

Development of RNAi-Based Therapeutics Using Dynamic Polyconjugate (DPC) Delivery Technology

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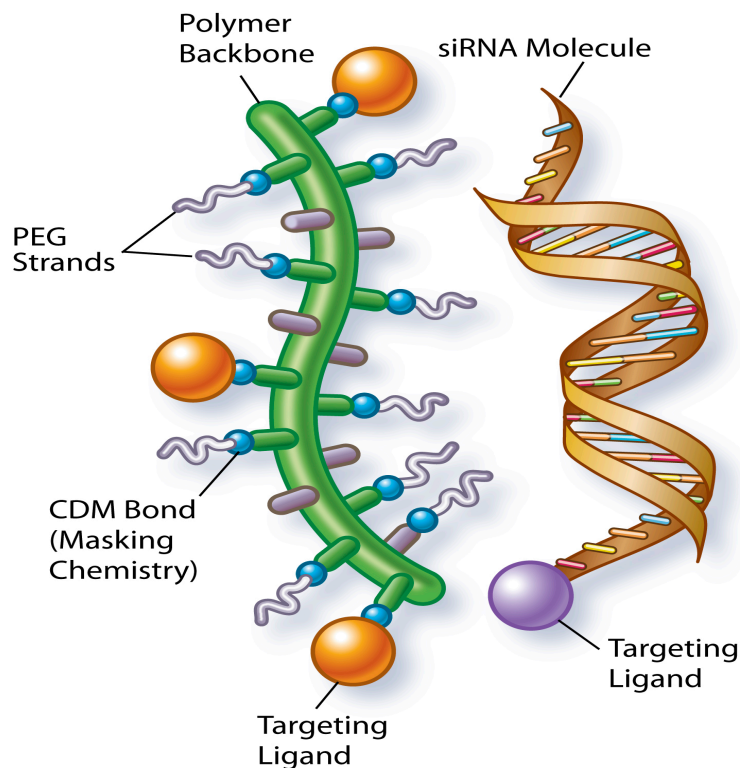
Presentation topics

- Introduction to DPC technology
- ARC-520 for treatment of chronic HBV infection
- ARC-AAT for treatment of liver disease associated with Alpha-1 Antitrypsin Deficiency (AATD)

Two component DPC technology for delivery of RNAi triggers

DPC

- amphipathic polymer for endosomal escape (can be peptide)
- Polymeric amines “masked” with pH-labile moiety, unmasked in endosome
- Slightly negatively charged
- Targetable
- Co-injected with RNAi trigger



RNAi Trigger

- Can be canonical siRNA or other format
- Targeting ligand (eg. cholesterol, GalNAc) attached to sense strand

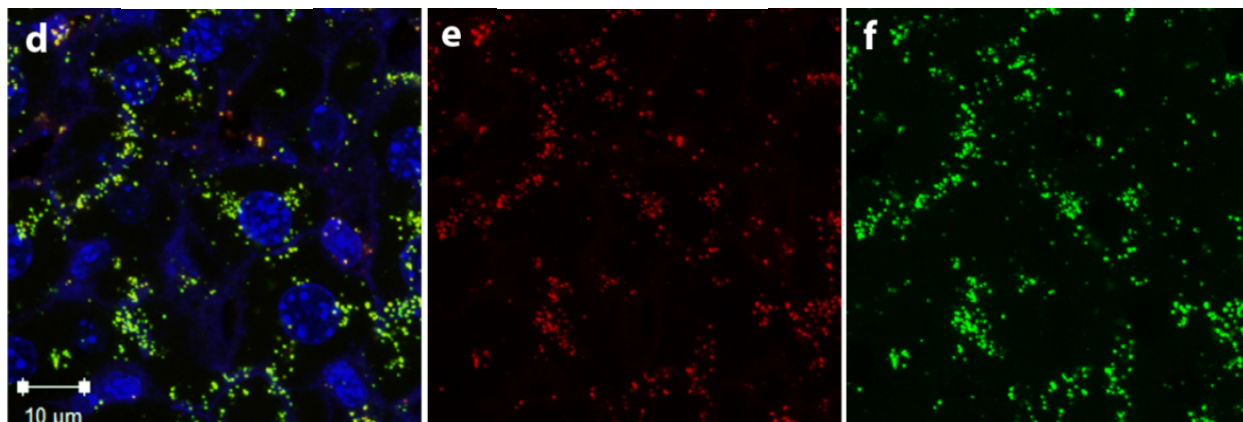
DPC and RNAi trigger do NOT form a complex - they are separately targeted to the tissue of interest

Co-localization of hepatocyte-targeted DPC polymer and cholesterol-siRNA in liver after co-injection

NAG-targeted
polymer

Merged

chol-siRNA



NAG =
N-acetylgalactosamine,
aka GalNAc

% chol-siRNA co-localized with NAG-polymer	% NAG-polymer co-localized with chol-siRNA
87%	70%

