Advances in RNAi Therapeutics at Arrowhead Pharmaceuticals

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Outline

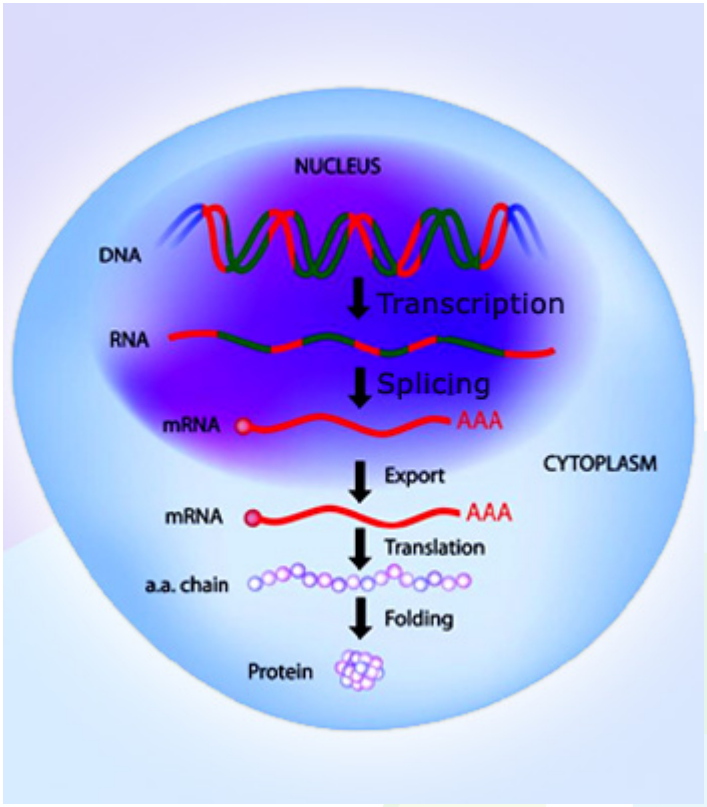
- RNA Interference
- Arrowhead Pharmaceuticals RNAi Platform Development
- Preclinical Candidates
  - ARO-AAT
  - ARO-HBV

Small Molecule Pharmaceuticals Target Proteins

- The central dogma of molecular biology
  - Transcription and translation
    - the information in genes flows into proteins

- Small molecule pharmaceuticals target proteins
  - Enzymes
  - Receptors

How about RNAi molecules?



DNA → mRNA → protein

Small molecule therapeutics

The Discovery of siRNA

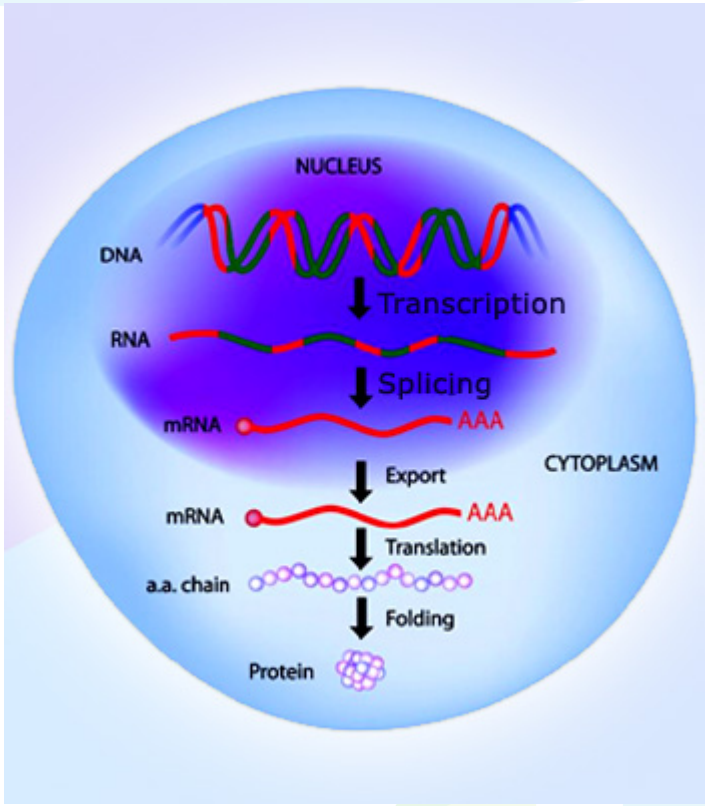
- In 1998, RNAi was discovered by Andrew Fire and Craig Mello.
- In 2001, siRNA was first used as a tool to silence genes in mammalian cells
- Awarded the Nobel Prize in Physiology or Medicine 2006
  - "for their discovery of RNA interference-gene silencing by double-stranded RNA"

Andrew Z. Fire

Craig C. Mello

RNAi Therapeutics – the Promise and Advantages

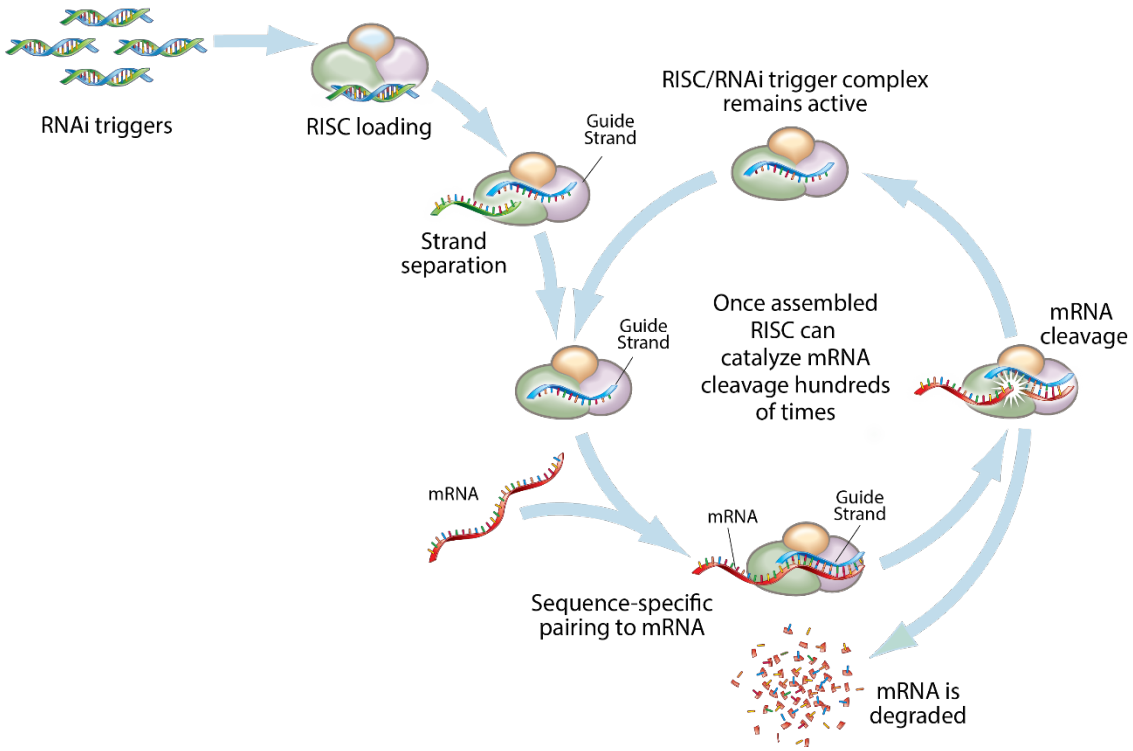
- RNAi
  - Cleave mRNA
    - Stop the translation process
    - Block the production of disease causing proteins
- The promise:
  - Treat and cure currently undruggable diseases
    - Genetic disorders, Cancer, infectious diseases, cardiovascular diseases, pulmonary diseases
- The advantage over small molecule therapeutics
  - Platform technology
  - Target specific cell type
  - Precision medicine
    - Only knockdown the target gene



