Arrowhead Research
CORPORATION

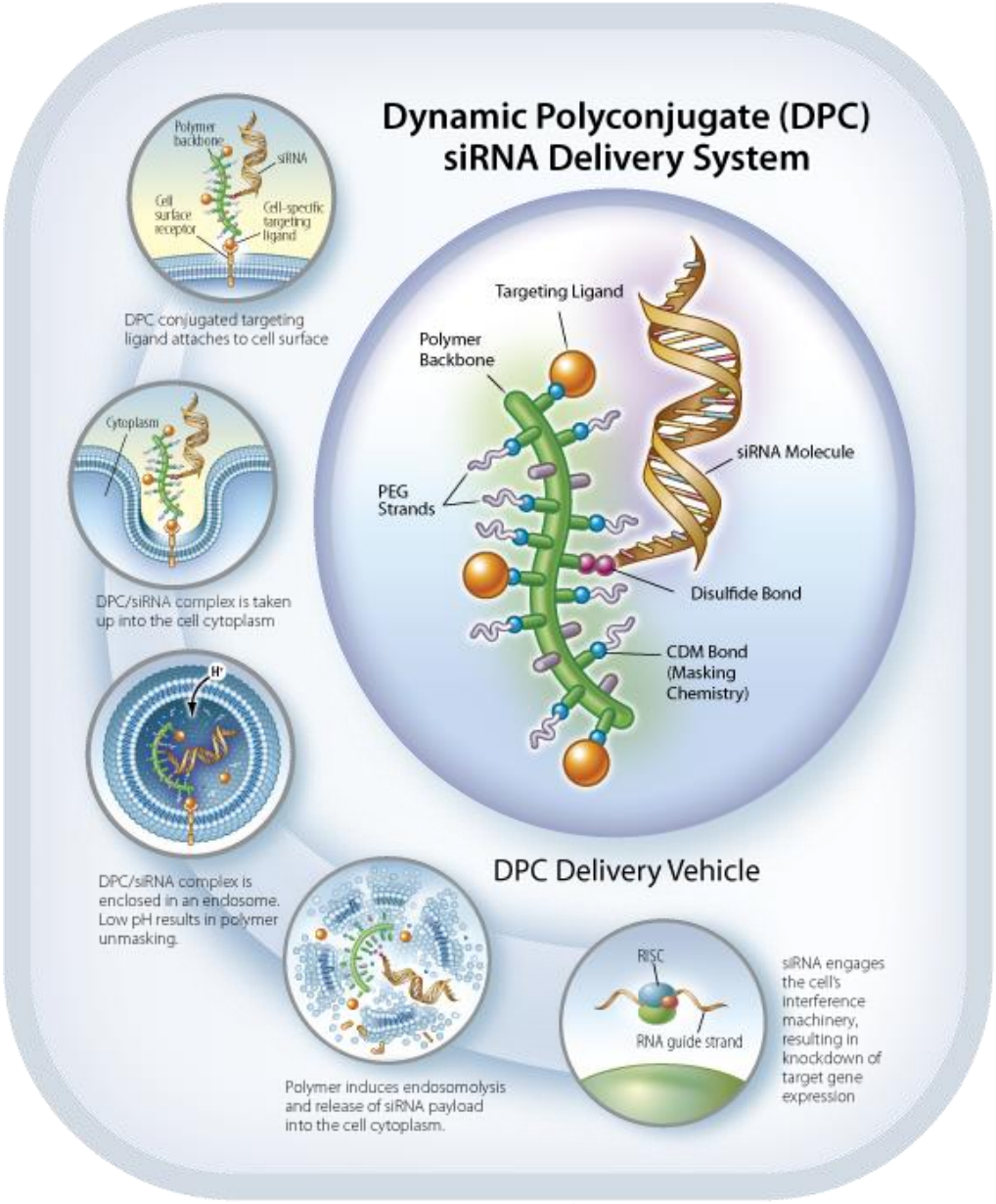
DPC Technology for Delivery of Therapeutic siRNAs

EuroTIDES

November 14, 2013

David Lewis, PhD
Chief Scientific Officer
Arrowhead Research Corporation

Dynamic Polyconjugate (DPC) technology for siRNA delivery *in vivo*

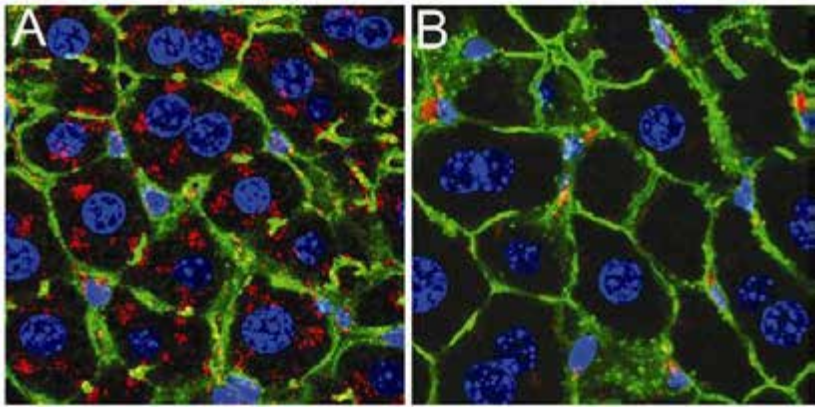


- **Composition and physical characteristics**
  - Endosomolytic, amphipathic polymer
  - Reversibly "masked" with pH-sensitive CDM linker attached to PEG or ligand
  - siRNA reversibly attached via disulfide
  - Very small, 5-15 nm in size
  - Slightly negatively charged
- **Cellular uptake is ligand-driven (N-acetyl galactosamine (NAG)) for hepatocytes)**
- **↓ pH in endosomes drives polymer unmasking**
- **Unmasked polymer disrupts endosomal membrane**
- **siRNA released to cytoplasm**

DPCs for targeted delivery to hepatocytes

N-acetyl galactosamine ligand (NAG)

ICR mice, T=60'



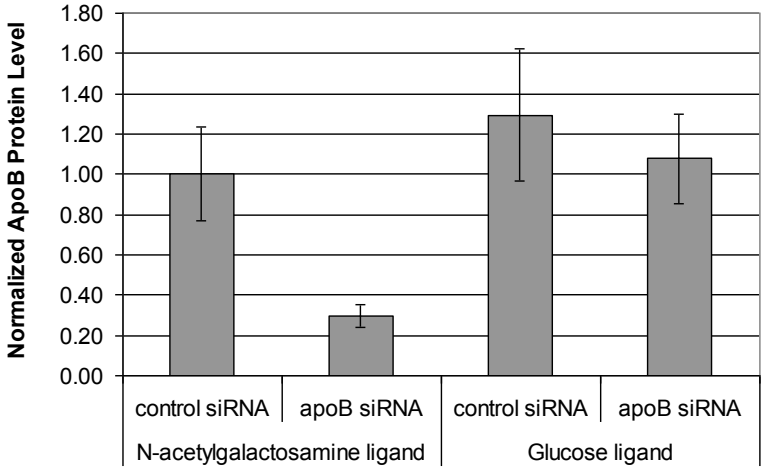
NAG ligand
(hepatocyte targeted)

glucose ligand
(non-targeted)

DPC-siRNA
nucleus
cell membrane

NAG is a ligand for the asialoglycoprotein receptor highly expressed on hepatocytes

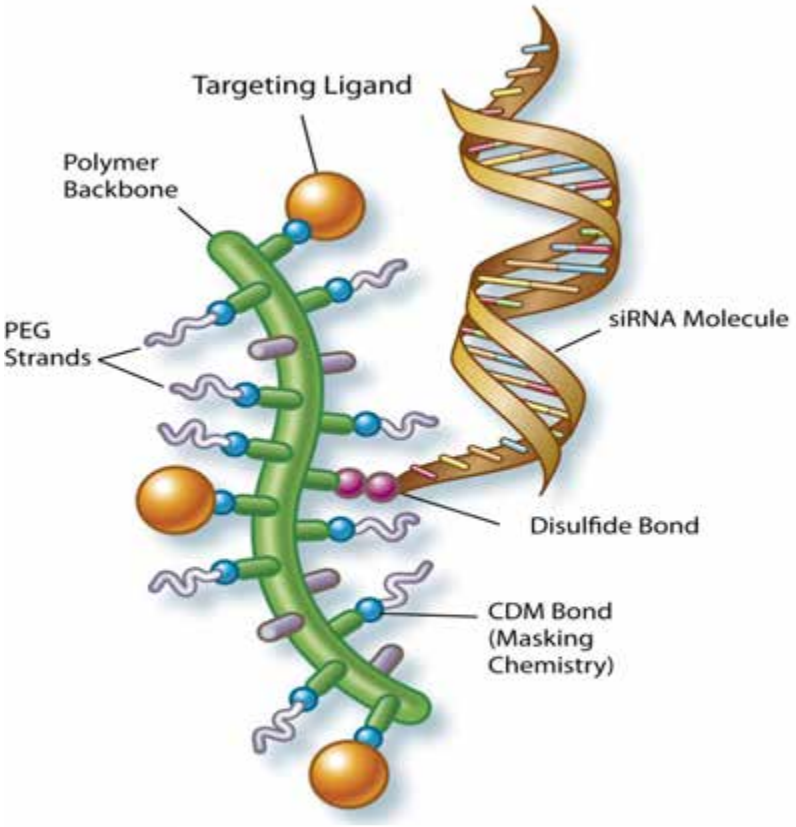
Hepatocyte-uptake of DPCs is ligand dependent



