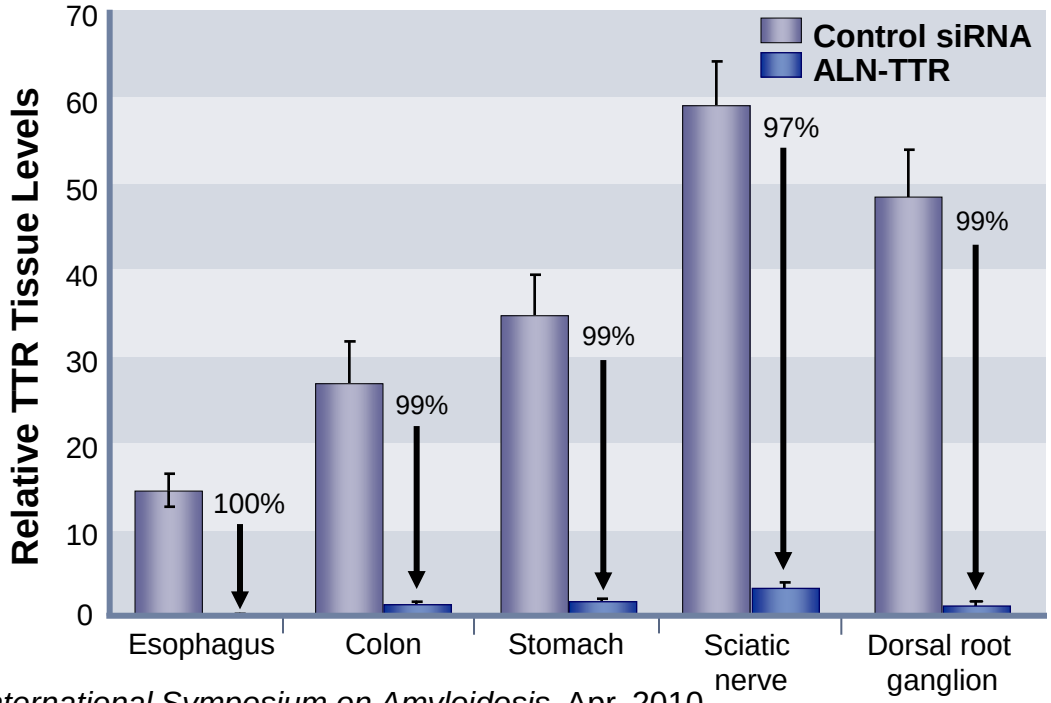
ALN-TTR Therapeutic Efficacy

V30M TTR Transgenic Mouse Model

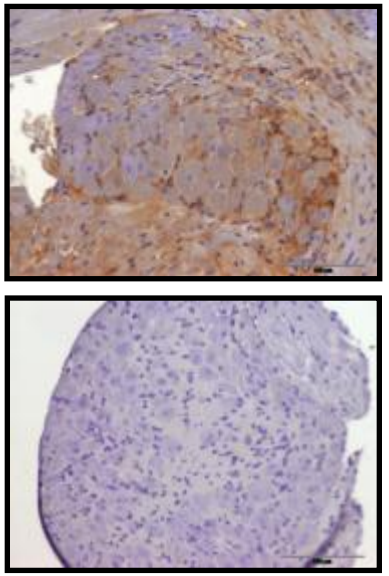
ALN-TTR promotes regression of pathogenic mutant human TTR deposits in peripheral tissues

- >90% Regression of existing V30M hTTR tissue deposits
- Multi-dose IV bolus of ALN-TTR or control siRNA, 3 mg/kg (d0, 14, 28, 42, 56, and 70)
- Quantitation of TTR deposition by immunohistochemistry (day 77)