DNA → mRNA → protein

RNAi

Target the Gene, Silence the Disease

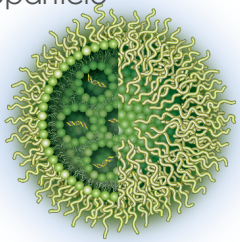


Therapeutic gene silencing with **RNA interference** is highly precise and efficient

A Long Journey for RNAi: Focused on the Vehicles Not Payloads

- Treated distinctly as two components: vehicles and payload
- The focus was on delivery vehicles for years in academia and industry
- The vehicles
  - Provided shielding for siRNA as in polymers and LNPs
  - Enabled rapid endosome escape as in polymers, LNP and DPC
- Lessons learned
  - Limit delivery mainly to liver
  - Observed toxicity from the delivery vehicles

Lipid Nanoparticle

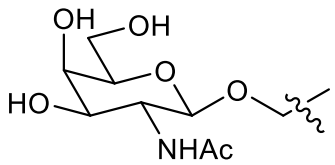


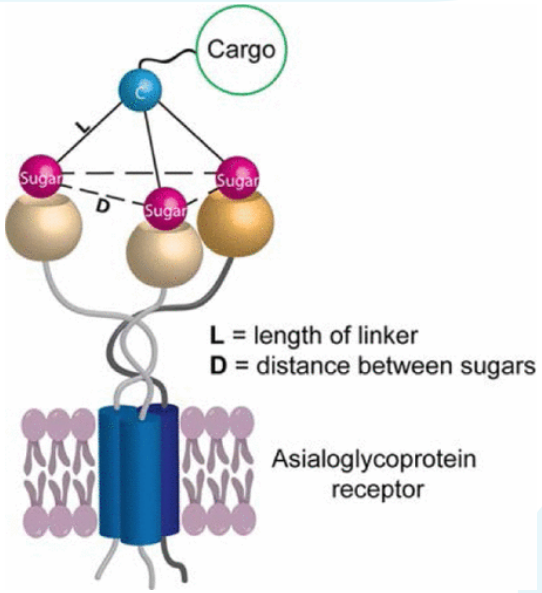
Various types of polymers

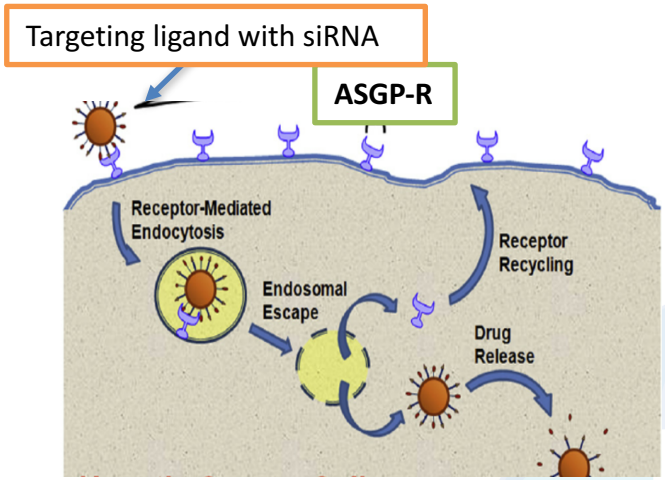
DPC-2

Direct Conjugation for Hepatocyte Delivery

- Asialoglycoprotein receptor (ASGP-R)
  - Tridentate receptor, overly expressed on the surface of hepatic cells, but minimally on extra-hepatic cells
  - Recycled every 15 mins
- Natural ligand to ASGP-R
  - N-Acetyl-Galactosamine (NAG)







Huang etc. *Bioconjugation, 2016*

The Challenge with Direct Conjugates – RNA Trigger

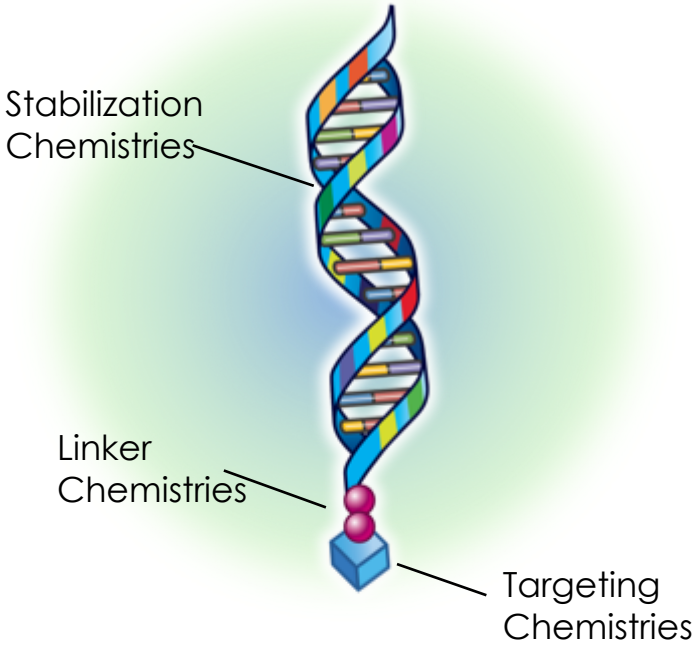
- **Trigger stability**
  - Survive long time in endosome
- The industry and academia moved on to stabilization of siRNA triggers
  - Fully modified at 2' positions (e.g., -F and –Ome)
  - Use of multiple phosphorothioates, instead of phosphates, including at terminal positions to increase nuclease resistance
  - Use of phosphate mimetics at 5'end of antisense strand
  - MOE
  - LNA
- **Trigger activities**
  - Not many triggers can survive endosome even with the increased stability
- Selection of triggers for in vivo study comes from cell based in vitro study
  - Lack of in vitro – in vivo correlation in direct conjugate approach
  - The industry approach:
    - Lower the bar on specificity to increase the trigger selection pool in the search for activity
      - Increase chances for off-target effects

Arrowhead: Holistic Approach

- Not to just modify RNA triggers, but select RNA triggers based on intrinsic characteristics
- Deep understanding, at molecular level, of critical factors in each step of RNAi:
  - RISC loading, mRNA cleavage, trigger metabolism, off target interactions
- This insight enables speed and high early success rates in our development programs
  - Sequence identification
  - Lead sequence modification and optimization to enhance potency
  - Minimize off-target effects

Arrowhead RNAi Platform: TRiM™

Simplicity, Specificity, and Activity



TRiM™ has rules and algorithms to optimize trigger sequence

- Limit cross reactivity with off target genes
- Maximize activity
- Maximize innate stability
- Rational use and placement of modifying chemistries
- RNAi chemistry insights and expertise have allowed us to see what others have not

Targeted RNAi Molecule

TRiM™ platform

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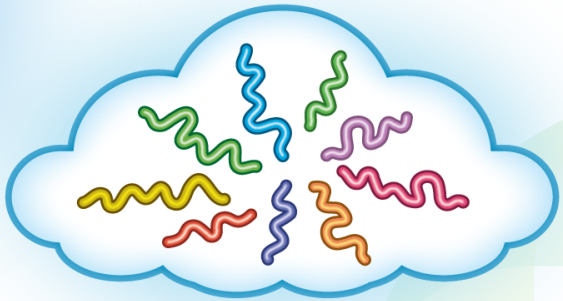
TRiM™ Chemical Modifications