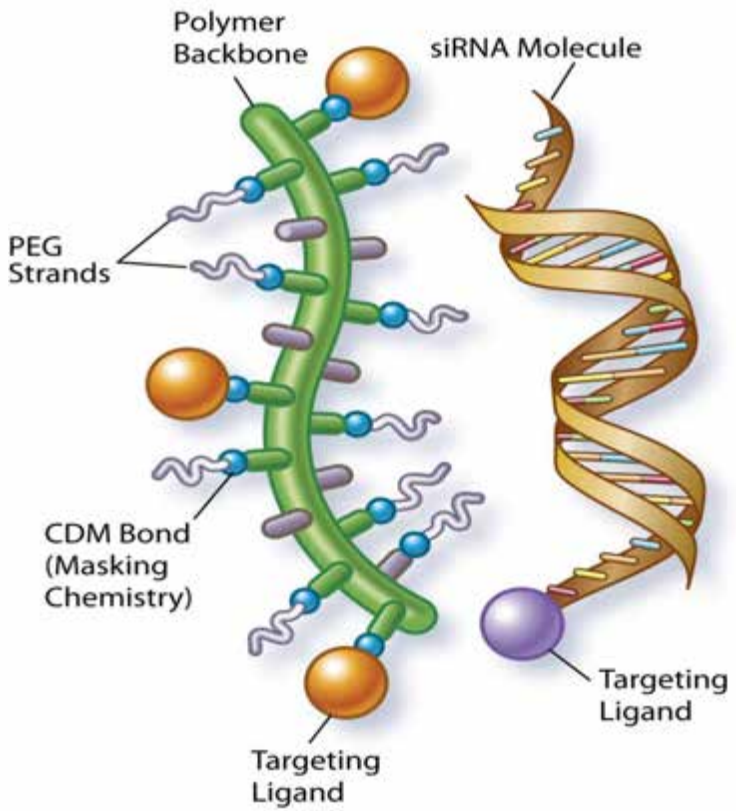
Target gene knockdown is ligand dependent

Rozema, Lewis et al. Proc Natl Acad Sci U S A. 2007 Aug 7;104(32):12982-7

DPC 2.0 – Separate targeting of the DPC polymer and siRNA



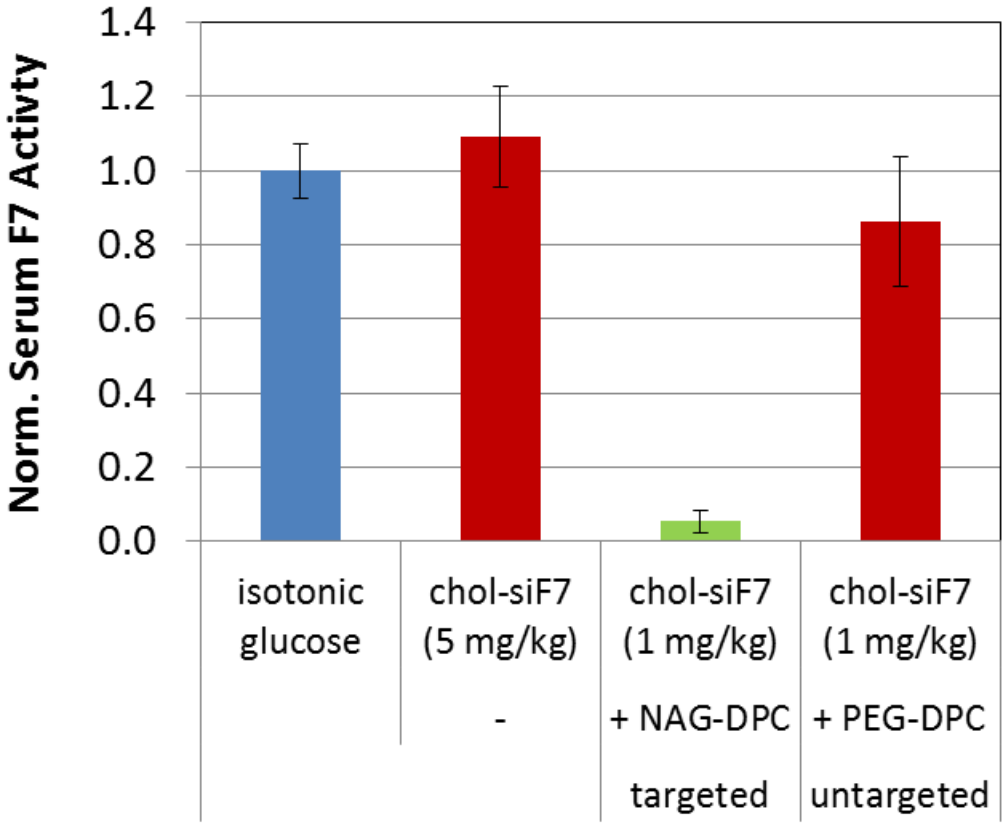
Prototypical DPC
Covalent attachment of siRNA to masked polymer



DPC + targeted siRNA
Masked polymer and siRNA are NOT attached and do NOT interact.
Targeted independently to the same cell after co-injection

Co-injection of hepatocyte-targeted NAG-DPC improves delivery of liver-tropic chol-siRNA

Target: Coagulation Factor 7



mice, single i.v. injection, 48 hr timepoint

Wong et al, Nucleic Acid Ther. 2012 Dec;22(6):380-90

Using peptides with membrane-lytic properties as DPC polymers

Melittin (2009) by Julian Voss-Andreae

Melittin peptide as a model

Naturally occurring peptide (component of bee venom)

Amphipathic with known membrane-lytic activity

DPC peptides

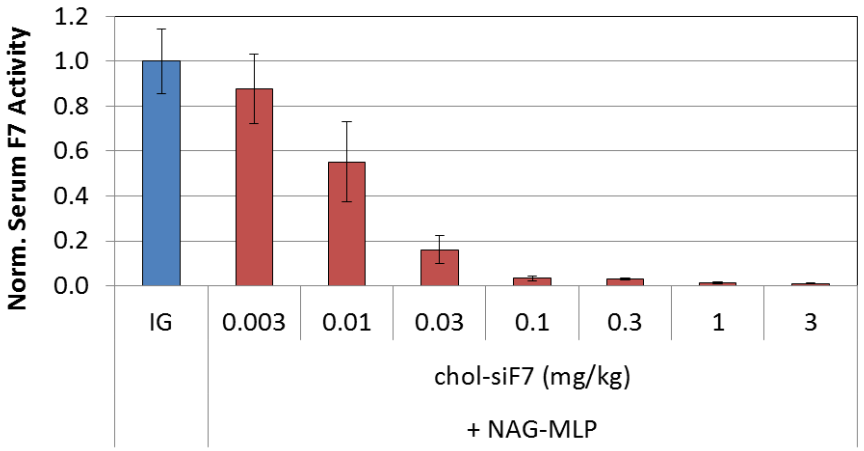
Synthetic membrane-lytic peptides (MLPs) modeled on melittin

DPC masking chemistry used to attach targeting ligands (eg. NAG) and mask membrane-lytic activity

>100 MLPs screened *in vivo* for chol-siRNA delivery efficacy

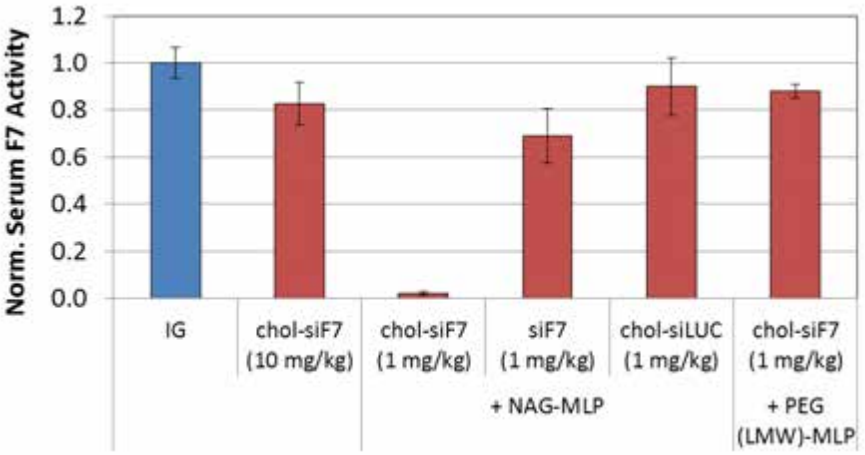
Co-injection of NAG-MLP and chol-siRNA

Chol-siRNA titration and requirements for target gene KD in liver



Co-injection of NAG-MLP with chol-siF7 enables highly efficient delivery

- ED_{50} = 0.01 mg/kg chol-siF7
- ED_{99} = 1 mg/kg chol-siF7



<u>Target gene knockdown requires</u>: Liver-tropic siRNA (cholesterol-siRNA) ***<u>and</u>*** hepatocyte-targeted DPC peptide (NAG-MLP)

mice, single IV dose 6 mg/kg NAG-MLP, 48 hr timepoint

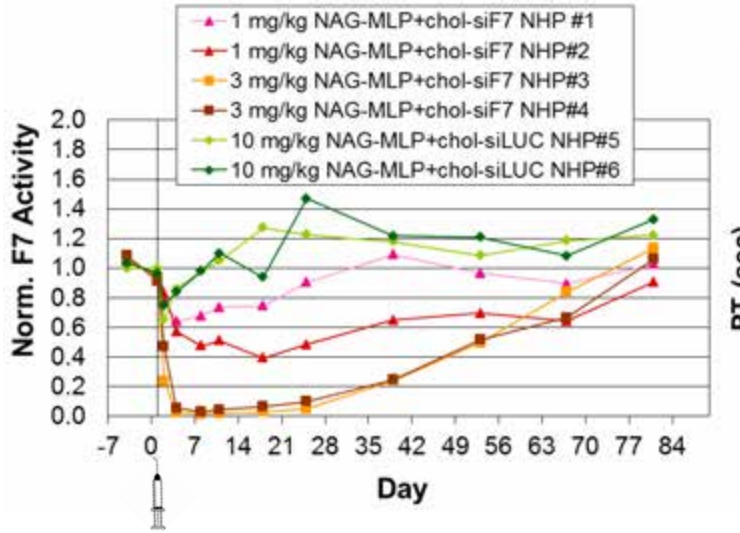
Wooddell et al, Mol Ther 2013 May; 21(5) 973-85