Dorsal Root Ganglion



Control siRNA

ALN-TTR

XII International Symposium on Amyloidosis, Apr. 2010

Collaboration with M. Saraiva

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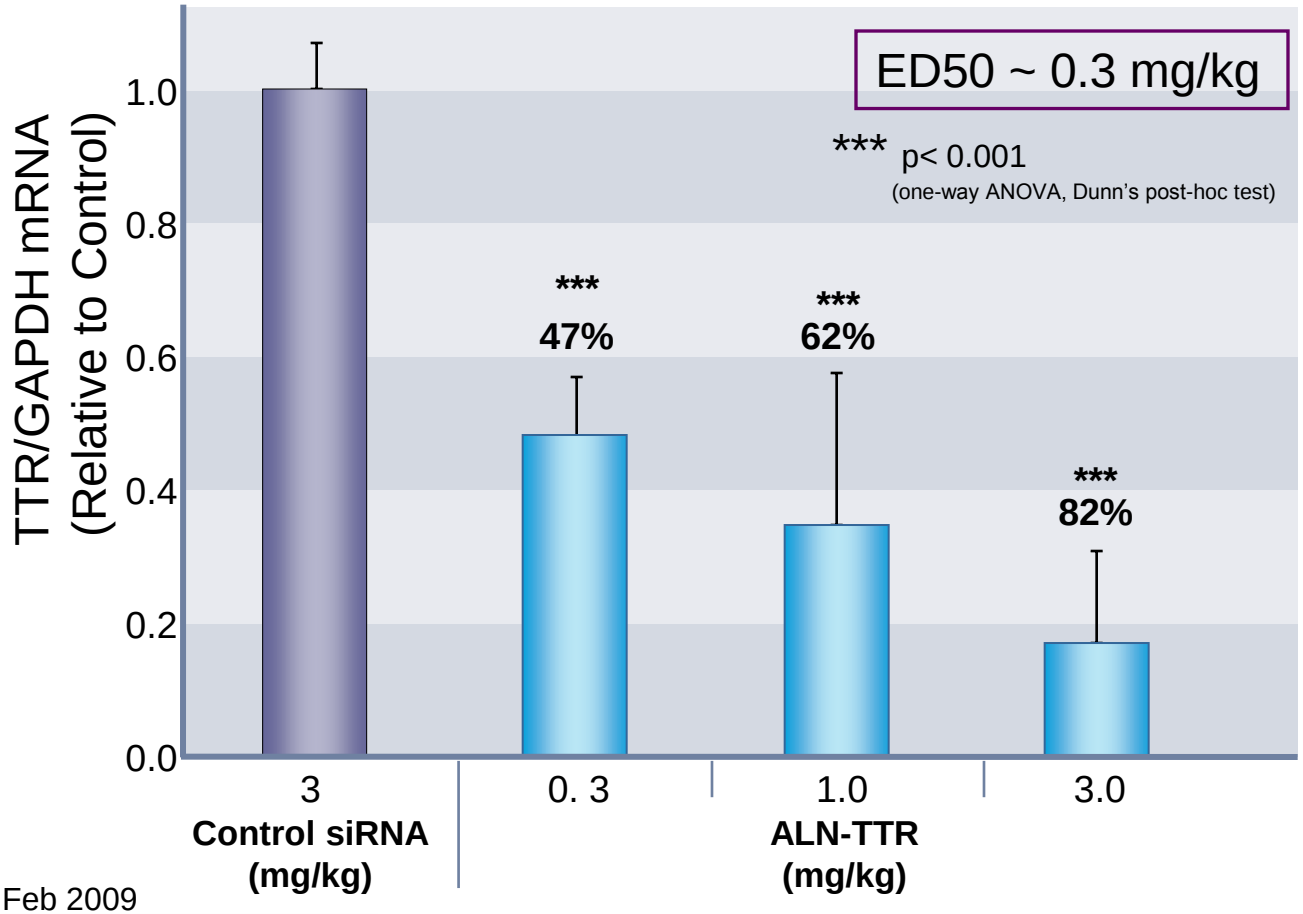
ALN-TTR Reduces TTR mRNA
Non-Human Primates

ALN-TTR shows dose dependent silencing of TTR mRNA

- Single i.v. infusion of ALN-TTR or control siRNA
- Liver mRNA levels measured 48hr post-dose

