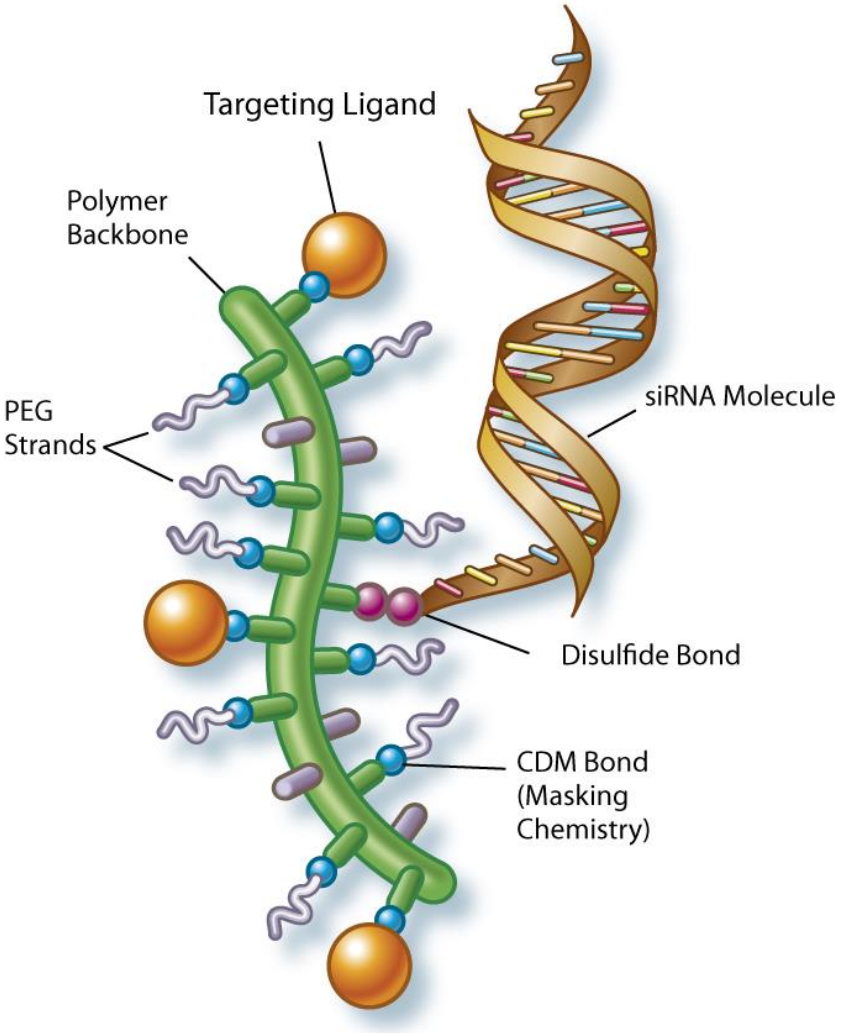
Arrowhead Research
CORPORATION

Development of an siRNA-based Therapeutic for Chronic HBV Infection Using DPC Technology