Efficacy in non-human primates

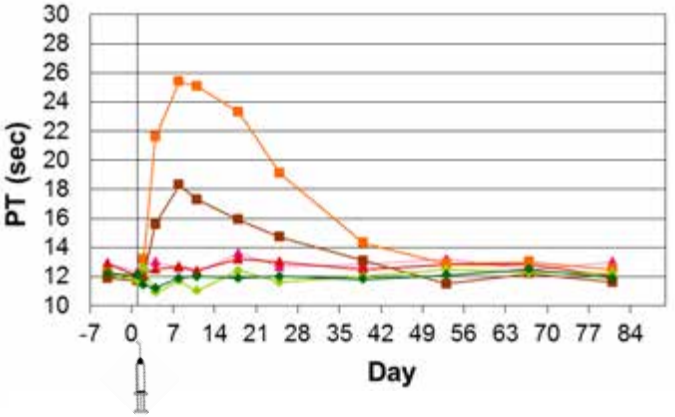
NAG-MLP dose titration + 2 mg/kg chol-siRNA, single iv dose

Target: Coagulation Factor 7

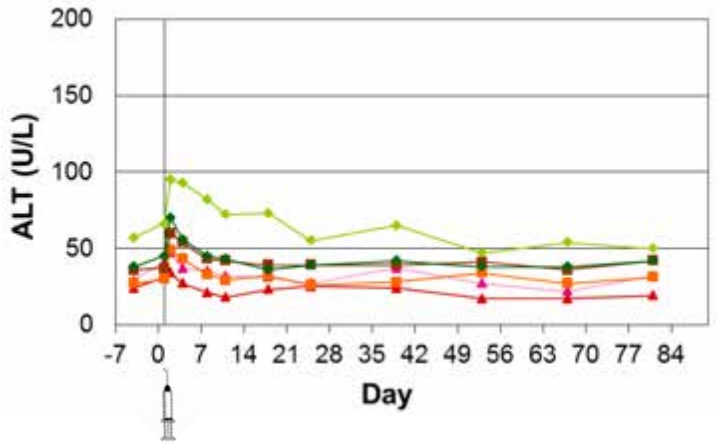
- **Highly efficacious**
  - *>99% KD at 3 mg/kg NAG-MLP @ 2mg/kg chol-siRNA*
  - *>80% KD for 5 weeks*
  - *↑ PT with F7 KD*

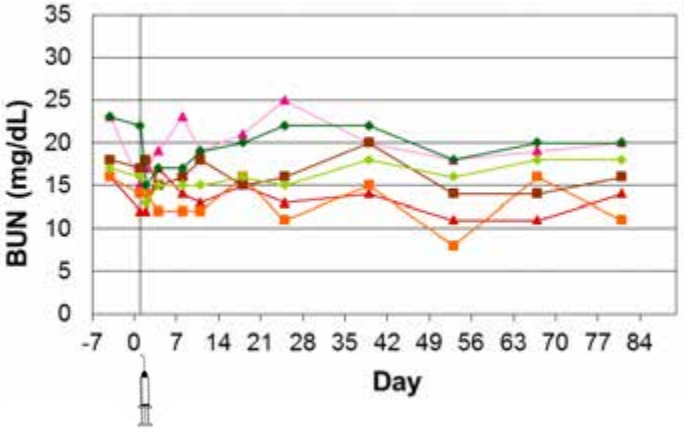


Increased clotting time due to F7 knockdown



- **No toxicity reached**
  - *No changes in clin chem markers*
  - *No changes in hematology*
  - *No changes in cytokine levels*





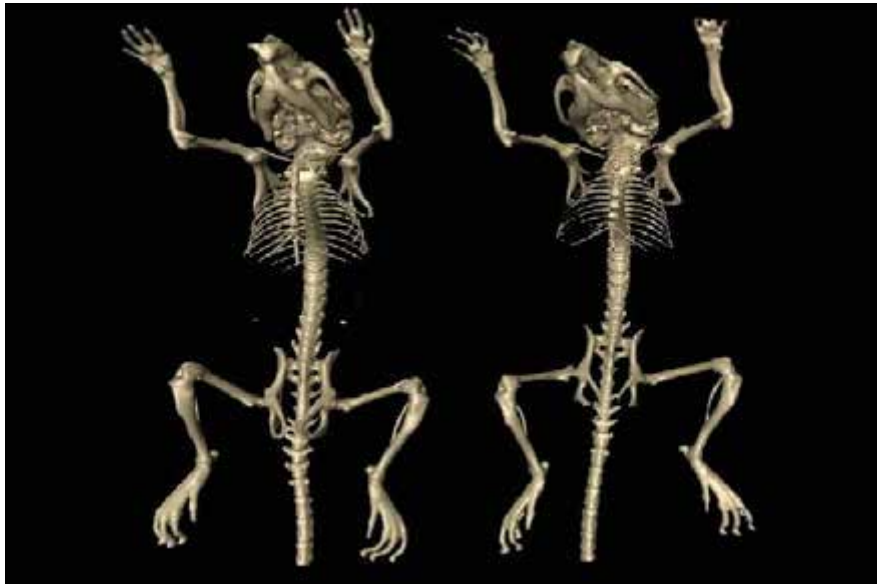
2 mg/kg chol-siRNA

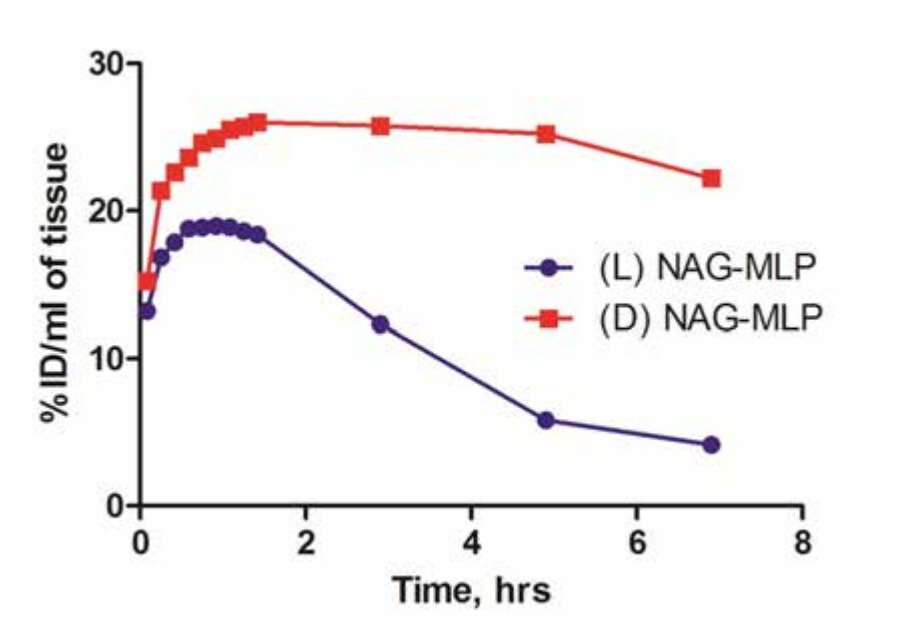
Wooddell et al, Mol Ther 2013 May; 21(5) 973-85

PET imaging of mice injected with ^{124}I-NAG-MLP

(L) NAG-MLP vs. non-biodegradable (D) NAG-MLP analog

NAG-MLP(L) NAG-MLP(D)





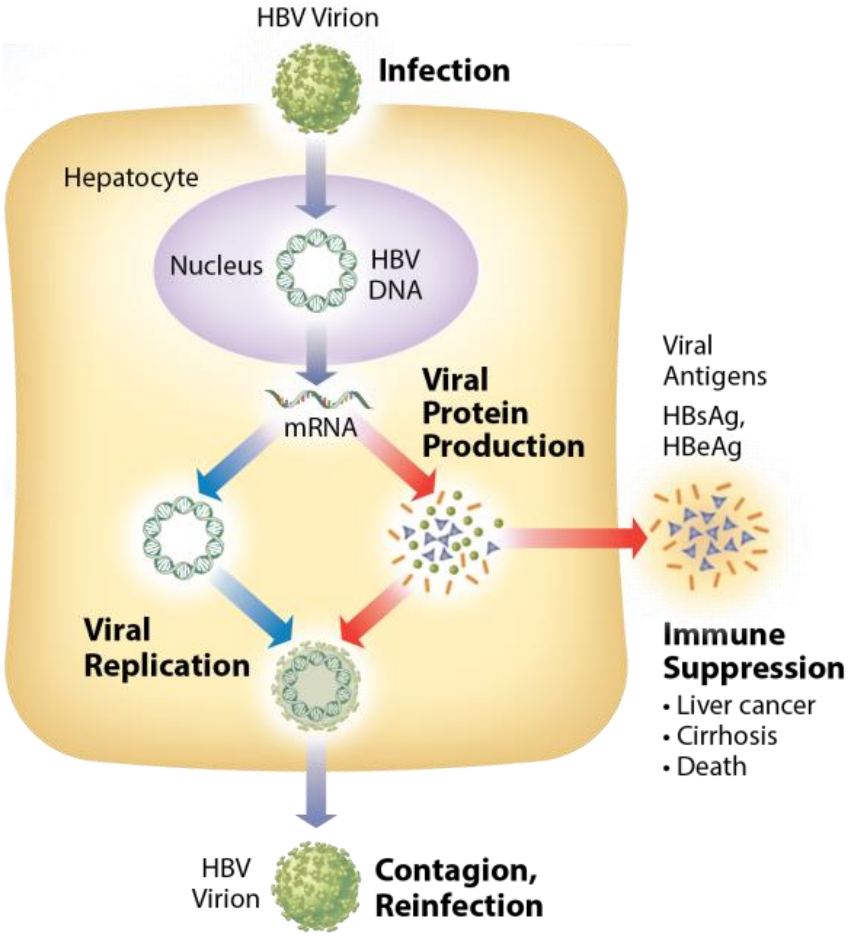
NAG-MLP (L) is rapidly metabolized in the liver and eliminated