Linker Chemistries

Stabilization Chemistries

Targeting Ligands



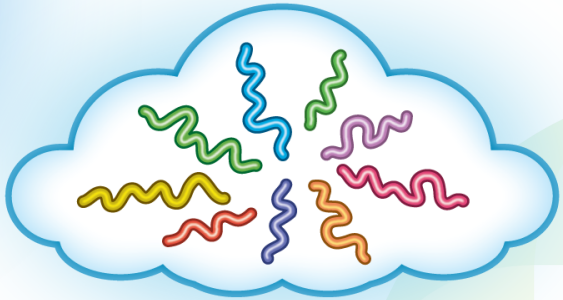
Structures to Enhance Pharmacokinetics

TRiM™ Chemical Modifications

Linker Chemistries

Stabilization Chemistries

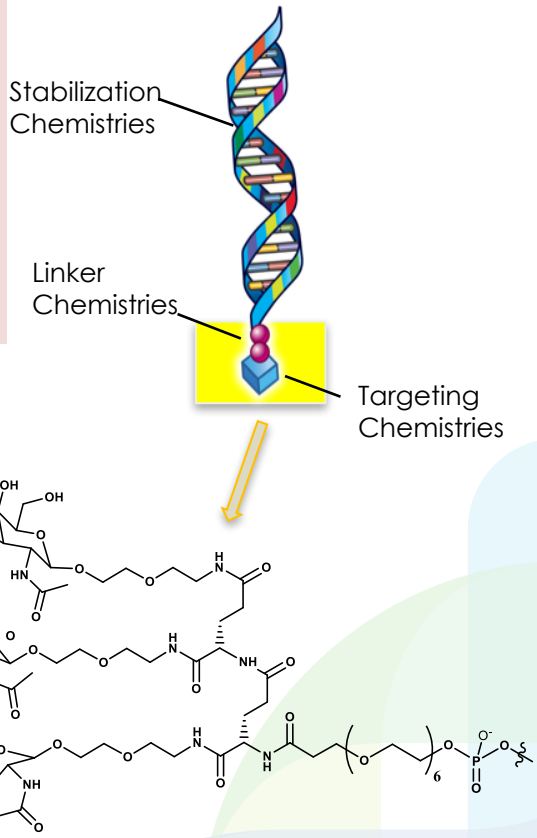
Targeting Ligands



Structures to Enhance Pharmacokinetics

Hepatic siRNA Discovery/Development

- **Key Design Elements in Hepatic Platform**
  - Subcutaneous dosing, monthly or less frequency
  - No need for active endosomal escape agent
  - Expectation of wide therapeutic index



- Uncover new triggers (see what others cannot see)
- Rational design of chemical modifications to improve
  - Stability in endosome and cytoplasm
  - Potency
- Targeting moiety Investigation:
  - NAG cluster
  - Linker chemistry
  - Overall ligand design
  - Topology

Two challenges: RNAi CHEMISTRY and DELIVERY

ARO-AAT

What is Alpha-1 Antitrypsin (AAT)

- AAT is an abundant serum protein
  - Primarily synthesized in the liver
  - About 10% made extrahepatically
- Physiological function includes:
  - Inhibition of neutrophil proteases to protect host tissues during inflammation
  - Especially important in the lung

Alpha-1 Antitrypsin Deficiency (AATD)

- Mutation in AAT gene (Z-AAT) leads to mis-folding of the protein and poor export from hepatocytes: low levels in circulation and accumulation in liver

Pathophysiology

Lung

Tissues susceptible to damage by neutrophil proteases: COPD

↓

Treated with AAT enzyme replacement therapy

Liver

Accumulation of mutant Z-AAT protein can cause cirrhosis and HCC

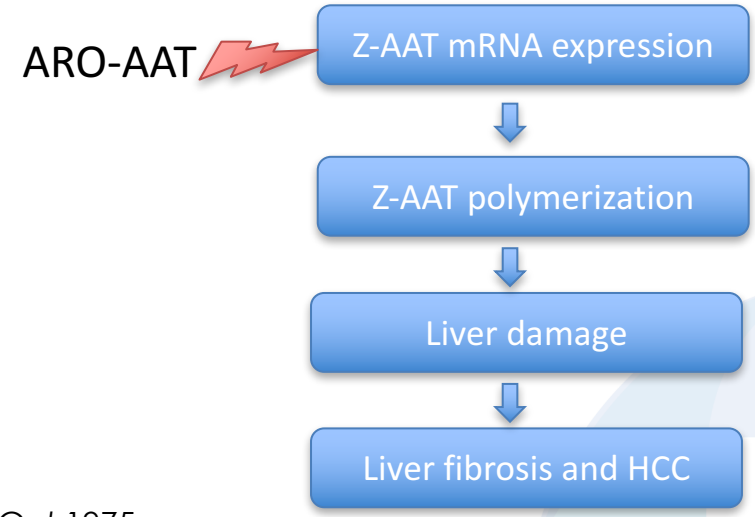
↓

Currently no treatment

- AATD is a large scale orphan disease
  - Alpha-1 Foundation estimates 100,000+ in the US
  - Approximately 100,000+ in Europe

RNAi for AATD: Mechanism of Action

- ARO-AAT designed to stop Z-AAT production by silencing AAT gene to:
  - Prevent accumulation of disease-causing protein in liver
  - Allow clearance of accumulated protein
  - Prevent repeated cycles of cellular damage
  - Reverse fibrosis associated with prior damage



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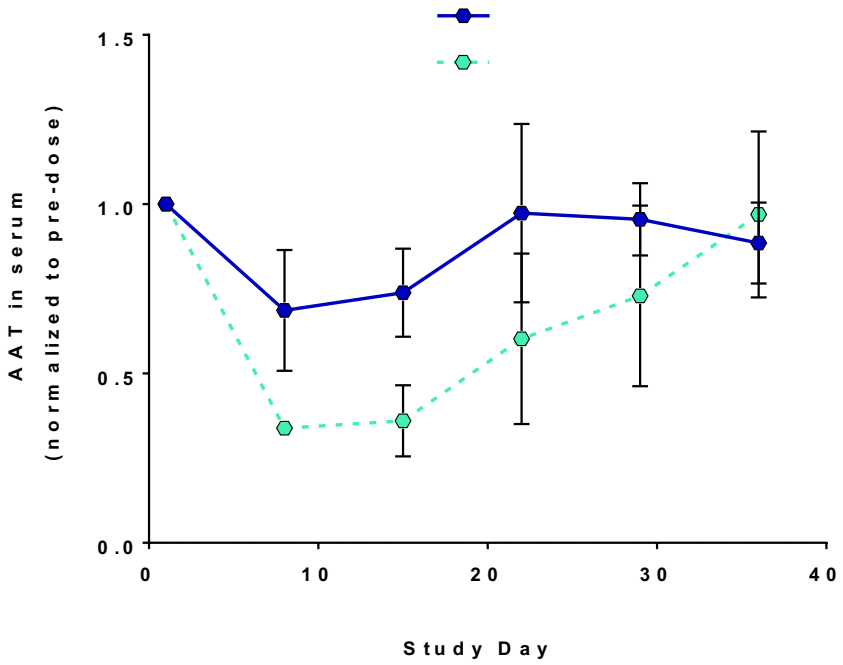
Feldmann G et al., *Gut* 1975