mouse

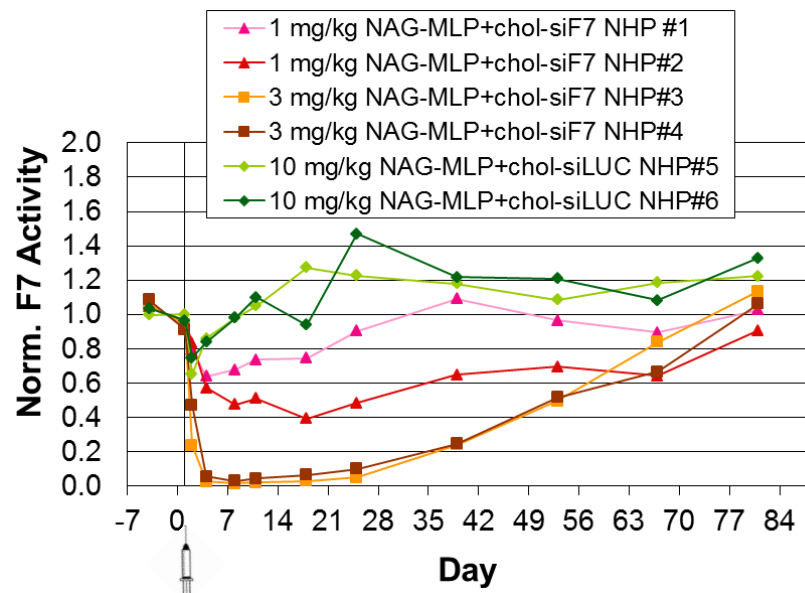
Efficacy in non-human primates

DPC (NAG-MLP) dose titration with constant 2 mg/kg chol-siRNA

Target: Coagulation Factor 7

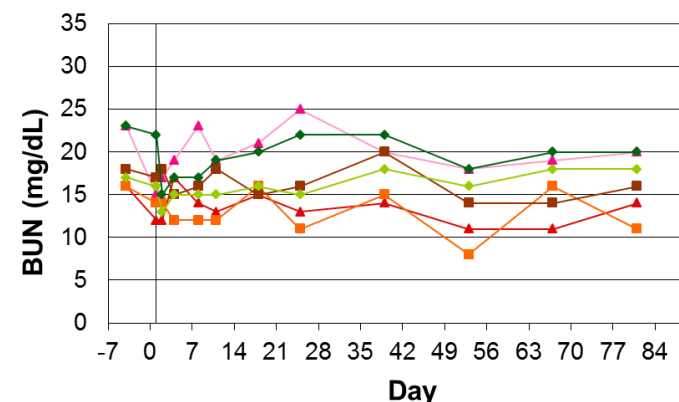
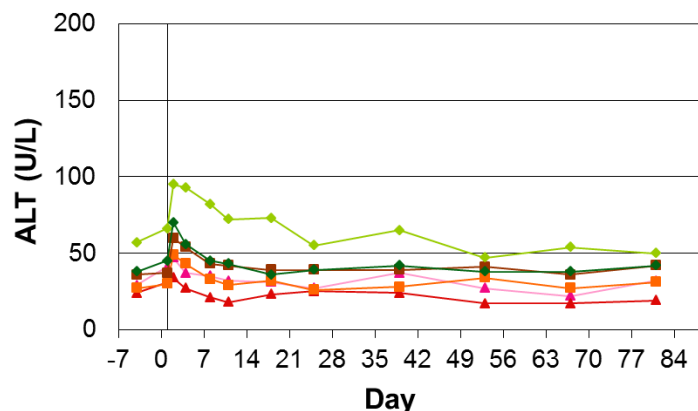
- Highly efficacious after single dose**

- >99% KD at 3 mg/kg NAG-MLP + 2mg/kg chol-siRNA
- >80% KD for 5 weeks



- Good safety profile**

- No changes in clin chem markers
- No changes in hematology
- No changes in cytokine levels