Dr. Jamie Weichert, Small Animal Imaging Facility, University of Wisconsin Carbone Cancer Center

Chronic Hepatitis B Virus infection: The need for better therapeutics

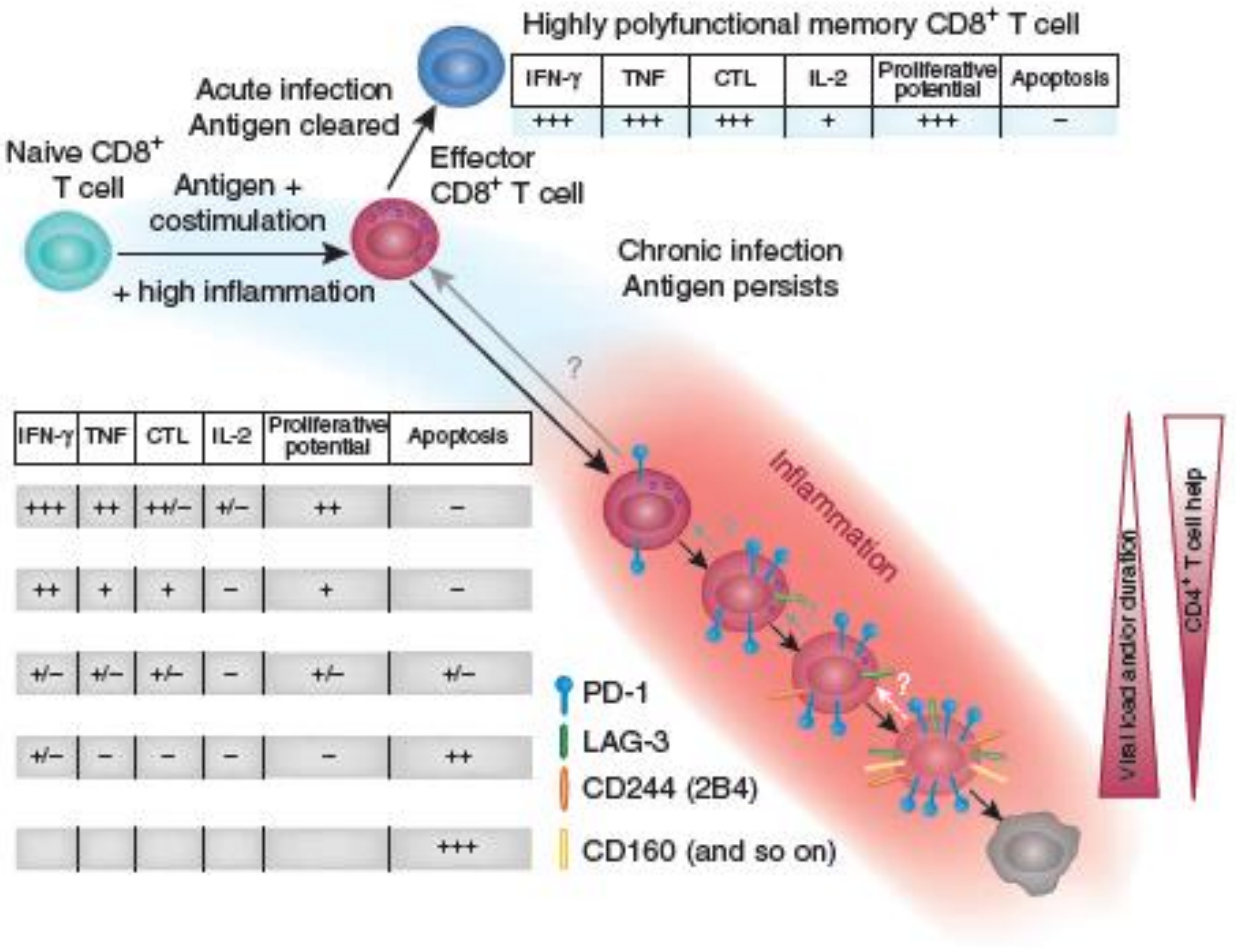
- 360 million people chronically infected worldwide
  - >500,000 deaths annually (cirrhosis and hepatocellular carcinoma)
  - Complex interplay between immune system and chronic infection with levels of viral proteins playing an integral role (T-cell exhaustion)
  - Surface antigen (HBsAg) seroconversion ≈ 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 thus require life-long treatment
  - PEG-Interferon (PEGASYS)
    - Can result in HBsAg seroconversion, but only 3-5% /yr (natural conversion rate is ~ 0.5%)
    - Significant side effects (flu-like symptoms, depression)

HBV infection cycle and immune suppression



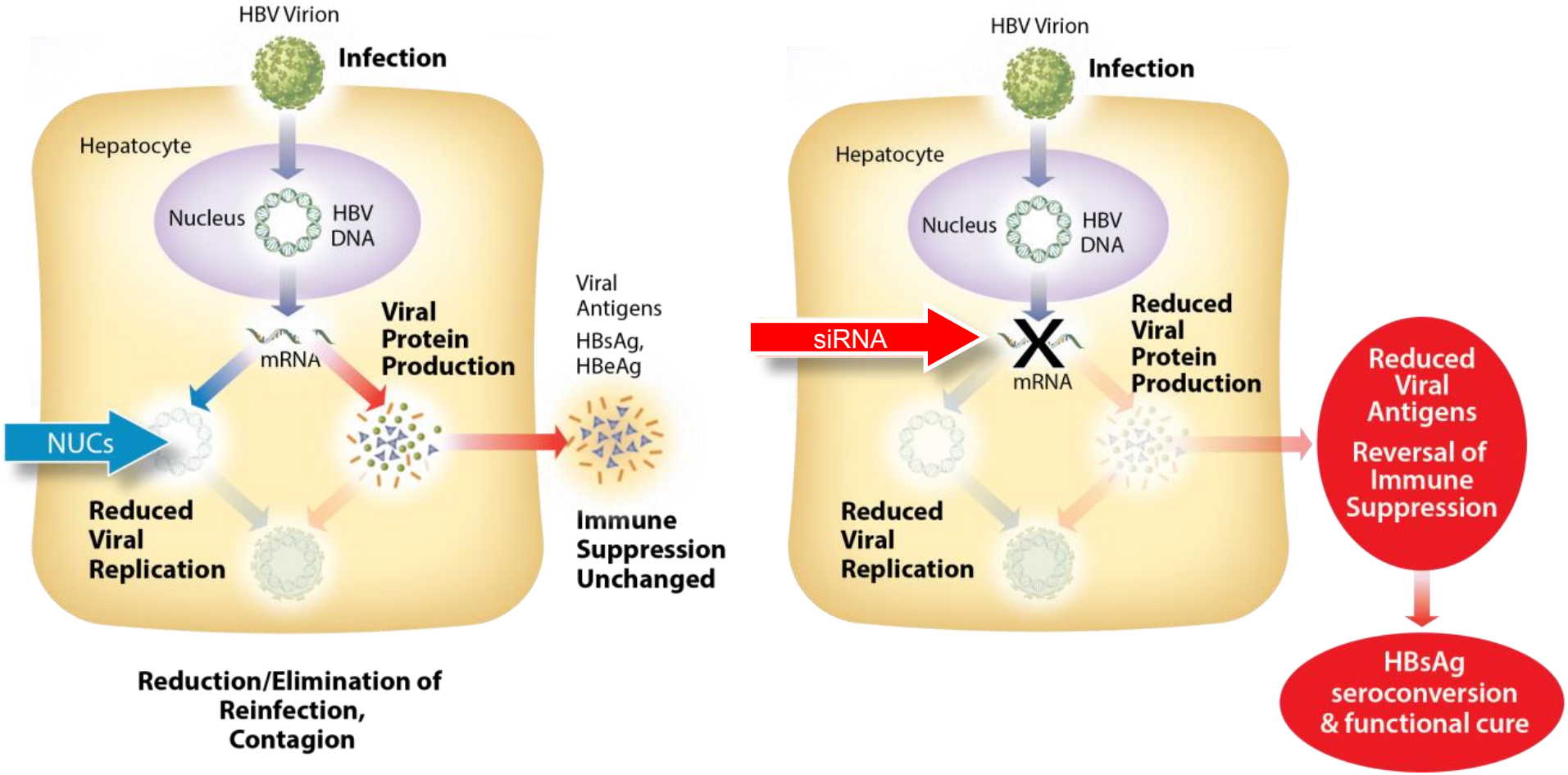
Sustained, high levels of viral protein production (esp. HBsAg) leads to immune suppression and chronicity.