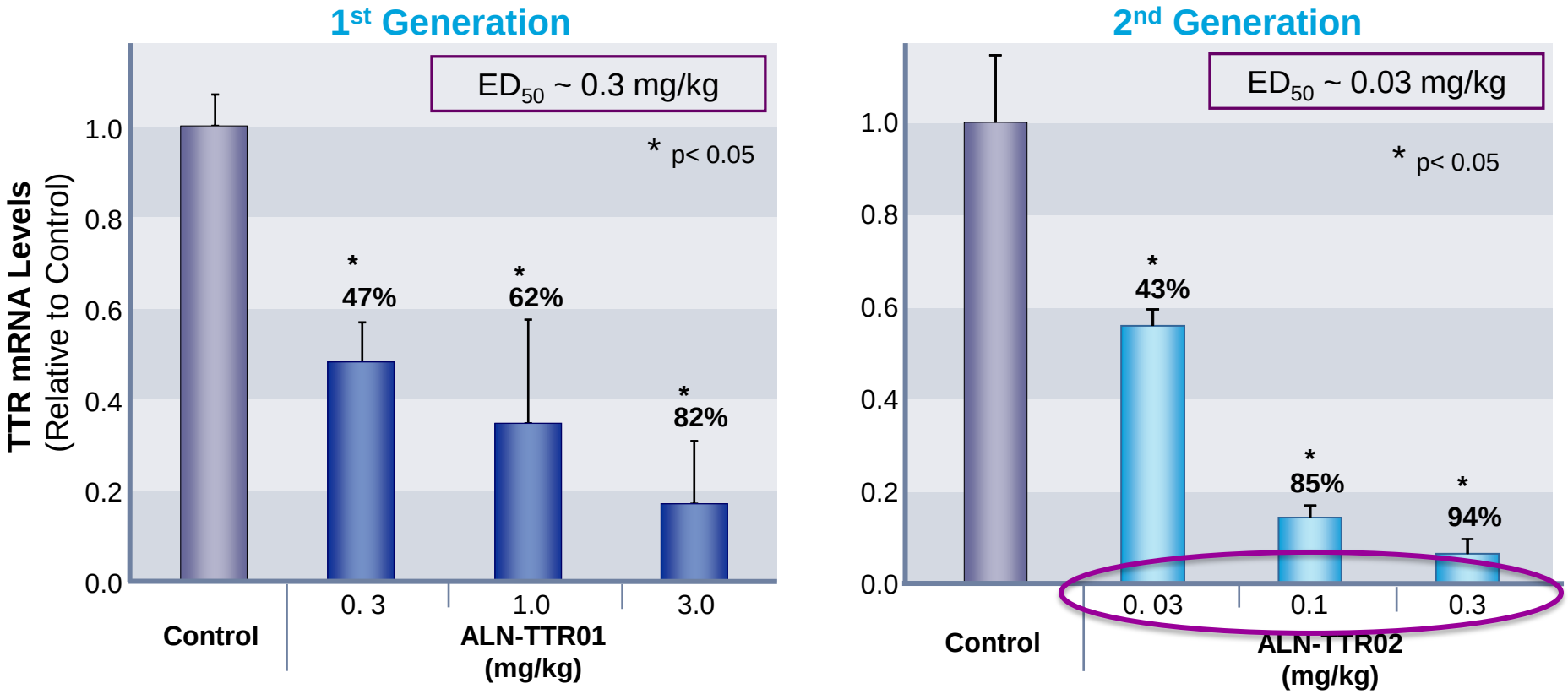


Keystone RNAi, Feb 2009

2nd Generation ALN-TTR Program
ALN-TTR02

ALN-TTR02 shows >10-fold improved *in vivo* efficacy

- Single i.v. infusion
- Liver mRNA levels measured 48 hr post-dose
- Potent, dose-dependent TTR silencing