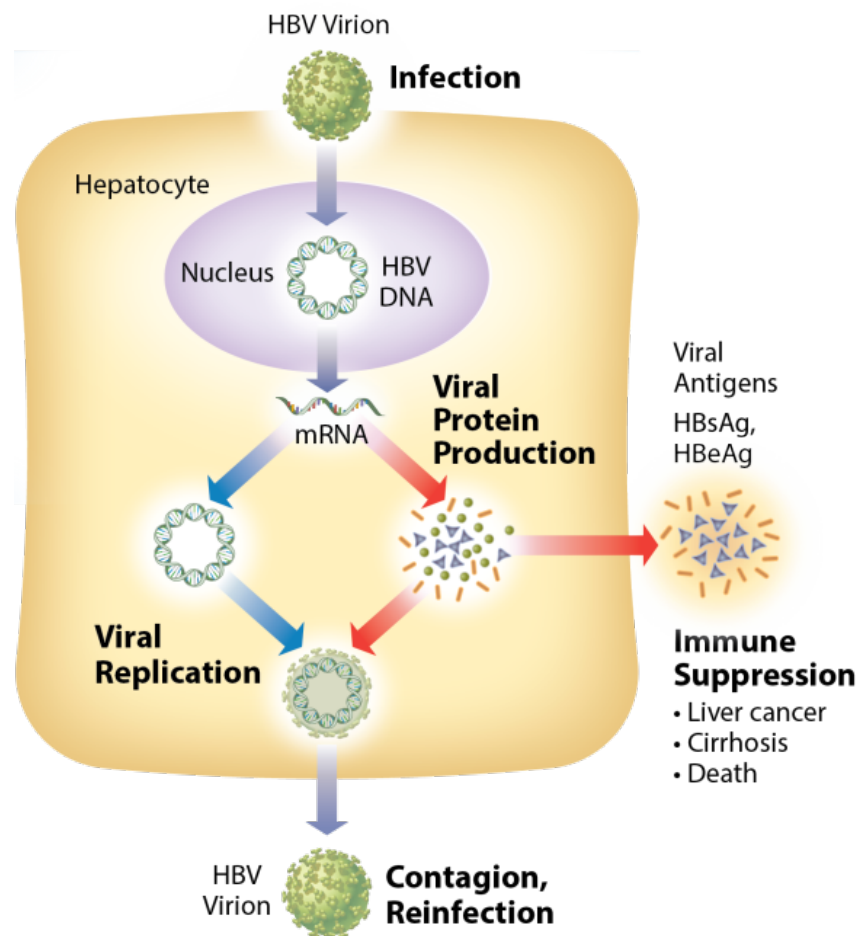
ARC-520

Treatment of chronic hepatitis B virus infection

Chronic hepatitis B virus infection: the need for better therapeutics

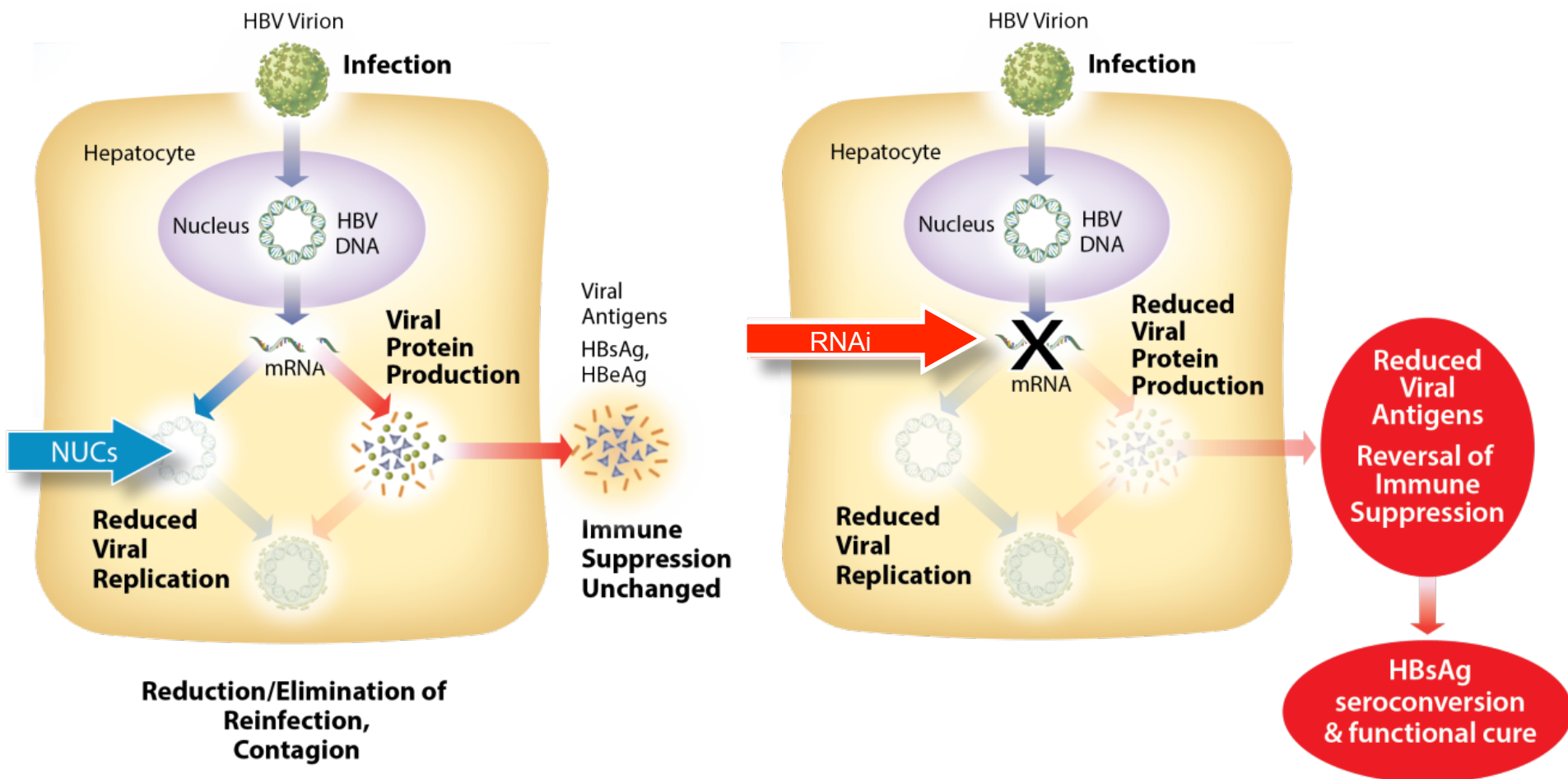
- 400 million people chronically infected worldwide
 - ~1M deaths annually (cirrhosis and hepatocellular carcinoma)
 - Complex interplay between immune system during chronic infection with levels of viral proteins playing an integral role (“T-cell exhaustion”)
 - Loss of surface antigen (HBsAg) or seroconversion $\approx$ functional cure
- Existing drugs (reverse transcriptase inhibitors, PEG-Interferon) are unsatisfactory
 - RT inhibitors “Nucs” (eg. tenofovir, entecavir, lamivudine)
 - Can improve patient outcomes
 - Do not significantly decrease HBsAg levels nor result in HBsAg seroconversion $\rightarrow$ life-long treatment required
 - PEG-Interferon (PEGASYS)
 - 48 week course
 - Can result in HBsAg seroconversion, but only 3-5% /yr (natural conversion rate is $\sim$ 0.5%)
 - Significant side effects (flu-like symptoms, depression)

HBV infection cycle and suppression of the immune response

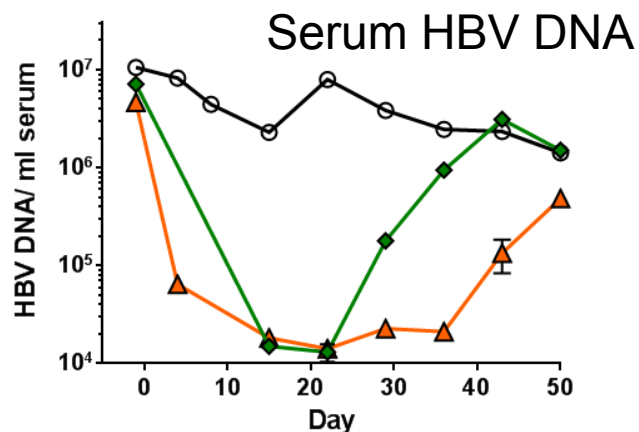
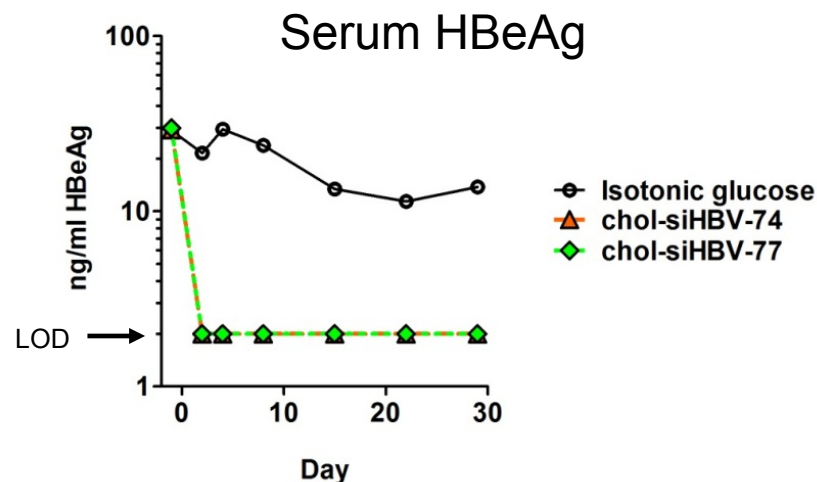
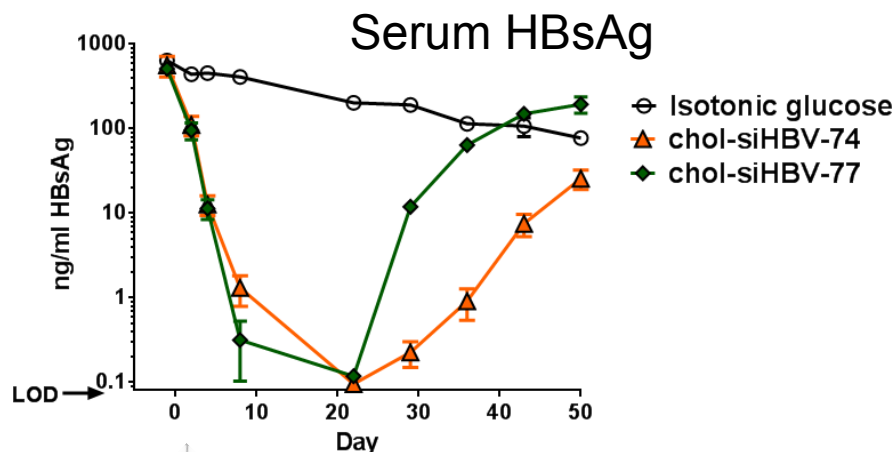


Sustained, high levels of viral protein production (esp. HBsAg) leads to immune suppression and chronicity.