T-cell exhaustion during chronic infection



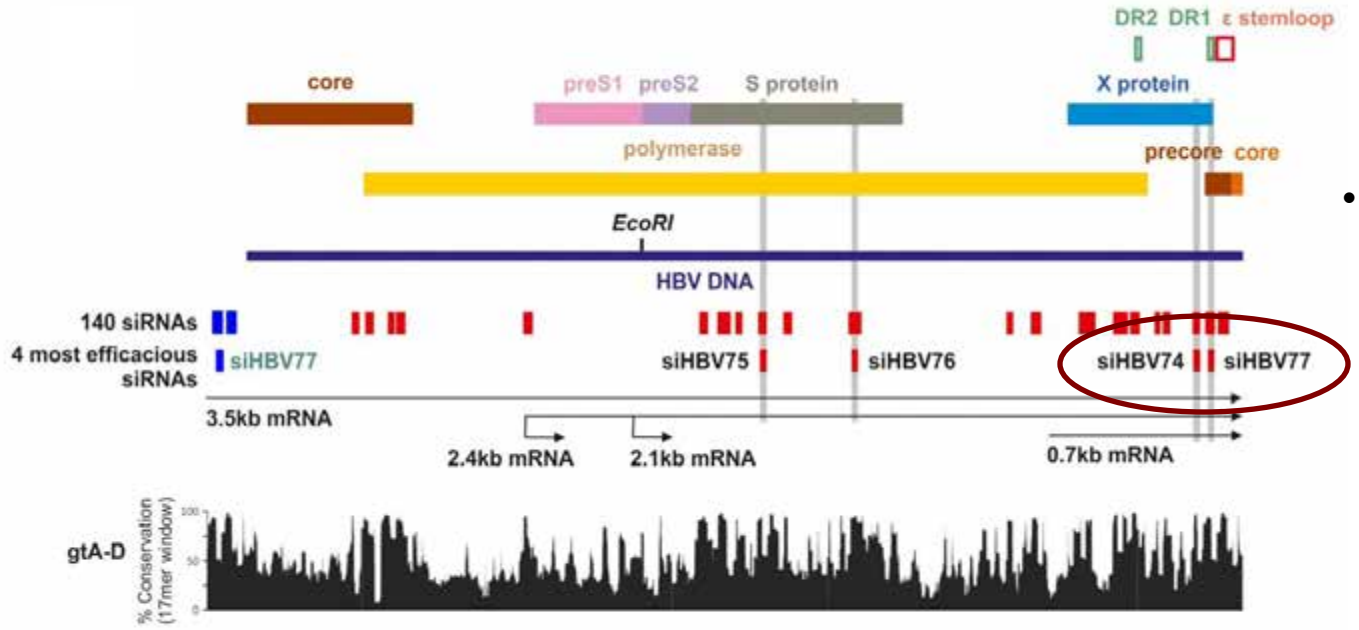
Wherry, Nat Immunol 2011 June; 12(6) 492-9

RNAi therapeutics for treatment of chronic Hepatitis B

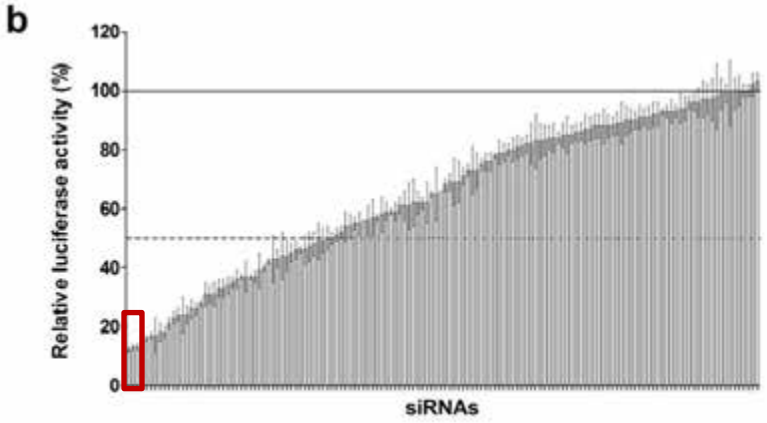


RNAi treatment for chronic Hepatitis B

siRNA design and in vitro screening



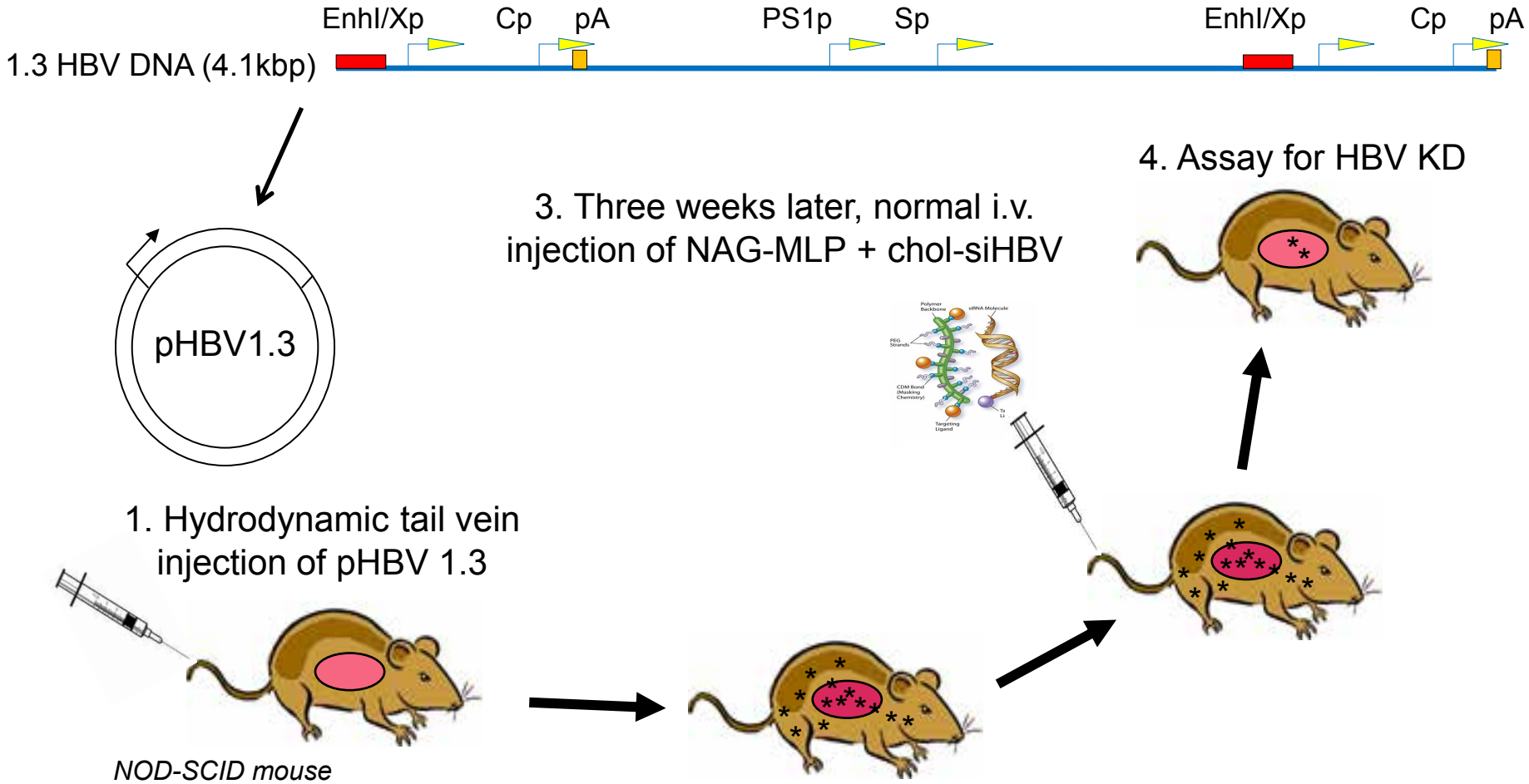
- Designed 140 siRNAs targeting conserved regions in GenBank HBV sequences (2,754)



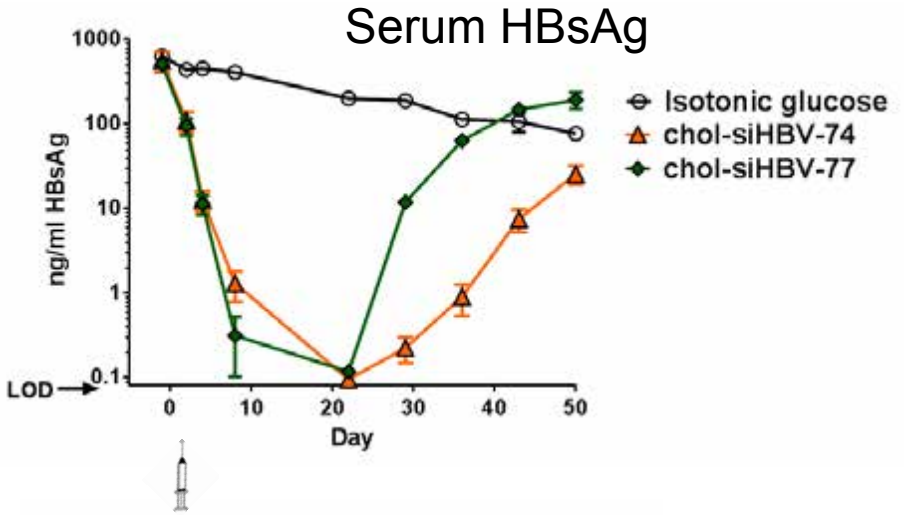
- Screened candidate siRNAs in a cell culture system
- 4 highly potent siRNAs chosen for further testing in animal models
- siHBV-74 and siHBV-77 chosen as leads

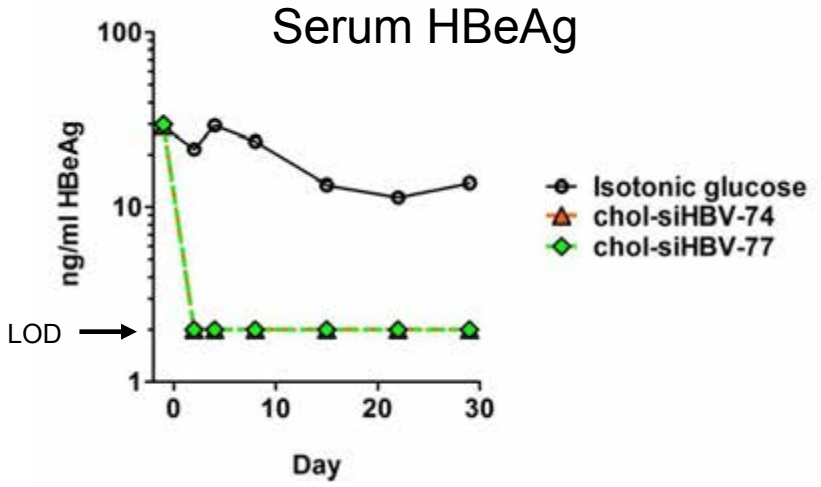
Roche-Kulmbach (Axolabs GmbH)