A Showcase of the Power of Chemistry Insight and Modification - ARO-AAT Discovery

AAT in mouse study at 5 mpk

AAT_trigger_1.1 Nadir 31.4% KD

AAT_trigger_1.2 Nadir 66.2% KD



- One round of chemical modification increased target gene knock down from 31% to 66%

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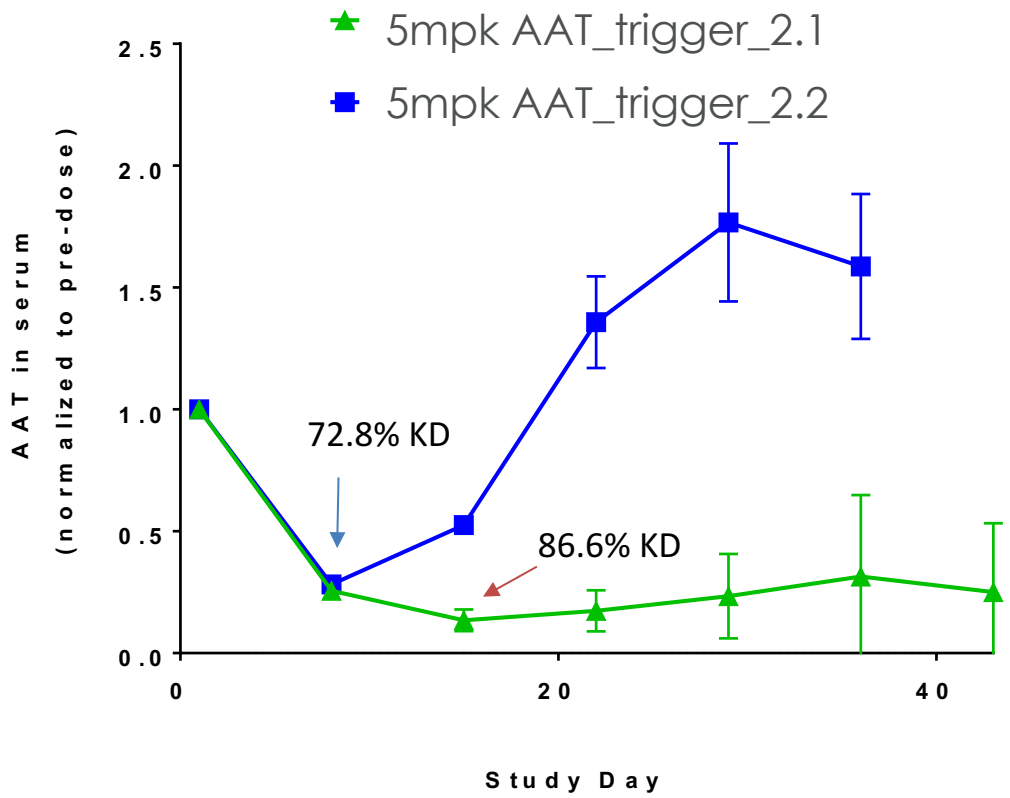
Sequence Selection Based on RNAi Chemistry Insights

AAT triggers in mouse study

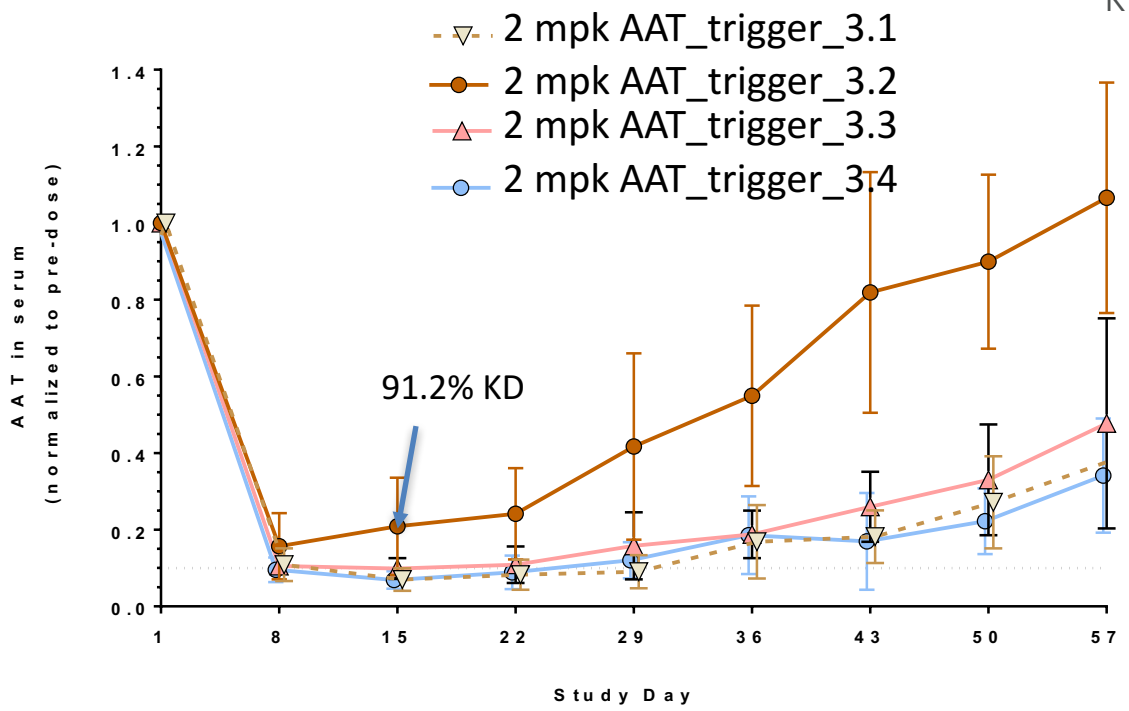


- Much increased AAT mRNA KD
- Improved duration

Lead Optimization Leads to ARO-AAT

- 91% serum AAT knockdown achieved
- Knockdown sustained for 3 weeks with one 2 mpk dose

RNAi triggers in mouse study



Chemical modifications led to deep reduction of AAT protein and long duration at dose of 2mg/kg