RNAi therapeutics vs. reverse transcriptase inhibitors (NUCs) for treatment of chronic HBV



Co-injection of chol-siHBVs with NAG-MLP in HBV mouse model



Strong reduction of serum viral markers using either chol-siHBV-74 or -77 with NAG-MLP

Decreased HBsAg

- 3-4 log reduction with both chol-siHBVs
- > 2 log reduction for 1 month

Decreased HBeAg to LOD

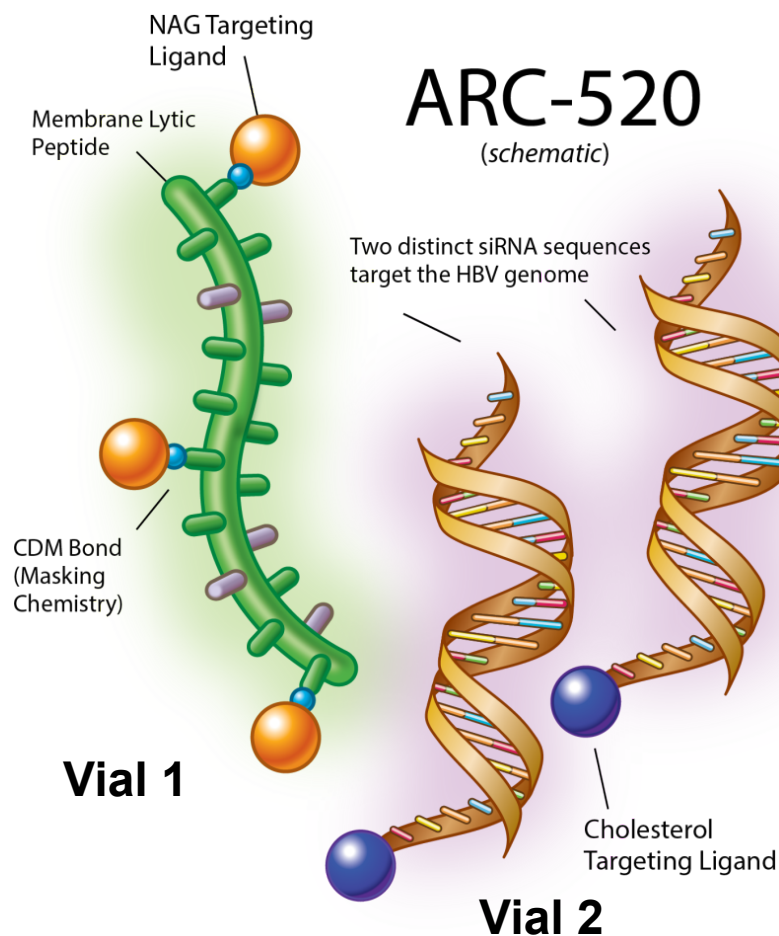
Decreased HBV DNA

- ~ 3 log reduction of HBV DNA for ~ 1 month ¹⁰

6 mg/kg NAG-MLP + 6 mg/kg chol-siRNA

Wooddell et al, Mol Ther 2013 May; 21(5) 973-85

ARC-520 for chronic HBV infection



ARC-520 is a two vial drug

- Vial 1: ARC-520 Excipient
 - Hepatocyte-targeted DPC peptide (NAG-MLP)
 - Lyophilized powder, stable
- Vial 2: ARC-520 API
 - 2 HBV chol-siRNAs in solution
 - Inclusion of two siRNAs gives broader genotype coverage

Liquid in Vial 2 is used to dissolve contents of Vial 1, drug administered IV

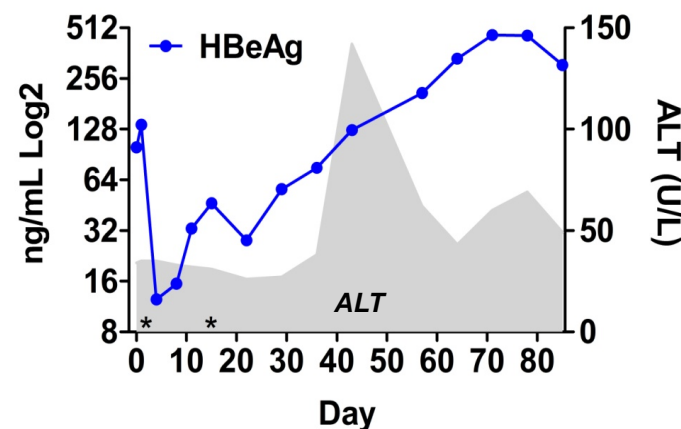
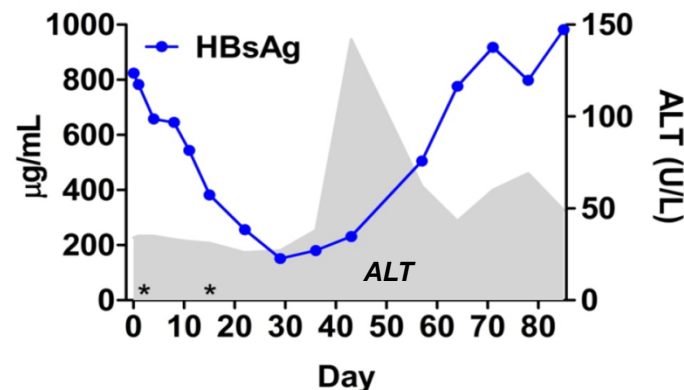
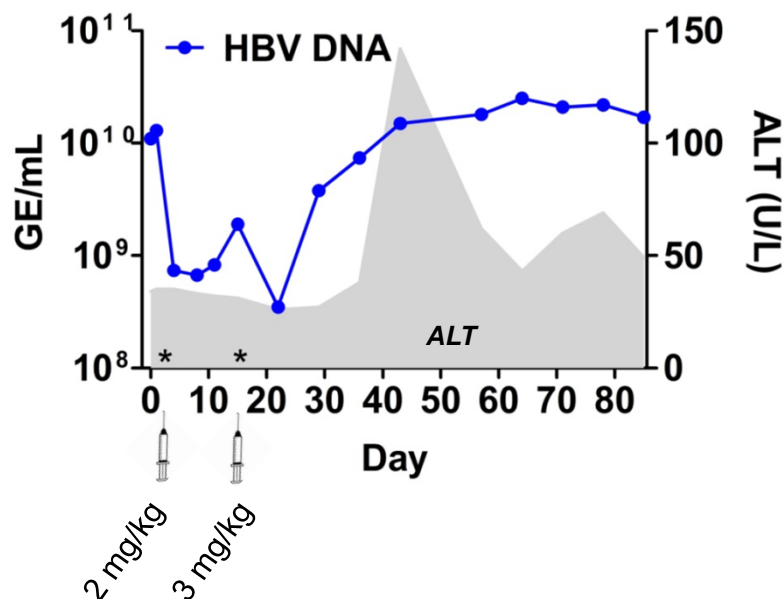
Testing ARC-520 in a chimpanzee chronically infected with HBV