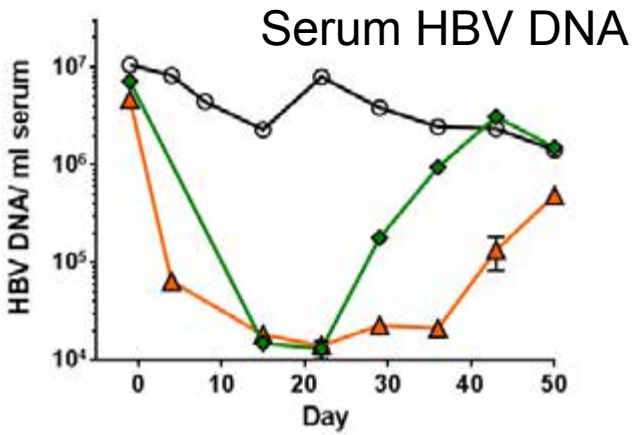
Non-transgenic mouse model for HBV infection



Co-injection of lead chol-siHBVs with NAG-MLP







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

Decreased HBsAg

– 3-4 log reduction with both chol-siHBVs

– > 2 log reduction for 1 month

Decreased HBeAg to LOD

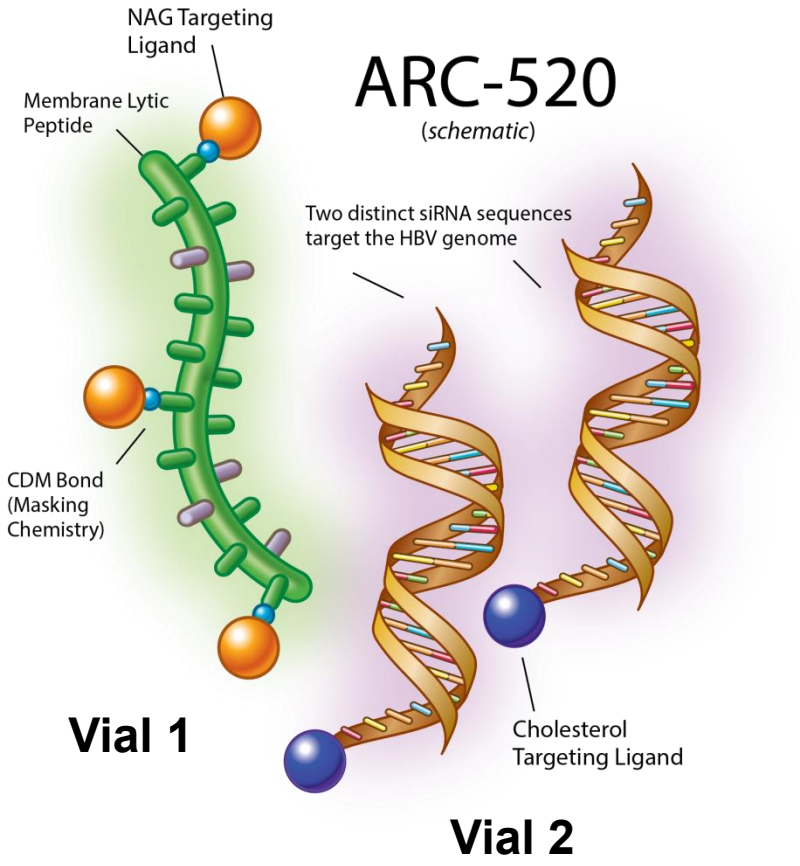
Decreased HBV DNA

– ~ 3 log reduction of HBV DNA for ~ 1 month

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

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

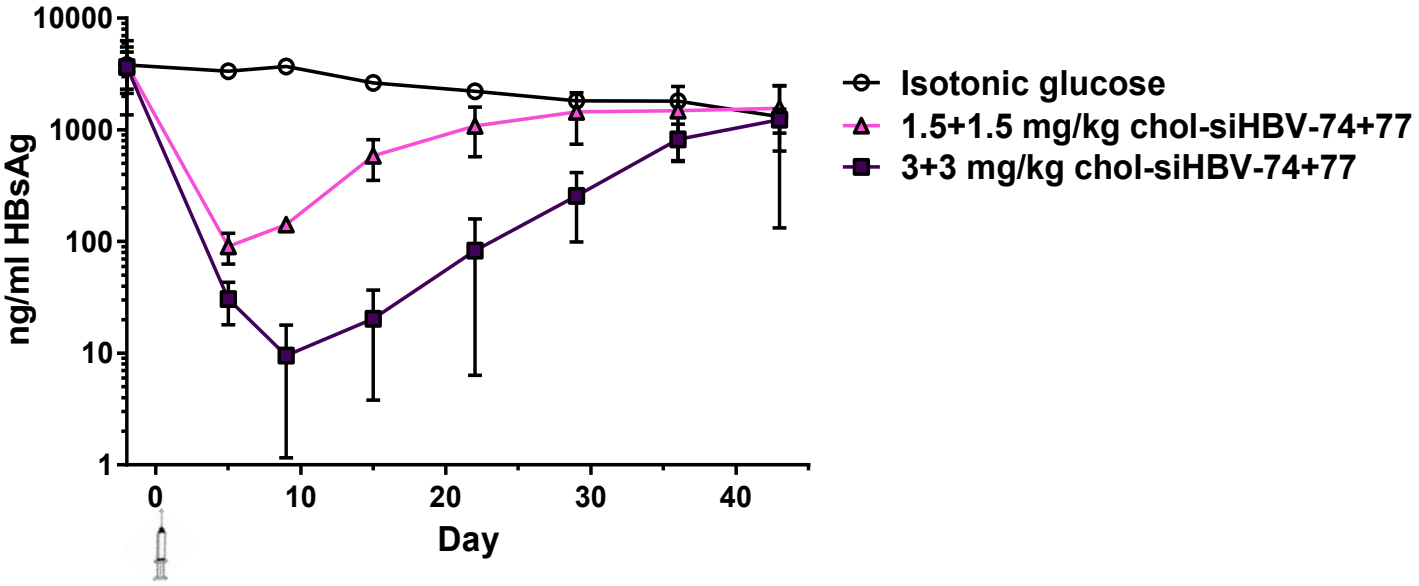
ARC-520 for chronic HBV infection



ARC-520 consists of 2 vials

- Vial 1: ARC-520 Excipient
  - Lyophilized powder
  - Contains a masked, hepatocyte-targeted peptide (NAG-MLP) that promotes endosomal escape of the HBV chol-siRNAs.
- Vial 2: ARC-520 API
  - Liquid
  - Contains the HBV chol-siRNAs.
  - Inclusion of two siRNAs gives broader genotype coverage

Dose response of NAG-MLP co-injected with chol-siHBV-74 + chol-siHBV-77 (ARC-520 drug)

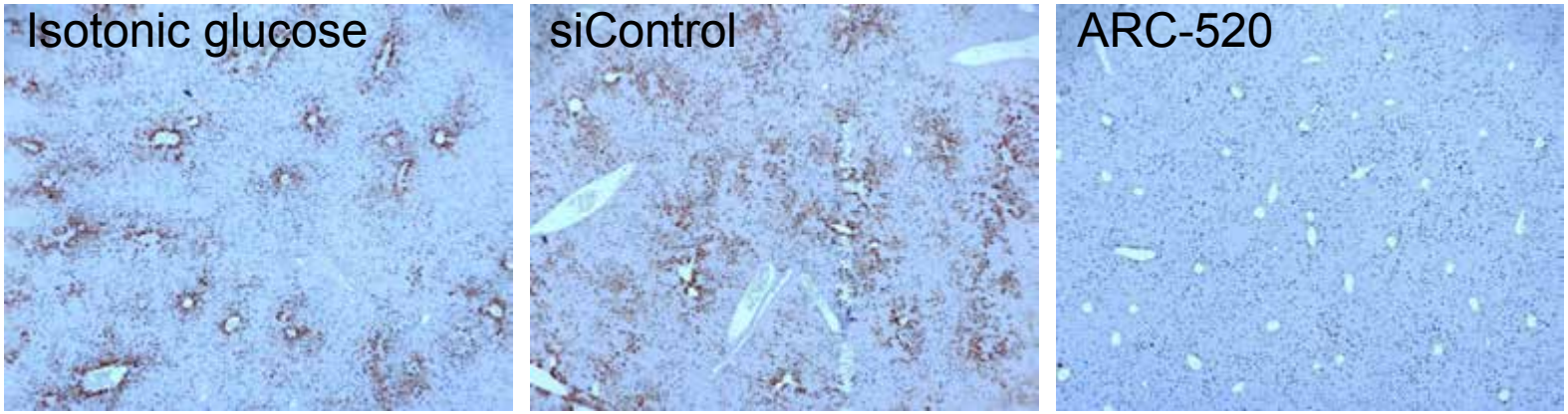


NAG-MLP (mg/kg)	Chol-siHBV-74 (mg/kg)	Chol-siHBV-77 (mg/kg)	Mean HBsAg reduction (NADIR)
3	1.5	1.5	1.5 ± 0.1 $\log_{10}$
6	3	3	2.7 ± 0.3 $\log_{10}$

Combination of both chol-siHBVs is effective and provides coverage for 99.6% of all known HBV genotypes

Effect of ARC-520 on HBV core antigen expression in livers of HBV transgenic mice