ASGCT 17th Annual Meeting
May 21, 2014

David L. Lewis, PhD CSO

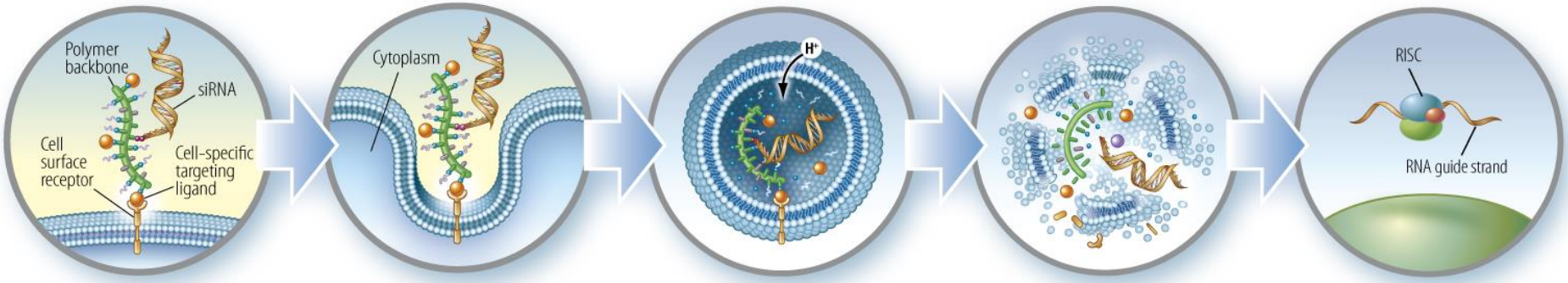
Dynamic PolyConjugate (DPC) technology for siRNA delivery



DPC

- Contains an amphipathic polymer for endosomal escape
- Polymeric amines are reversibly “masked” with pH-labile CDM attached to PEG or targeting ligand
- siRNA is attached to polymer through cleavable disulfide linker
- 5-15 nm in size

Mechanism of DPC-mediated siRNA delivery



DPC conjugated targeting ligand attaches to cell surface

DPC and siRNA are taken up

DPC/siRNA complex is enclosed in an endosome. Low pH results in polymer unmasking.

Polymer induces endosomolysis and release of siRNA payload into the cell cytoplasm.

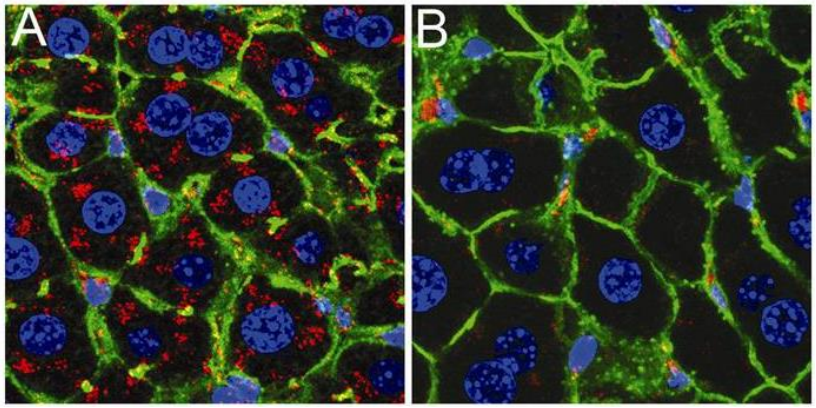
siRNA engages the cell's interference machinery, resulting in knockdown of target gene expression

DPCs for targeted siRNA delivery to hepatocytes

Targeting ligand: N-acetyl galactosamine ligand (NAG)

NAG is a ligand for the asialoglycoprotein receptor expressed on hepatocytes

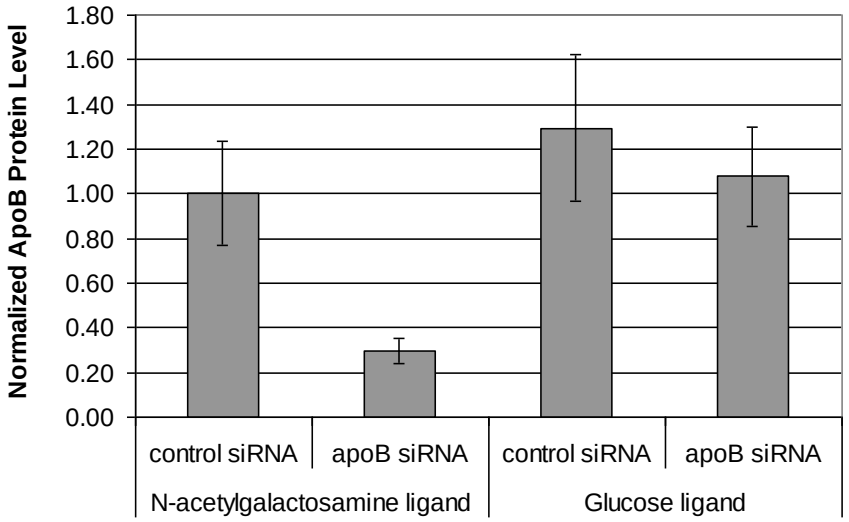
ICR mice, t=60'