- Key historical attributes
 - 36 year old female, weight 113 pounds
 - Chronic HBV since 1979
 - Liver biopsy shows near 100% staining for HBV
 - Exceptionally high titers of circulating DNA (high viral load) and HBsAg (10^{10} vs. 10^7 in average patient)
 - Genotype B with mismatch for one of the two siRNAs in ARC-520
 - Only 8% of genotype B sequences in GenBank are mismatches for this siHBV

Reduction in HBV after treatment with ARC-520

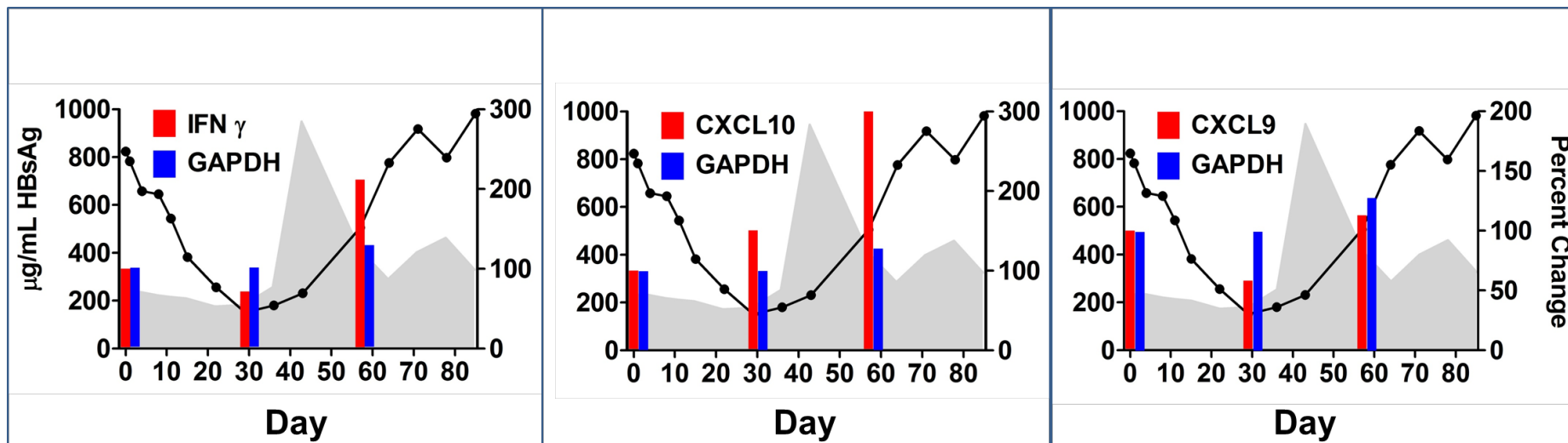
Elevated liver enzymes observed 4 weeks post-last dose

chimpanzee



- Reduction in HBV DNA, HBsAg, HBeAg
- An increase in ALT was observed near the HBsAg nadir.
- The increase occurred 4 weeks **AFTER** the last dose of ARC-520.
→ Not drug toxicity-related
- T-cell activation?

Intrahepatic cytokine/chemokine mRNA



- Liver core biopsies taken on Days -6, 29 and 57
- RT-qPCR performed to determine mRNA levels of IFN γ and the IFN γ -inducible genes CXCL10 (IP10) and CXCL9 (Mig)

- IFN γ $\uparrow$ 210%
- CXCL10 $\uparrow$ 310%
- CXCL9 $\uparrow$ 280% from preceding biopsy

Induction of IFN γ and downstream genes characteristic of T-cell activation

ARC-520 Phase 2

- Trial design

- Chronic HBV patients (HBeAg-neg) on the RTI Baraclude (entecavir)
- Randomized, double-blind, placebo controlled, single IV dose escalation with over the counter oral antihistamine prior to receiving ARC-520
- Dose levels: 1, 2, 3 mg/kg; possible expansion to 4 mg/kg
- 8 patients/cohort (2 placebo, 6 ARC-520)
- Primary objectives: safety in patients; evaluate depth and duration of HBsAg decline
- no discontinuations due to AEs, no SAEs, or AEs rated as severe, no signs of hypersensitivity, no dose limiting toxicities

- Interim results

- 3 mg/kg cohort still blinded and final analysis not yet available
- No clinically significant, treatment emergent changes in measurements of organ toxicity, vital signs, physical exam or ECG rated. All AEs reported to date (n=4) have been mild or moderate and rated as unrelated to study drug by the investigator
- HBsAg KD observed: 1 mg/kg mean nadir = -39% (range -22 to -57)
2 mg/kg mean nadir = -51% (range -46 to -59)
 - Detailed results to be presented at AASLD Nov. 2014

ARC-AAT

Treatment of liver disease associated with Alpha-1
Antitrypsin Deficiency (AATD)

Alpha-1 antitrypsin deficiency

- AATD is a large scale orphan disease
 - Alpha-1 foundation estimates 100,000 in the US
- Mutation in AAT gene 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