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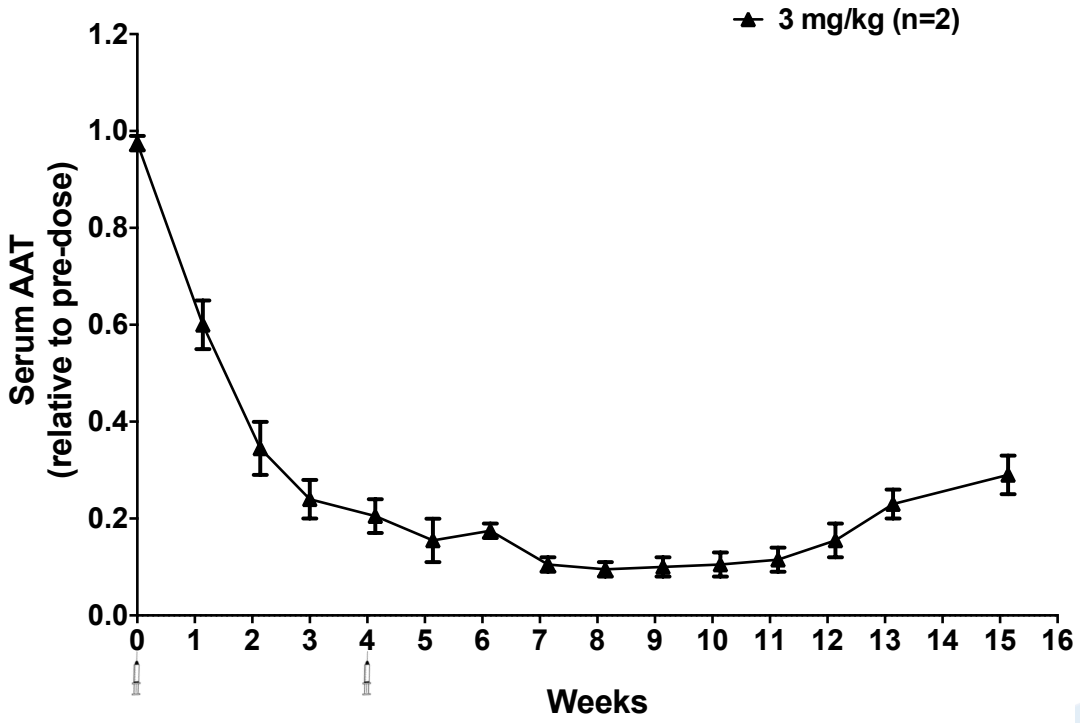
ARO-AAT Provides Durable AAT knockdown:
Multi-dose in NHP, dosed subcutaneously

- 92% maximum serum AAT knockdown achieved in cynomolgus monkeys
- Knockdown sustained for 7+ weeks following second dose



Durable knockdown supports once monthly or less frequent dosing

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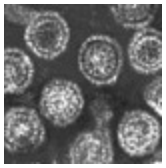
ARO-AAT Safety Evaluation and Next Steps in Drug Development

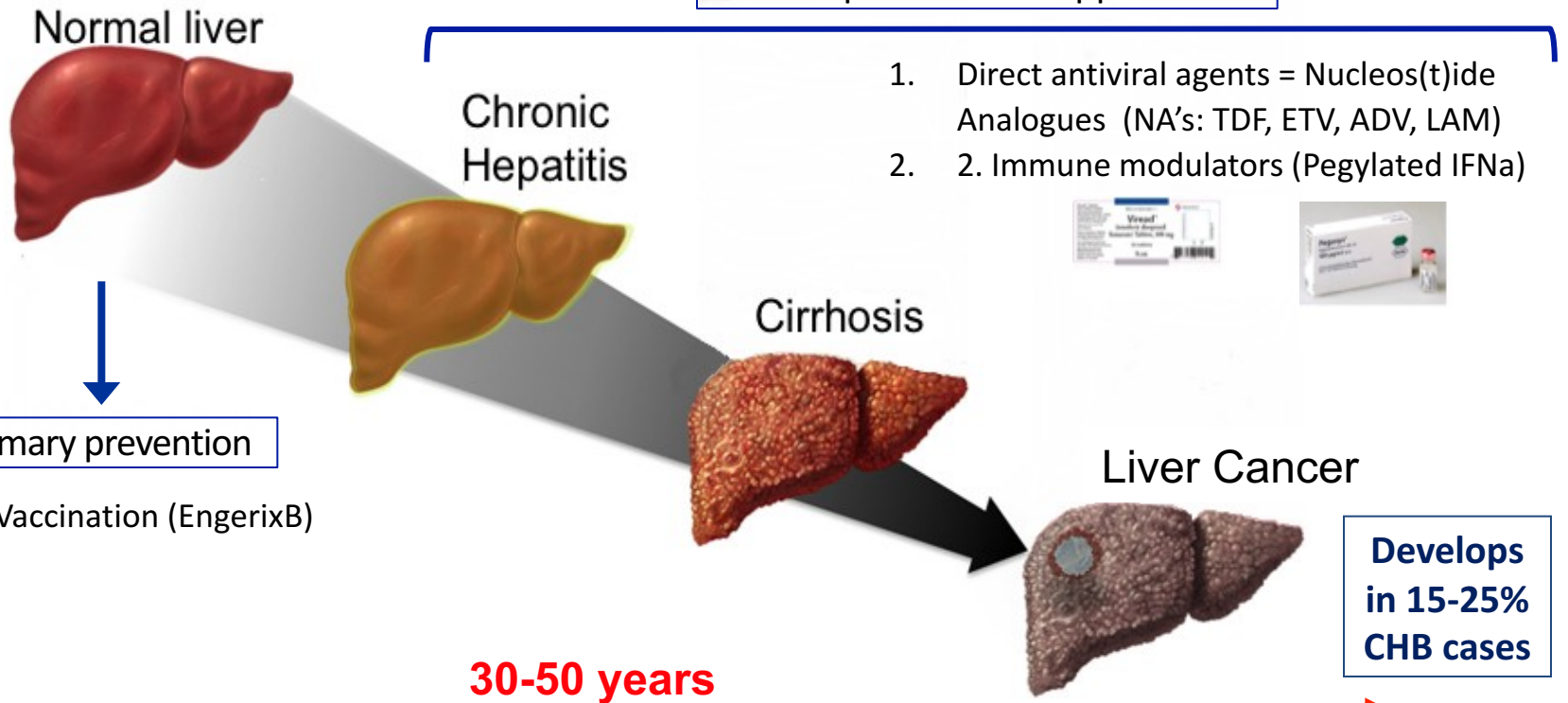
- Preliminary toxicology studies appear to show that ARO-AAT is well tolerated
- Potential for significant therapeutic index
- GMP production completed
- Phase 1 study is scheduled early 2018

ARO-HBV

Chronic hepatitis B (CHB): Disease pathogenesis

2 billion infected with HBV → **250 million living with chronic HBV infection**





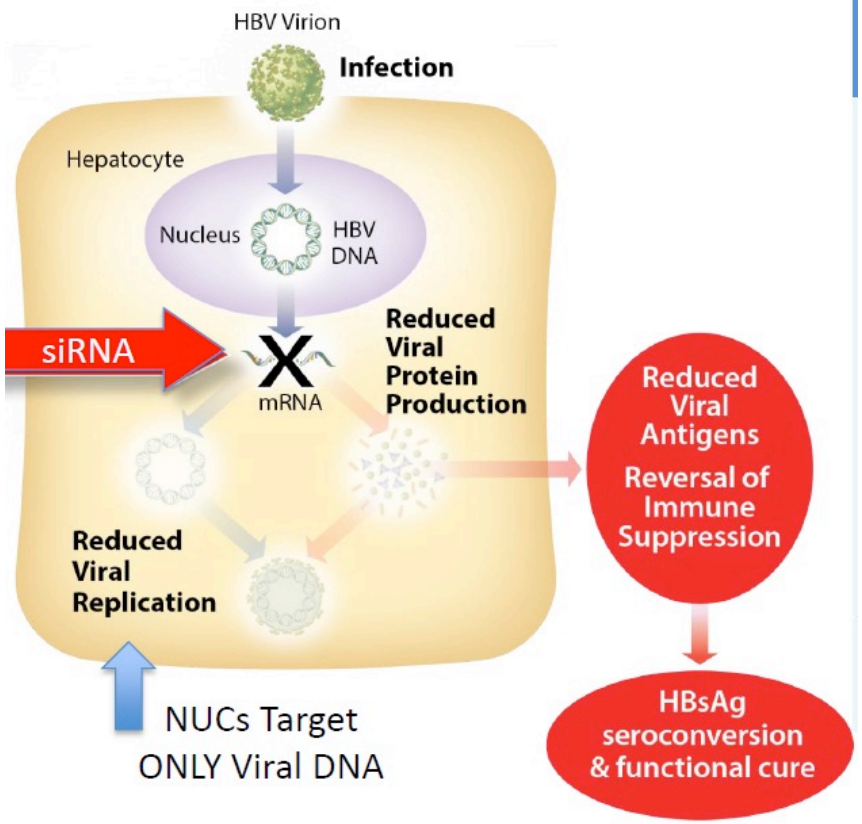
Chronic HBV infection occurs following:

- 95% neonate infections
- 10% adult infections

Neither preventative vaccination nor viral suppression influence CHB cure

Liaw YF et al, N Engl J Med. 2004; Chang et al, Hepatology 2010; Marcellin et al, Lancet 2013; Hosaka et al, Hepatology 2013; Kim et al, Cancer 2015; Papatheodoridis et al, J Hepatol 2015; Seeger, Zoulim, Mason; Fields Virology; 2007-2013

Small Molecule Drugs vs RNAi Therapeutics



Silence Entire HBV Genome

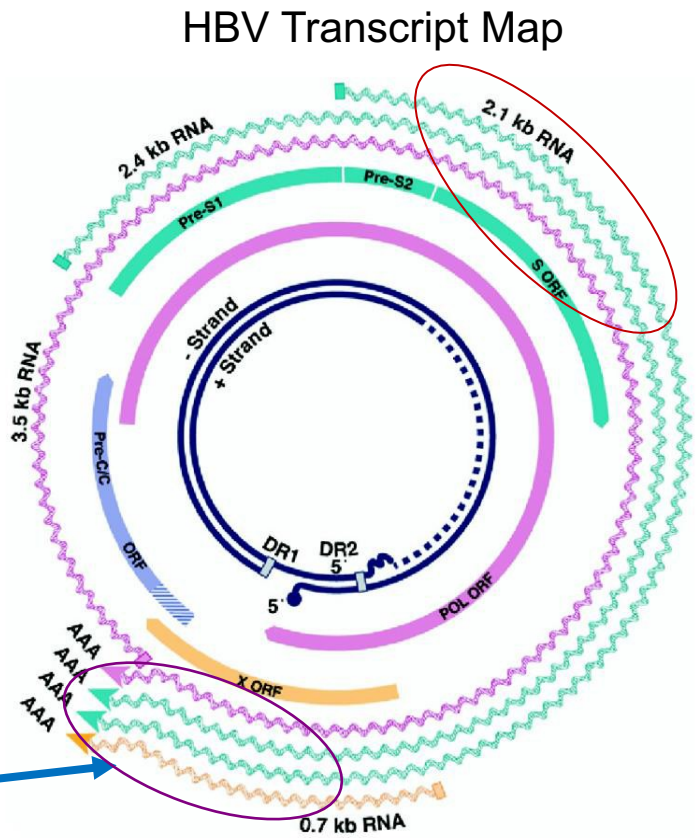
1. "HBsAg Theory"
   - Reducing HBsAg enables host immune system de-repression and long term control of virus
2. Destabilizing Viral Function
   - Silencing all antigens could destabilize normal viral function
   - Enable host immune system de-repression and long term control of virus

POTENCIAL TO ENABLE A FUNCTIONAL CURE

Importance of Integrated DNA as mRNA Source has Changed RNAi Strategy

- All HBV transcripts, including pregenomic RNA, overlap and terminate with the same polyadenylation signal
- A single siRNA targeting this common region can reduce all HBV transcripts derived from cccDNA

Single siRNA can reduce all mRNA from cccDNA but can miss integrated-derived mRNA



Ghany & Liang (2007), *Gastroenterology* **132**: 1574-1585

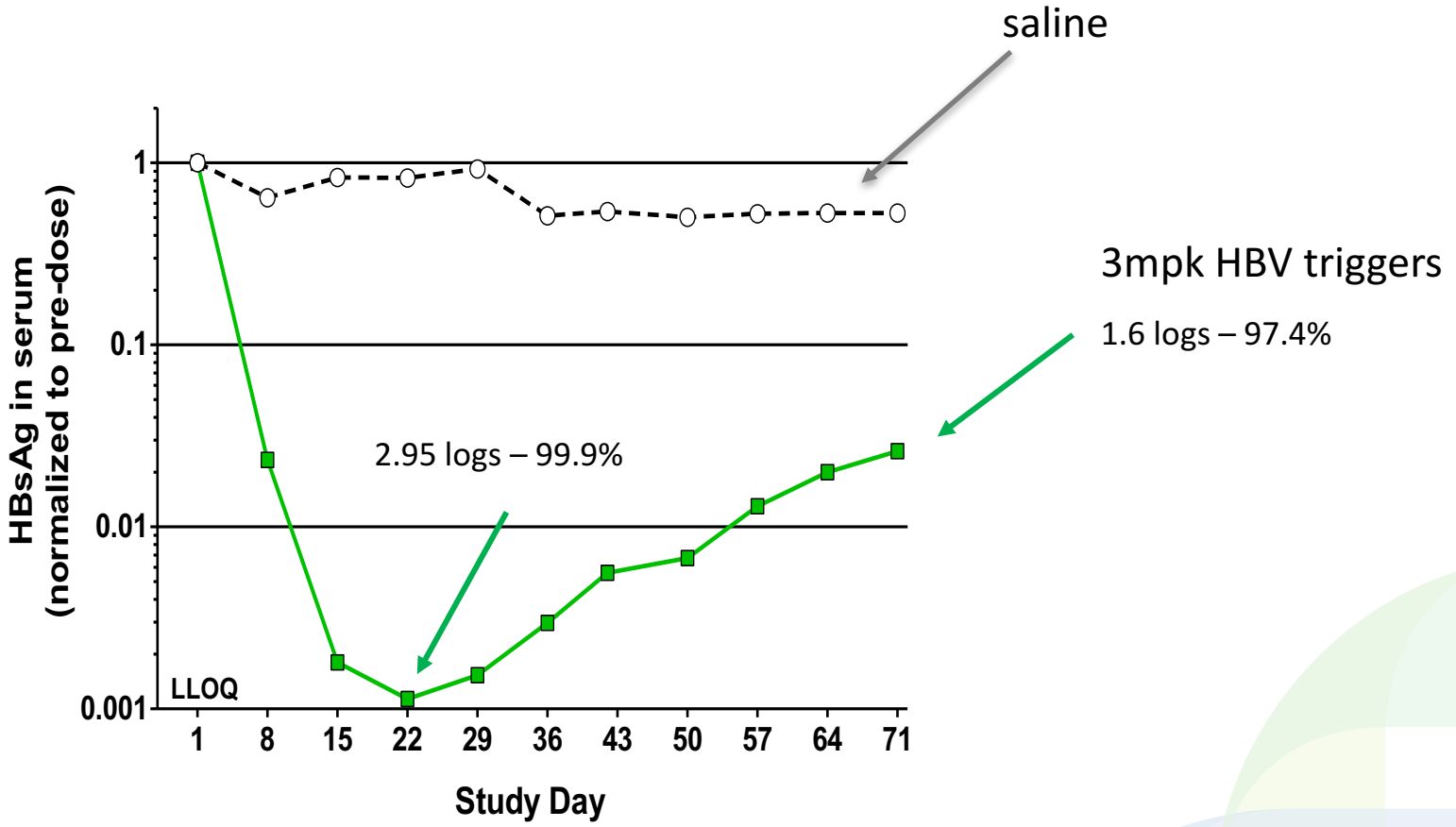
ARO-HBV: Key Design Elements

- Subcutaneous dosing, monthly or less frequent
- No need for active endosomal escape agent
- **Addresses full HBV transcriptome**
  - **Works for cccDNA *and* integrated-derived transcripts**
- Multiple triggers to avoid resistance development
- Powerful HBsAg reduction
- Wide therapeutic index
- Efficacy and safety in HBV patients