NAG ligand
(hepatocyte targeted)

glucose ligand
(non-targeted)

DPC-siRNA
nucleus
cell membrane

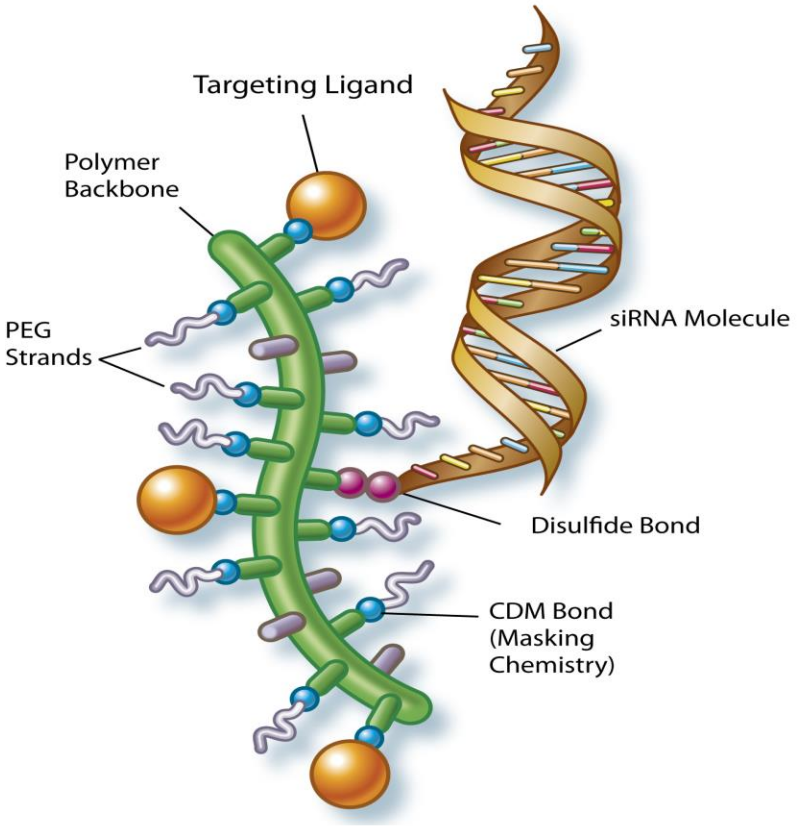


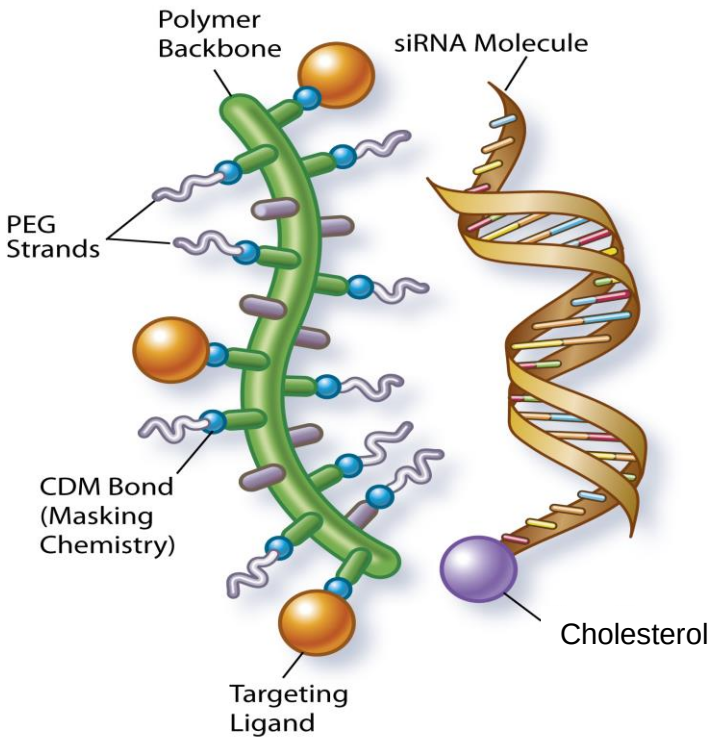
Hepatocyte-uptake of DPCs is ligand dependent