Controlled with enzyme replacement therapy

Liver

Accumulation of mutant Z-AAT protein causes clinical liver disease



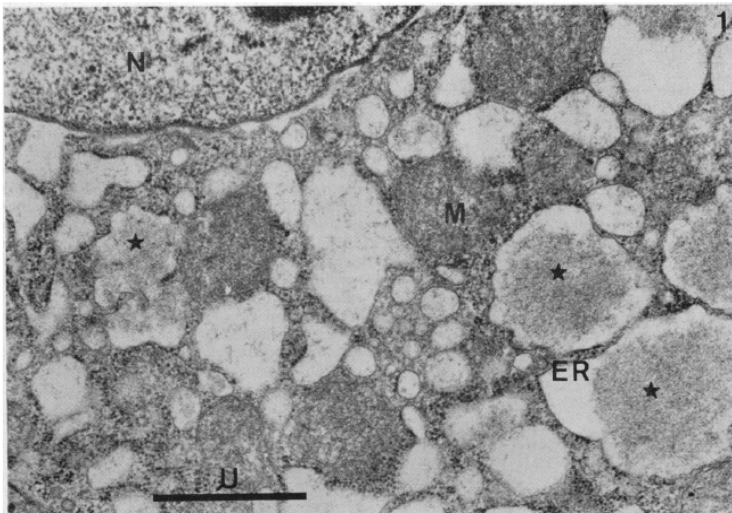
No current treatment

ARC-AAT mechanism of action

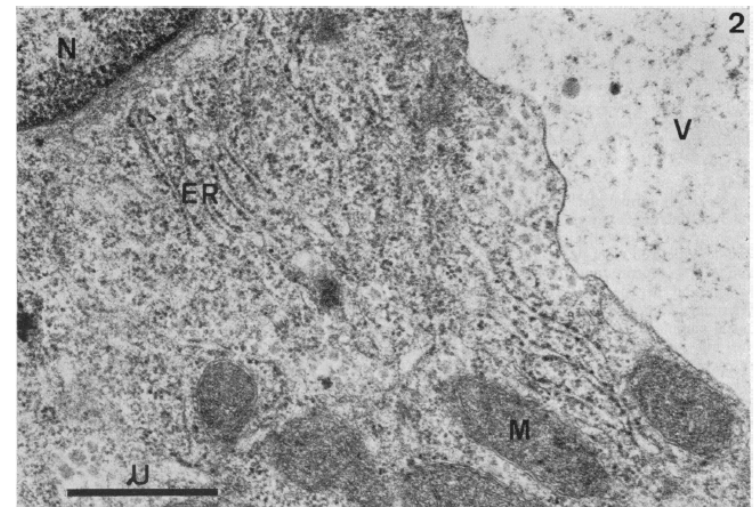
ARC-AAT designed to stop Z-AAT production by silencing AAT gene to:

- Prevent accumulation of disease causing protein in liver
- Allow clearance of accumulated protein
- Prevent repeated cycles of cellular damage and liver tissue repair
- Reverse fibrosis associated with prior damage by allowing repair

PiZZ phenotype (diseased)



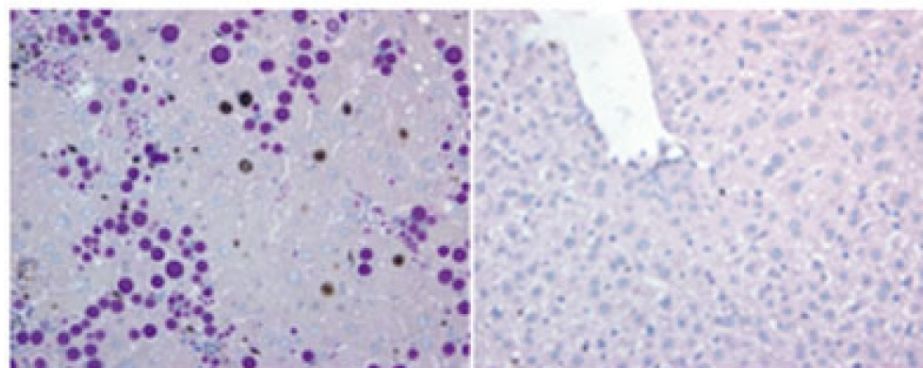
Pi null phenotype (normal)



PiZ model mirrors human adult disease

The transgenic PiZ mouse model expressing the human Z-mutant AAT gene (Z-AAT) recapitulates the human AATD-associated liver phenotype:

- Hepatocytes produce high levels of human Z-AAT
- Hepatocytes are not able to efficiently process and secrete the Z-AAT
- Z-AAT forms polymers that accumulate in large “globules” within the hepatocytes
- These globules stress the hepatocytes, eventually leading to fibrosis and hepatocellular carcinoma