BRI Translational Genetics Series, Feb. 2011

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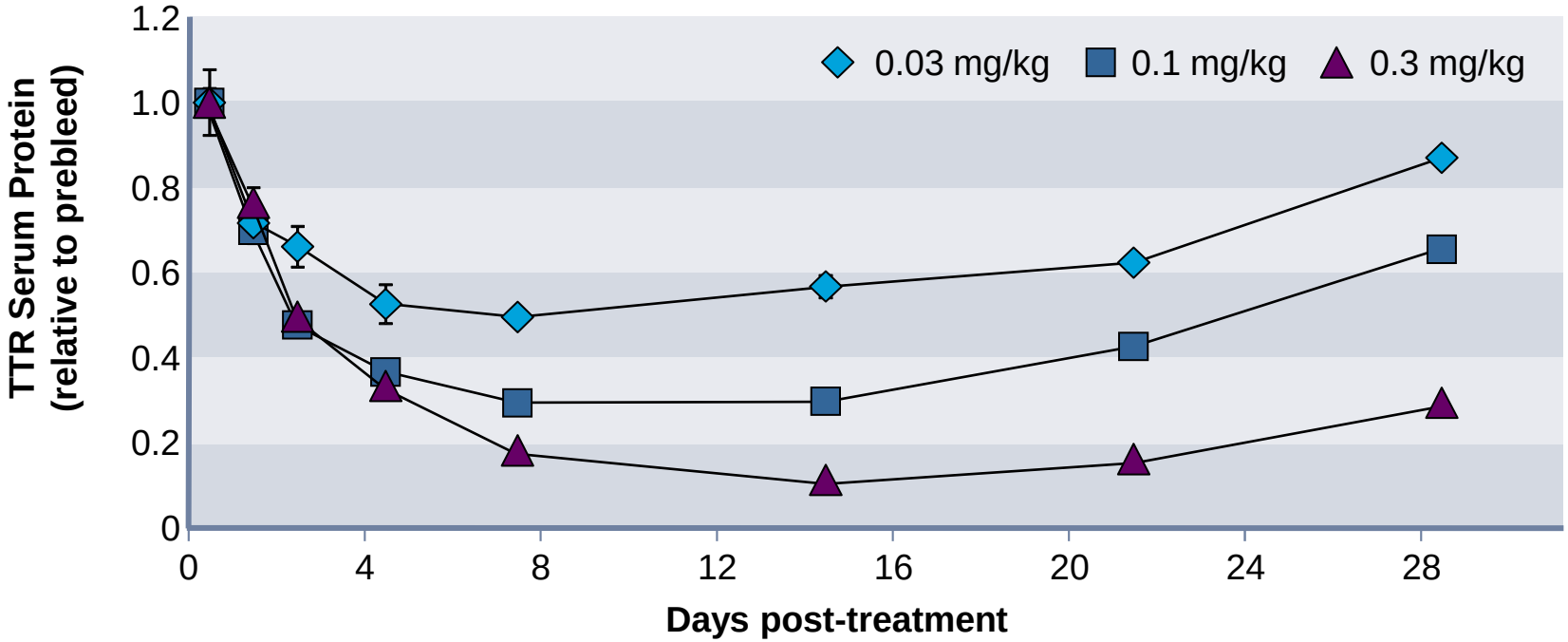
Suppression of TTR Protein by ALN-TTR02 is Durable
Non-Human Primates

ALN-TTR02 demonstrates durable, dose-dependent suppression of TTR serum protein levels greater than 28 days in NHP

- Single 15 min IV infusion (0.03, 0.1, 0.3 mg/kg)
- Serum TTR protein levels measured by ELISA on days 0, 1, 2, 4, 7, 14, 21, and 28



- ALN-TTR02 ED_{50} ~ 0.03 mg/kg, ED_{80} ~ 0.2 mg/kg
- Maximal TTR protein suppression at ~ 7-14 days

BRI Translational Genetics Series, Feb. 2011

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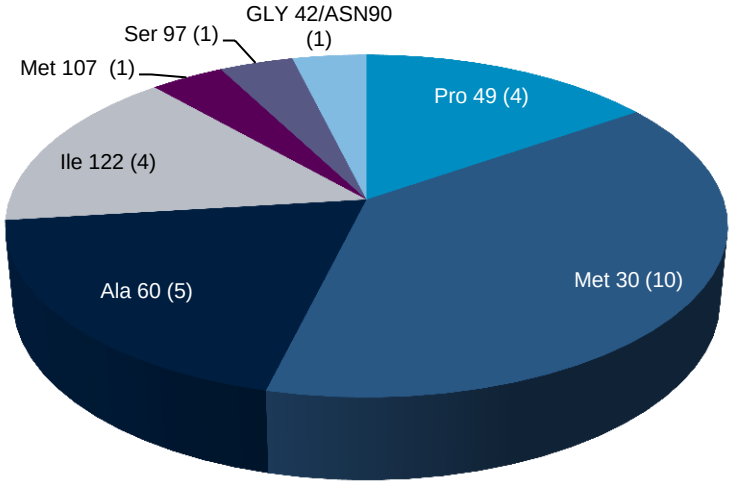
ALN-TTR-NT