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 the siRNA to liver





Prototypical DPC

- Covalent attachment of siRNA to masked polymer

DPC polymer + targeted siRNA

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

Using peptides with membrane-lytic properties as DPC 2.0 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

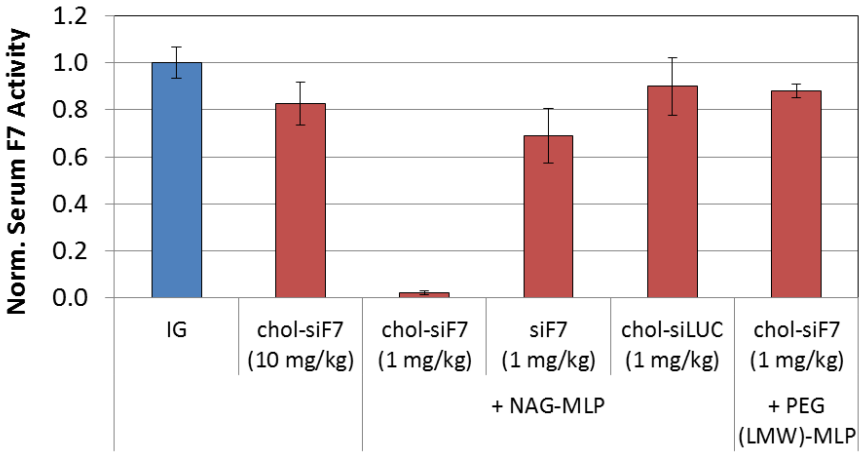
CDM used to attach targeting ligands (eg. NAG) and reversibly mask membrane-lytic activity

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

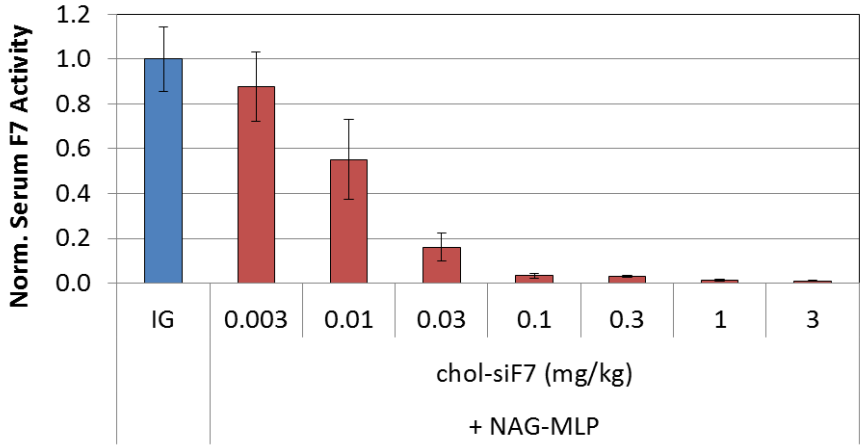
Co-injection of NAG-MLP and chol-siRNA in mice