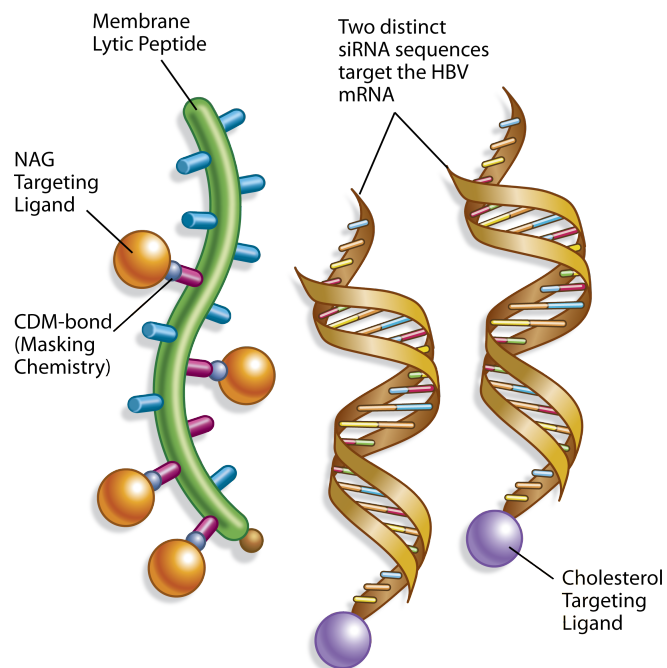
Male Piz

Male WT

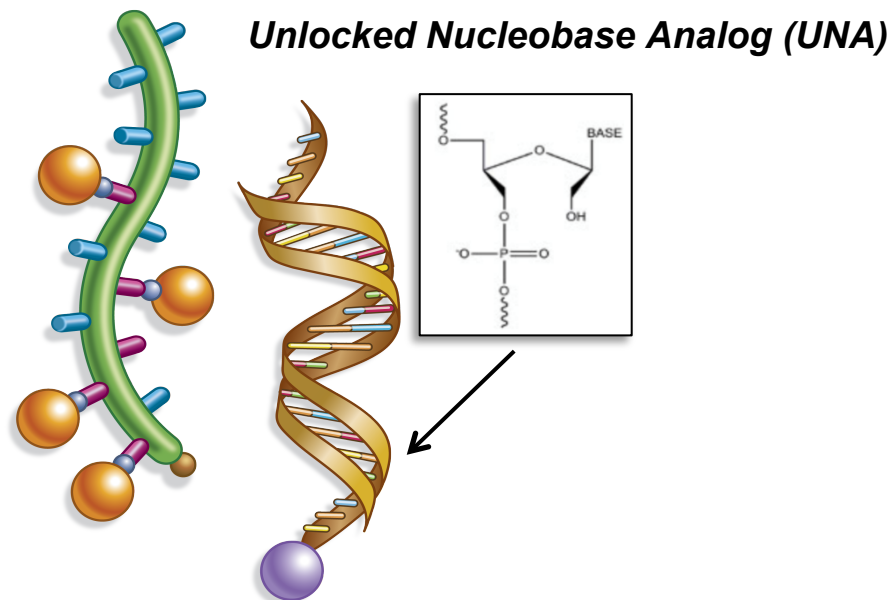
ARC-AAT vs. ARC-520

Same DPC, different RNAi trigger

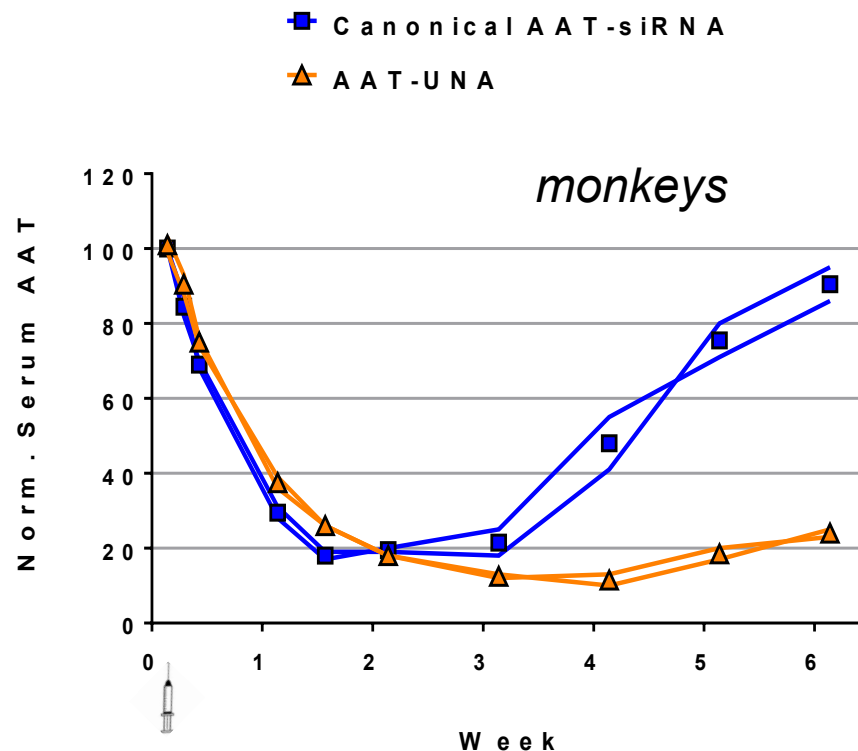
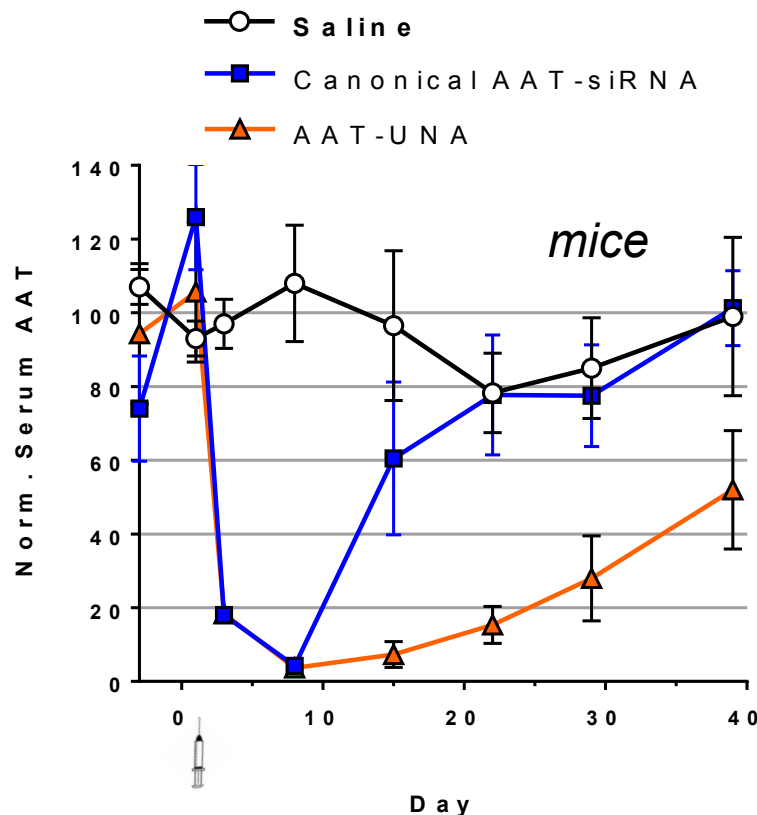
ARC-520 for chronic HBV infection



ARC-AAT for AATD associated liver disease



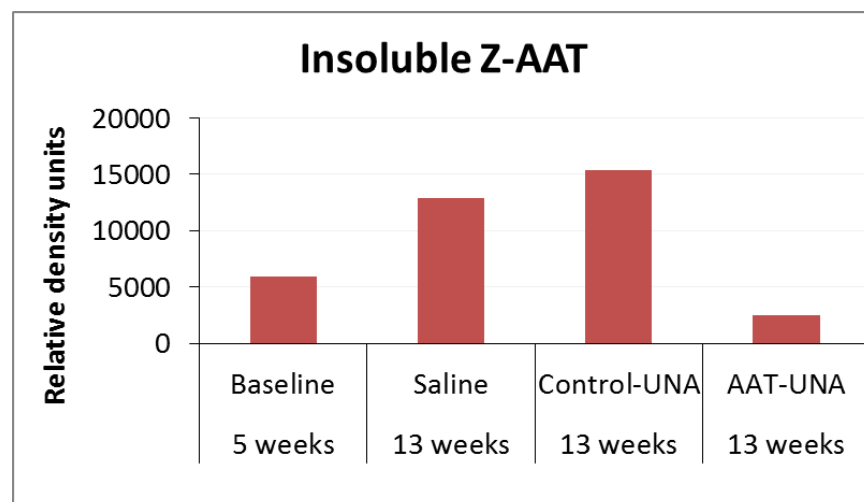
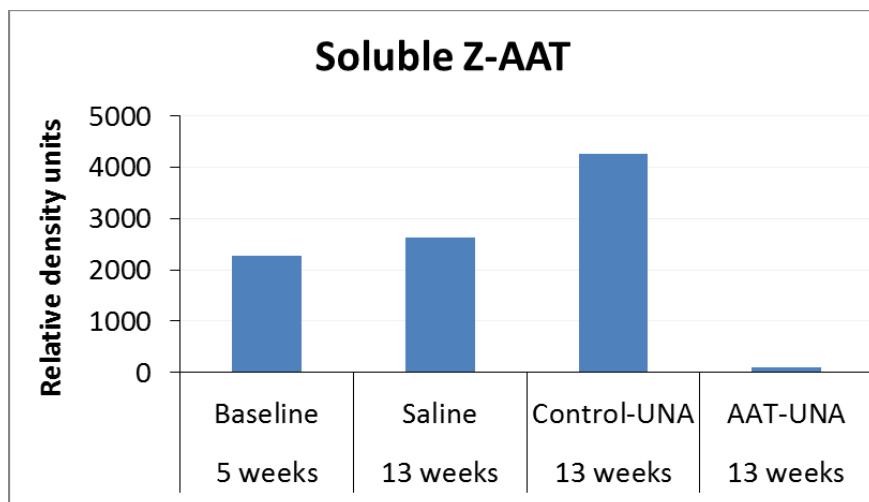
Incorporation of a single UNA in AAT RNAi trigger sequence results in improved KD duration



Max knockdown: UNA $\approx$ canonical siRNA
 Duration of effect: UNA $>$ canonical siRNA

ARC-AAT reduces Z-AAT in livers of PiZ mice

- PiZ mice were dosed 4 times q2w with saline, or ARC-AAT containing control UNA or AAT-UNA beginning at 5 weeks of age (baseline).
- After 8 weeks, soluble (monomeric) and insoluble (polymeric) Z-AAT in the liver were measured.