Anti-HBcAg immunostain



Strong reduction of core antigen in ***ALL*** liver hepatocytes in HBV transgenic mice receiving ARC-520

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

Testing ARC-520 in a chimpanzee chronically infected with human HBV

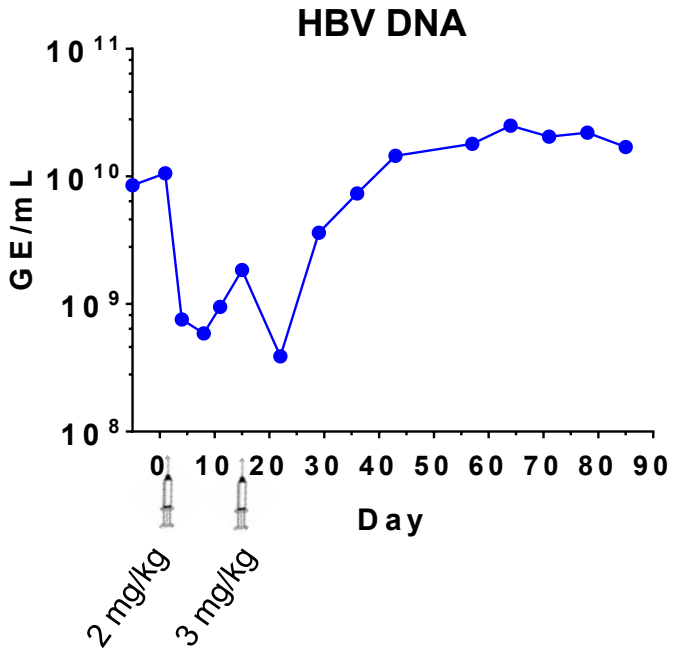
- Chimpanzee key historical attributes
  - 36 year old female, weight 113 pounds (51 kg)
  - Chronic HBV since 1979
  - Liver biopsy shows near 100% staining for HBV
  - Exceptionally high titers of circulating HBV DNA (10^{10} vs. 10^{7} in average patient) and HBsAg

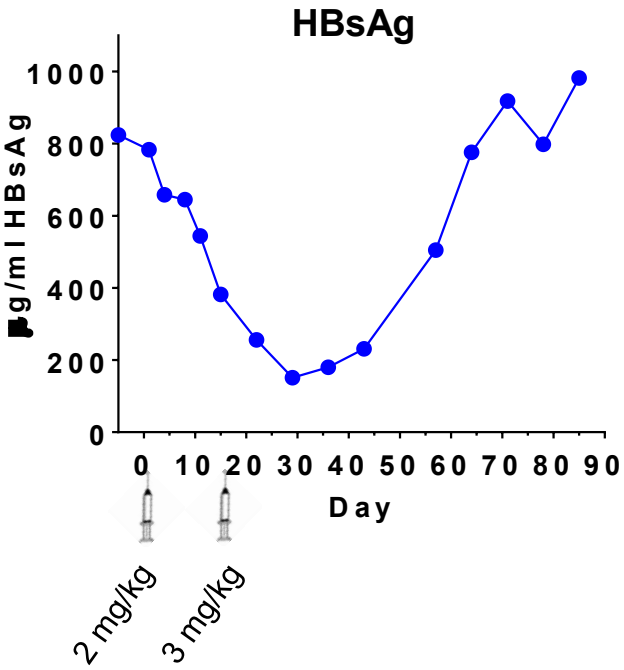
- Study design
  - Goals:
    1. Demonstrate KD by monitoring HBV markers
    2. Look for signs of immune system reactivation
  - Treatment:

    2 mg/kg ARC-520 on Day 1, followed by 3 mg/kg ARC-520 on Day 15
  - Monitor serum HBV markers, routine safety labs
  - Perform liver core biopsy at monthly intervals
    - Assessment of intrahepatic cytokine and chemokine transcript levels

Dr. Robert Lanford, Texas Biomedical Research Institute

Reduction in HBV after administration of ARC-520 in a chronically infected chimp





HBV DNA

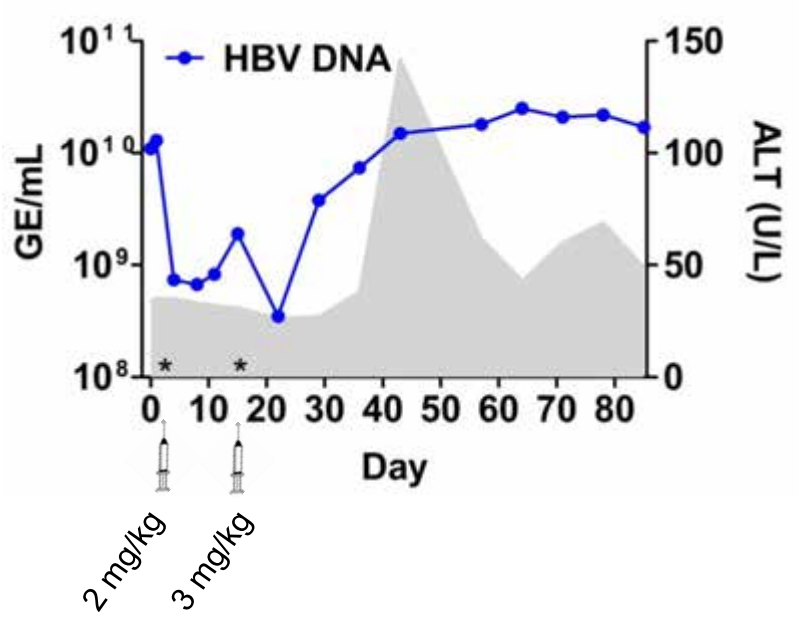
- 17-fold reduction on Day 4
- 36-fold reduction following second dose

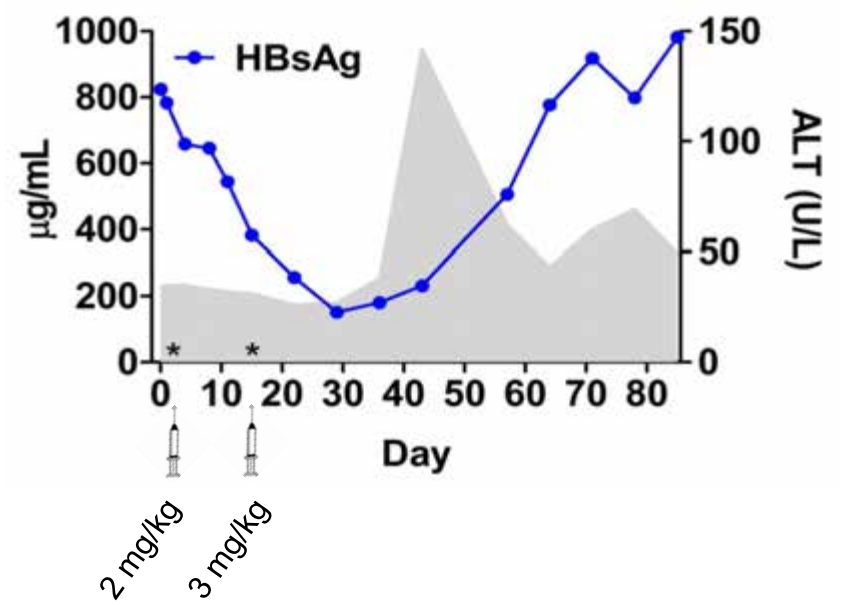
HBsAg

- Gradual reduction
- >80% reduction by Day 29

Dr. Robert Lanford, Texas Biomedical Research Institute

Elevated liver enzymes observed after HBsAg decrease

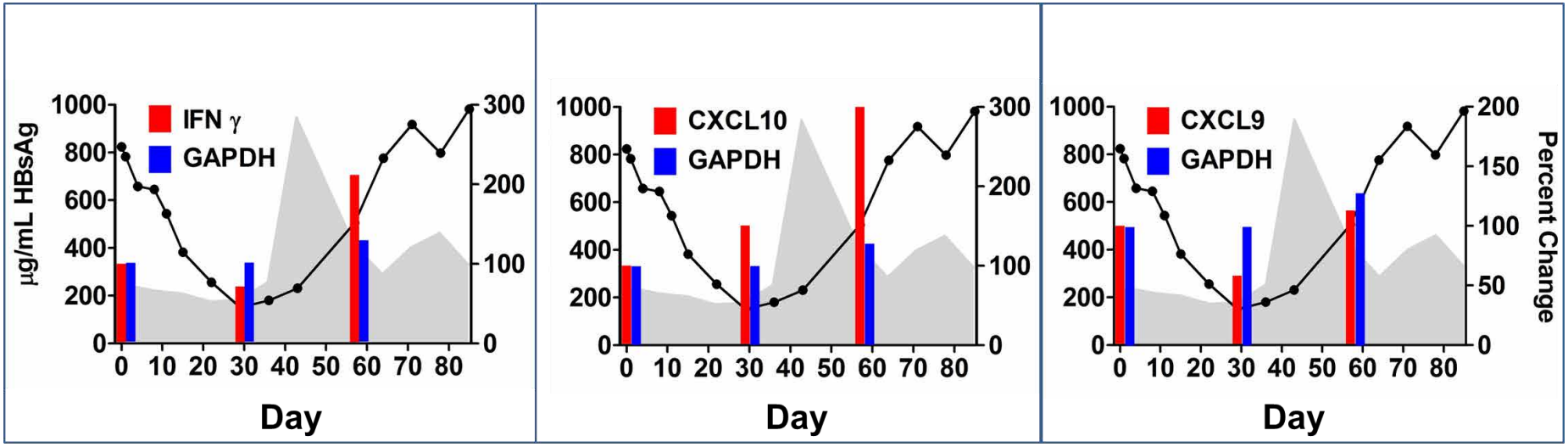




- An increase in ALT was observed near the HBsAg nadir.
- The increase occurred 4 weeks ***AFTER*** the last dose of ARC-520.
  → Likely not drug-related.
- T-cell reactivation?