Using ARWR Hepatic Platform for HBV Drug Development

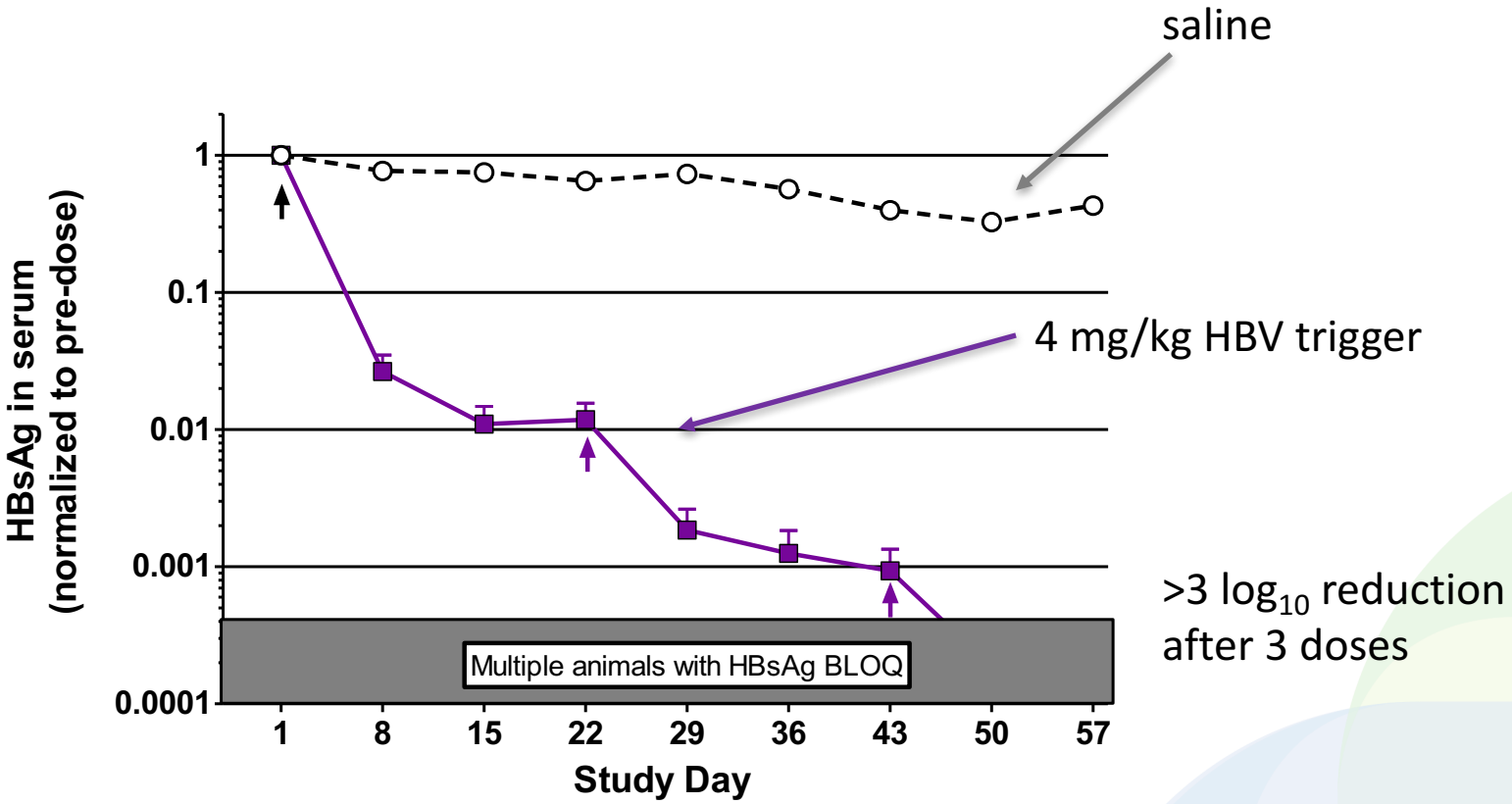
- Utilized the established ARWR hepatic TRiM platform
- Focused on RNAi chemistry
- Discovered potent RNAi molecules
  - Demonstrate deep KD of HBsAg, HBV DNA, HBeAg
  - Have the potential to provide a cure for millions of people suffering from the disease

We Modeled Integration in a New, Mutated pHBV Transfected Mouse



HBsAg knockdown is deep and prolonged despite loss of x trigger site

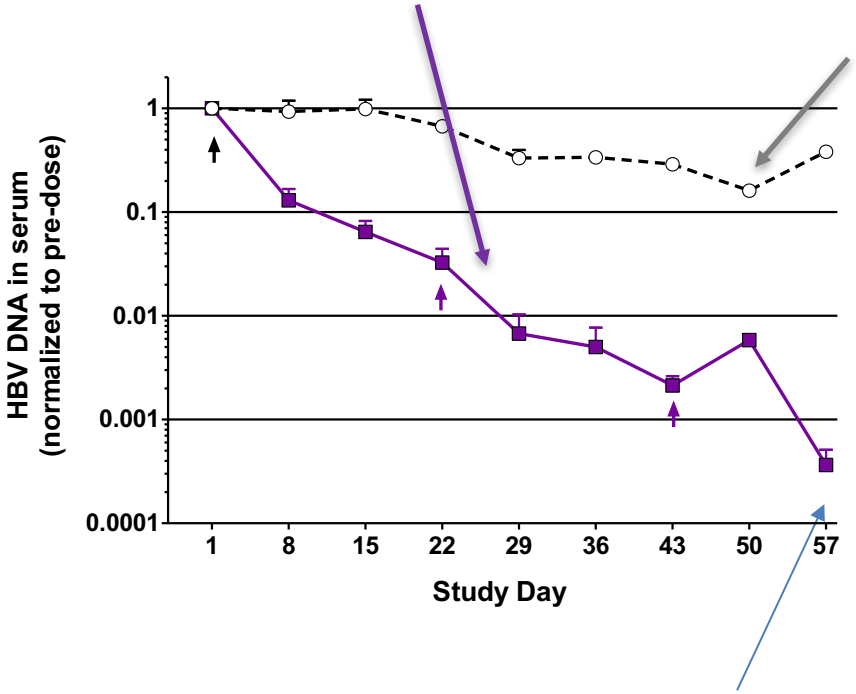
Multiple Dosing in Intact pHBV Mice Reduces HBsAg Below Level of Quantitation



.....With Deep Knockdown also Observed for HBeAg and HBV DNA

4 mg/kg HBV triggers (Days 1, 22 and 43)