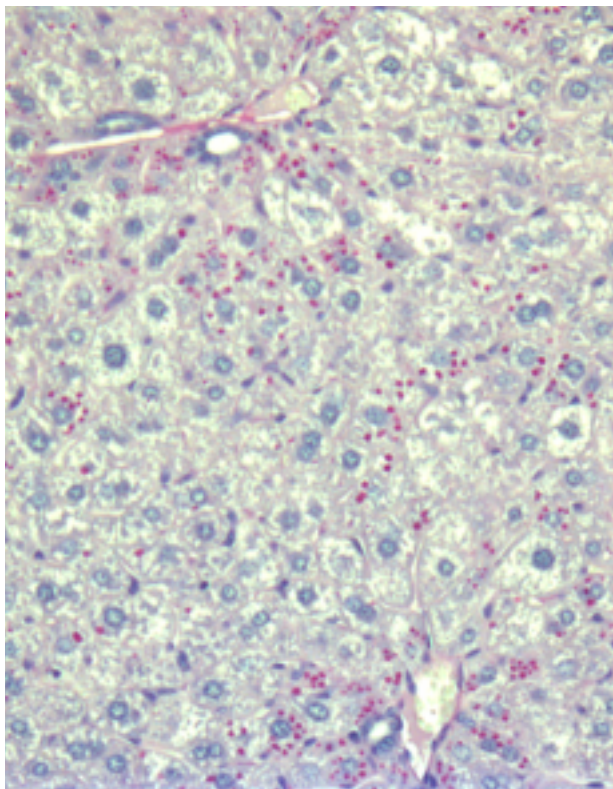


ARC-AAT reduces soluble and insoluble forms of Z-AAT in livers of PiZ mice

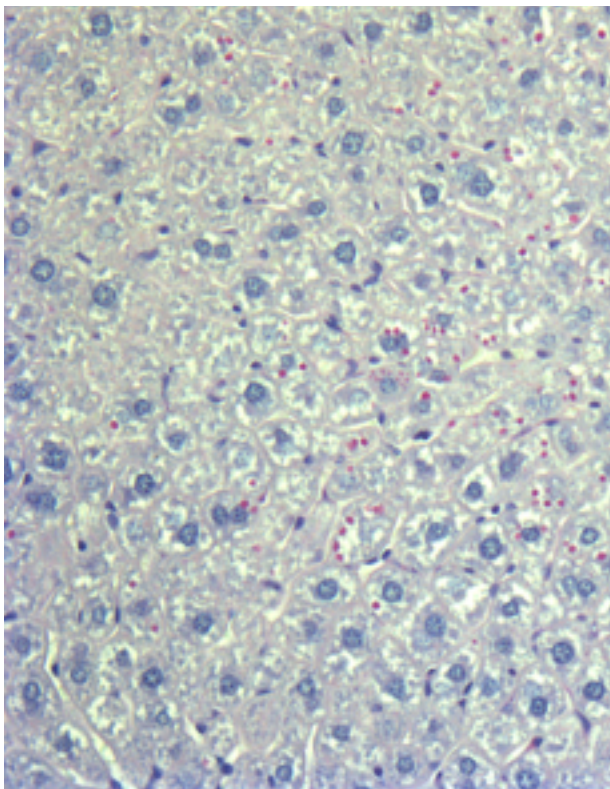
8 mg/kg

Reduction in Z-AAT liver globules

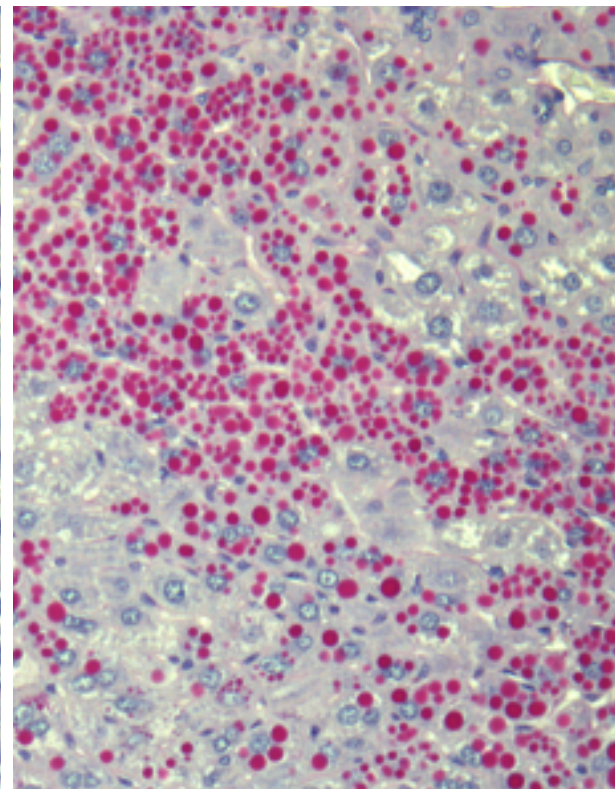
Baseline
5 weeks old



AAT-UNA q2w
13 weeks old



Saline
13 weeks old



Liver globule burden is reduced after two months of
q2w ARC-AAT treatment

ARC-AAT: Phase I clinical plan

- Regulatory filing planned by EOY 2014
- Single ascending dose study
- Healthy volunteers and AATD patients
- **Primary Objective:**
 - Determine the safety and tolerability of escalating doses of ARC-AAT
 - Evaluate the pharmacokinetics of different doses
- **Secondary Objective:**
 - Evaluate the depth and duration of decline in serum total alpha-1 antitrypsin levels
 - Time for serum alpha-1 antitrypsin levels to return to baseline

Summary

- Two molecule DPC platform allows effective delivery of RNAi triggers to liver
- ARC-520 for chronic HBV infection
 - Preclinical studies show deep KD in mouse model after single injection
 - Effects observed in chronically HBV infected chimpanzee
 - KD of HBV DNA and viral proteins
 - ALT flare observed near HBsAg nadir – immune reactivation?
 - Clinical phase 2 interim results show KD of sAg in chronic HBV patients after single dose
- ARC-AAT for AATD-associated liver disease
 - Effective at reducing liver burden of Z-AAT globules in mouse model
 - Use of UNA in RNAi trigger extends duration of effect in mice and monkeys
 - Regulatory filings for Phase I in healthy volunteers and AATD patients planned EOY 2014

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