Dr. Robert Lanford, Texas Biomedical Research Institute

Intrahepatic cytokine/chemokine mRNA



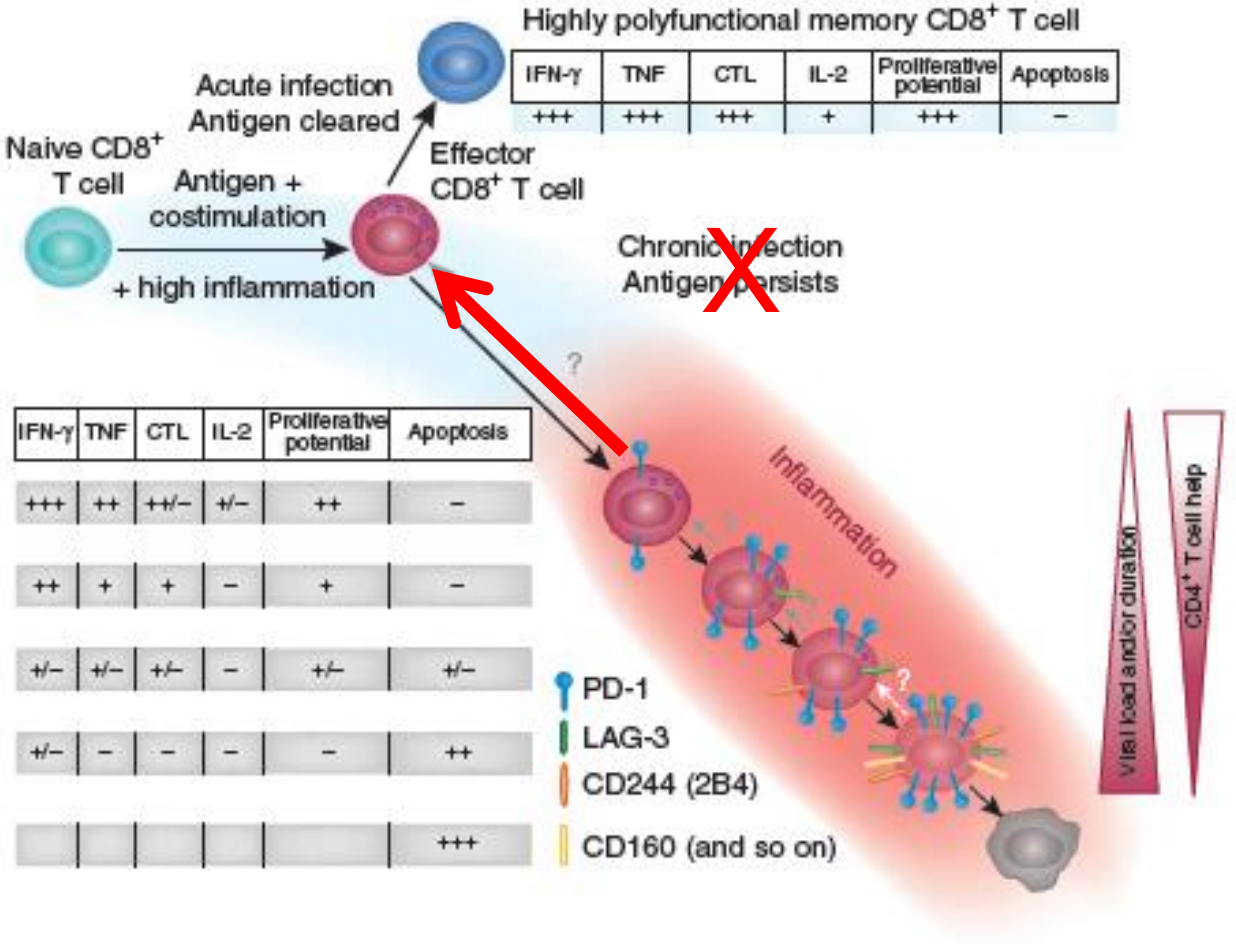
- Liver core biopsies taken on Days -6, 29 and 57
- RT-qPCR performed to determine levels of IFNγ and IFNγ-inducible genes CXCL10 (IP10) and CXCL9 (Mig)

- IFNγ ↑ 210%
- CXCL10 ↑ 310%
- CXCL9 ↑ 280% from preceding biopsy

Data consistent with a T-cell reactivation after HBsAg reduction

Dr. Robert Lanford, Texas Biomedical Research Institute

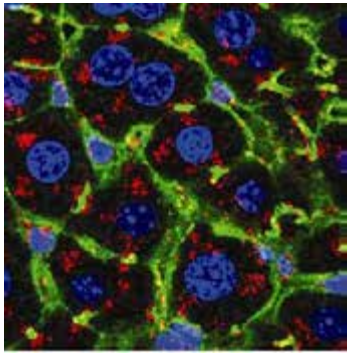
T-cell exhaustion during chronic infection



Wherry, Nat Immunol 2011 June; 12(6) 492-9

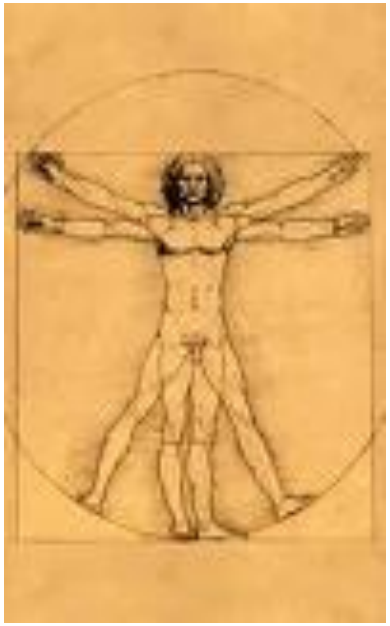
Summary: ARC-520 pre-clinical data

- IV injectable drug containing NAG-MLP and 2 chol-siHBVs:
  - NAG-MLP enables efficient chol-siRNA endosomal release and is biodegradable
  - Inclusion of two chol-siHBVs provides broad genotype coverage (99.6% of all known HBV sequences)
- Highly effective in HBV mouse models with multi-log reduction of HBV mRNAs, proteins, and DNA with long DoE (~1 month) after single injection
- Treatment of a chronically infected chimpanzee with human HBV reveals:
  - Significant, rapid reductions in viral load and viral antigens including HBsAg
  - Good tolerability with respect to clinical chemistries, CBC, cytokines, and clinical observation
  - Increase in liver transaminases observed 4 weeks post-last dose, and near the HBsAg nadir
  - Increase in liver INFγ and INFγ-induced genes is consistent with T-cell reactivation



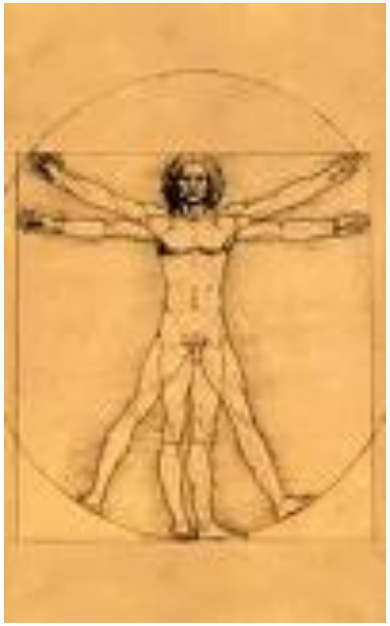
ARC-520 Phase I FIH clinical trial - design

- Phase I trial design
  - Randomized, double-blind, placebo controlled, single IV dose escalation
  - Normal healthy volunteers
  - Six cohorts: 0.01, 0.1, 0.3, 0.6, 1.2, 2 mg/kg
  - 6 subjects/cohort (2 placebo, 4 drug)
  - No pre-treatment
  - Assessment of safety and tolerability, PK



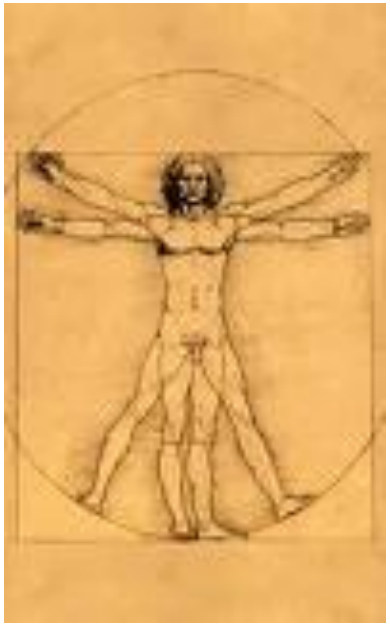
ARC-520 Phase I clinical trial – preliminary results

- Phase I trial preliminary results
  - Study remains blinded and data has not been locked.
  - Enrollment completed for all 6 cohorts (all 36 subjects).
  - All subjects received the full assigned dose without discontinuation.
  - Laboratory results have not indicated toxicity in any organ including liver, kidney or muscle (heart, skeletal) in any subject.
  - No SAEs.
  - AEs included upper respiratory infection (7), headache (7), lightheadedness (2), localized urticarial rash (1).



Clinical Plan for ARC-520

- Phase IIa trial in chronic HBV patients
  - single dose pilot efficacy study in chronic HBV patients on RT inhibitor therapy
  - Plan to apply for ethics and regulatory permission in fall 2013
  - Dosing planned to initiate Q1 2014
  - Will assess safety and measure levels of HBsAg, HBeAg and anti-HBsAg antibody and other signs of immune reactivation
- Phase IIb multi-dose pilot efficacy study in chronic HBV patients
  - Multi-site study
  - Will follow safety and measures of viral load, antigenemia, anti-HBsAg antibody production and HBsAg seroconversion



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Thank you!


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