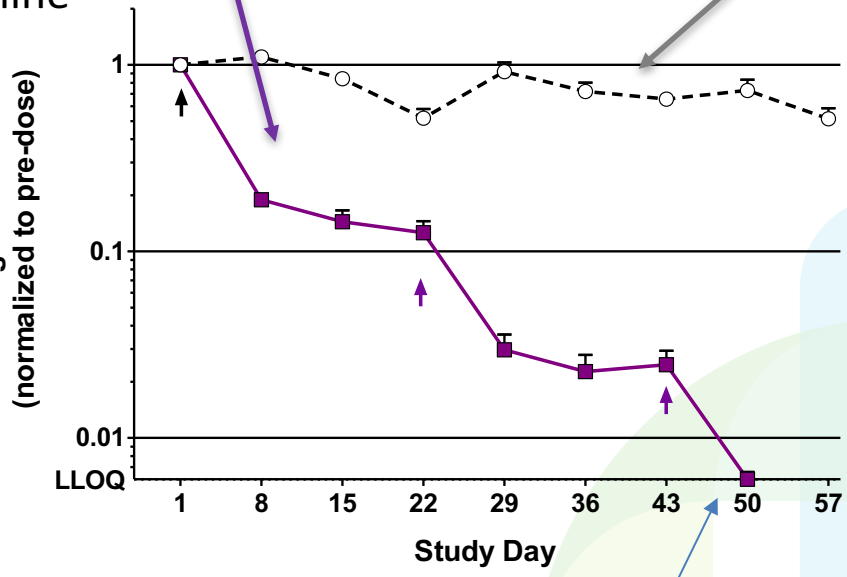
saline



3.44 $\log_{10}$ =
>99.9% reduction

4 mg/kg HBV triggers (Days 1, 22 and 43)

saline



2.2 $\log_{10}$ =
99.4% reduction
to LLOQ

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ARO-HBV Preliminary Safety Evaluation and Next Steps in Development

- Preliminary toxicology studies appear to show that ARO-HBV is well tolerated
- Potential for significant therapeutic index
- Phase 1 clinical study scheduled to start 1Q 2018

Targeting New Tissues Using TRiM™ Platform

ARO-Hif2 – for the treatment of renal cell carcinoma

- Up to 85% KD in rodent tumor model
- iv administration
- Solid tumor targeting
- Non-GLP tox studies planned
- Broaden tumor model testing

ARO-Lung1

- Deep KD in rodent models
- Inhaled administration
- Large animal studies and disease models underway
- Non-GLP tox studies underway

TRiM™: A Dynamic Platform

The result of a drive toward simplicity

Superior activity and targeting capabilities without engineered endosomal escape

Growing libraries of targeting agents, linkers, stabilization chemistries, and PK enhancers enable modular approach…but in a streamlined structure of decorated RNA, that we believe will provide:

- Simplified manufacturing at reduced cost
- Multiple routes of administration
- Faster time to clinical candidates
- Wide safety margins
- Promise of taking RNAi to tissues beyond the liver

Evolution from biologic complexity to small molecule precision and execution

Pipeline

Drug	Indication	Pre-clinical	Pre-IND	Phase 1	Phase 2	Phase 3
ARO-AAT	Alpha-1 Antitrypsin Deficiency			CTA filed Q4 2017		
ARO-HBV	Hepatitis B			CTA filed Q4 2017		
ARO-APOC3	Hypertriglyceridemia		CTA planned Q4 2018			
ARO-ANG3	Hypertriglyceridemia		CTA planned Q4 2018			
ARO-Lung1	Undisclosed		CTA planned Q4 2018			
ARO-HIF2	Renal Cell Carcinoma		CTA planned 2019			
ARO-LPA	Cardiovascular Disease		Partnered with Amgen			
ARO-AMG1	Cardiovascular Disease		Partnered with Amgen			

Arrowhead R&D Facility in Madison, Wisconsin

Newly renovated (2016) laboratories,
>40,000 total sq. ft. space

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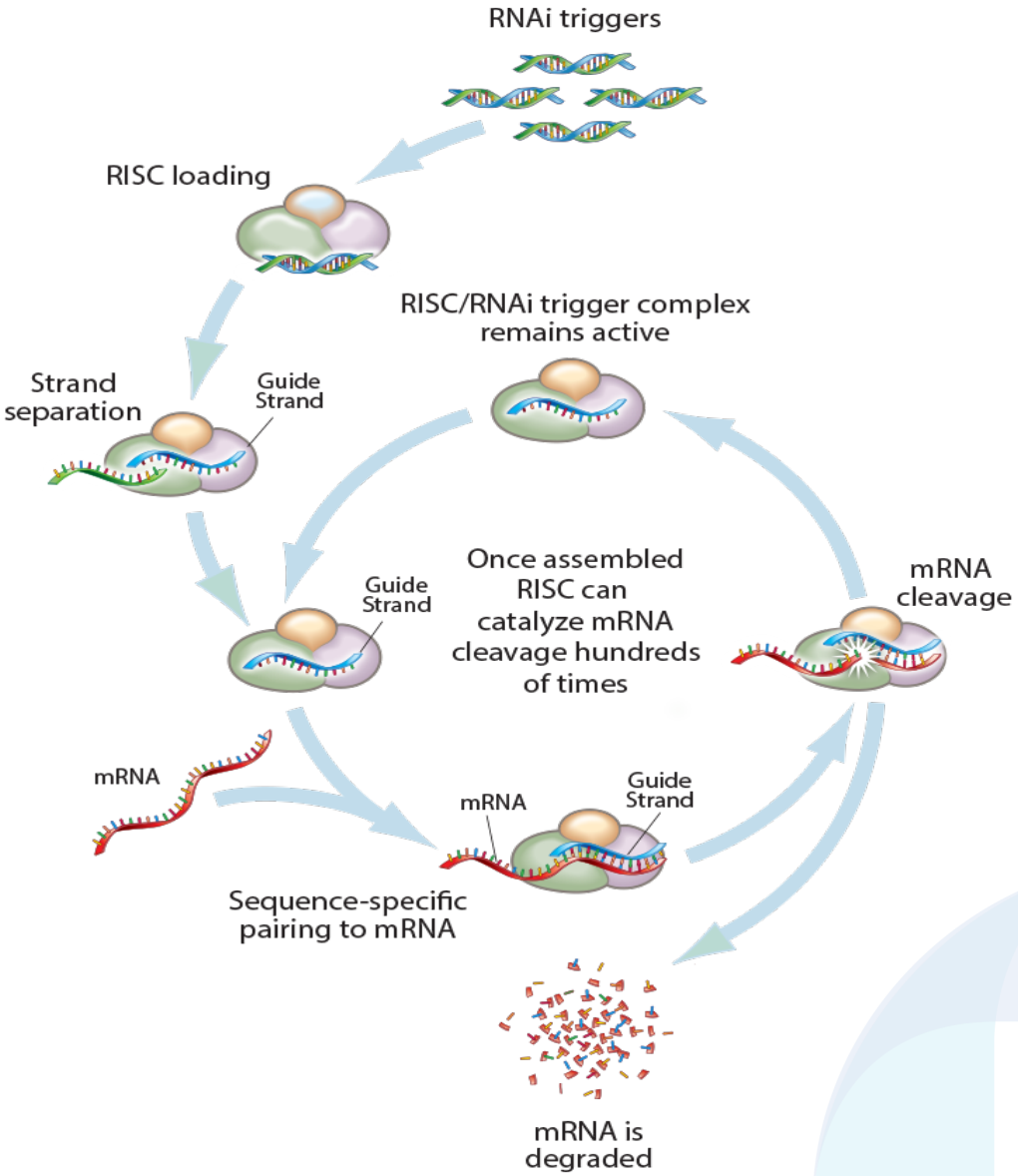
Arrowhead Team

Backup Slides

After RNAi Triggers Get Into Cytoplasm



Hepatitis B Virus Life Cycle