Study Design / Demographics / Mutations

Study Design / Objective

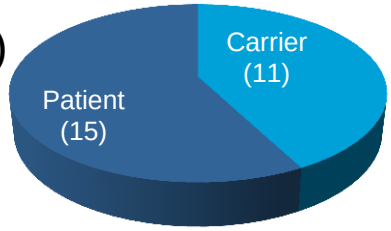
- Non-intervention / natural history study
- Assess variability of serial serum TTR levels in patients and carriers using:
  - ELISA-based assay
  - Isoelectric focusing gel (IEF)
  - LCMS/MS
- Weekly blood draws
  - 4 consecutive weeks at clinic or at subject's home
- PI: Dr. John Berk (Boston University)



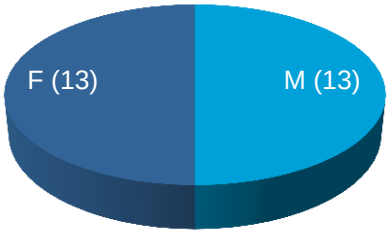
ALN-TTR-NT-001 Mutations

Status/Demographics

- 27 subjects enrolled
- 26 completers



Disease State



Gender

Coefficient of Variation for Serum TTR

All versus V30M Only

Group included in analysis	Visits included in analysis	Number of Subjects	Number of Observations	Mean	Variance Component Estimate of Within Subject SD		Variance Component Estimate of Between Subject SD
Carrier (All)	All visits	11	44	194.9	33.2	17%	42.6
Patient (All)	All visits	15	57	207.9	47.8	23%	52.3
Overall (All)	All visits	26	101	202.2	42.0	21%	47.7
Carrier (V30M)	All visits	4	16	230.7	26.5	11%	55.1
Patient (V30M)	All visits	6	24	230.2	19.0	8%	65.5
Overall (V30M)	All visits	10	40	230.4	22.3	10%	58.2

Summary

- Serum TTR shows relatively modest intrapatient variability (~10-20% fluctuation from week to week)
- LCMS/MS analysis on V30M and V122I samples in progress
- Additional key factors to be analyzed in assessment of response to ALN-TTR include:
  - » Serum RBP
  - » Vitamin A levels
  - » Kinetics of TTR/RBP/Vitamin A suppression

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ALN-TTR01 Phase I Study

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), Sweden (O. Suhr), France (D. Adams) and UK (P. Hawkins)
- Primary objective
  - » Safety and tolerability
- Secondary objectives
  - » Characterize plasma pharmacokinetics
  - » Assess preliminary pharmacodynamic activity
    - – Serum TTR, RBP, Vitamin A levels
      - » TTR measured by ELISA and LCMS/MS

ALN-TTR01 Phase I Study (Cont'd)

Study Design

- Treatment regimen
  - » Single 15-minute ALN-TTR01 i.v. infusion
  - » Premedication with corticosteroids, H1/H2 blockers, acetaminophen
  - » Dose levels: 0.01-1.0 mg/kg
- Status
  - » Initiated Q3 '10; actively enrolling
  - » To present safety and pharmacodynamic data at VIIIth International Symposium on Familial Amyloidotic Polyneuropathy in Kumamoto, Japan on November 21st

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TTR Program
Summary and Next Steps

ALN-TTR01

- In Phase I clinical trial, actively enrolling
  - » Targets wild-type TTR and all mutant forms of TTR
  - » LNP formulation
  - » Potent reduction of TTR *in vitro*
  - » Potent and durable silencing of TTR *in vivo*
    - – Wild-type TTR in rodents and non-human primates
    - – Mutant human V30M TTR in transgenic mouse
  - » Therapeutic efficacy measured by reduced TTR pathogenic tissue deposition in V30M transgenic mouse

ALN-TTR02

- Drug candidate using same siRNA as ALN-TTR01 formulated in second-generation LNP with enhanced potency, in development; IND/CTA filing in 2011

Acknowledgments
TTR Amyloidosis Program

Scientific Collaborators

- Maria Saraiva, Institute of Cellular and Molecular Biology, Porto, Portugal
- Yukio Ando and Hiro Jono, Kumamoto University, Japan

ALN-TTR01 Clinical Investigators

- Teresa Coelho
  - » Hospital Geral de Santo Antonio, Porto, Portugal
- Ole Suhr
  - » Umea University Hospital, Umea, Sweden
- David Adams
  - » CHU Hospital Bicetre, Le Kremlin-Bicetre, France
- Tim Mant/Philip Hawkins
  - » Quintiles Drug Research Unit at Guy's Hospital, London

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