Requirements for target gene KD in liver and chol-siRNA titration

single dose



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



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

48 hr timepoint

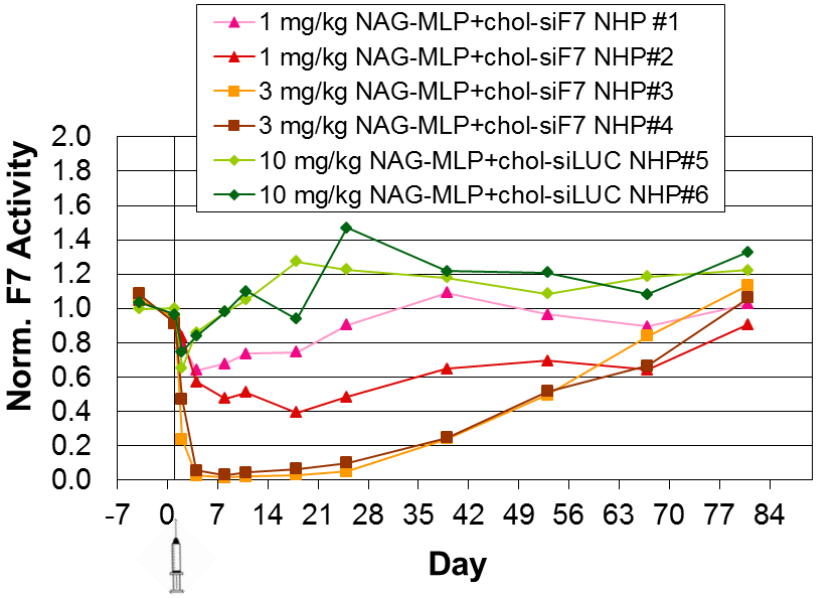
Wooddell et al, Mol Ther 2013 May; 21(5) 973-85

Efficacy in non-human primates

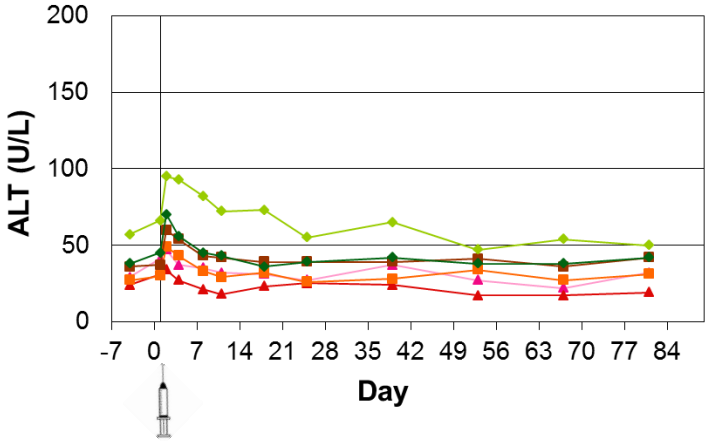
NAG-MLP dose titration + 2 mg/kg chol-siRNA, single iv dose

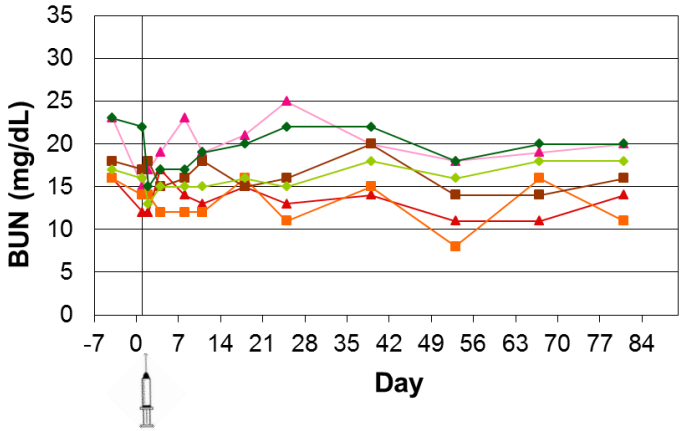
Target: Coagulation Factor 7

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



- **No toxicity**
  - *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

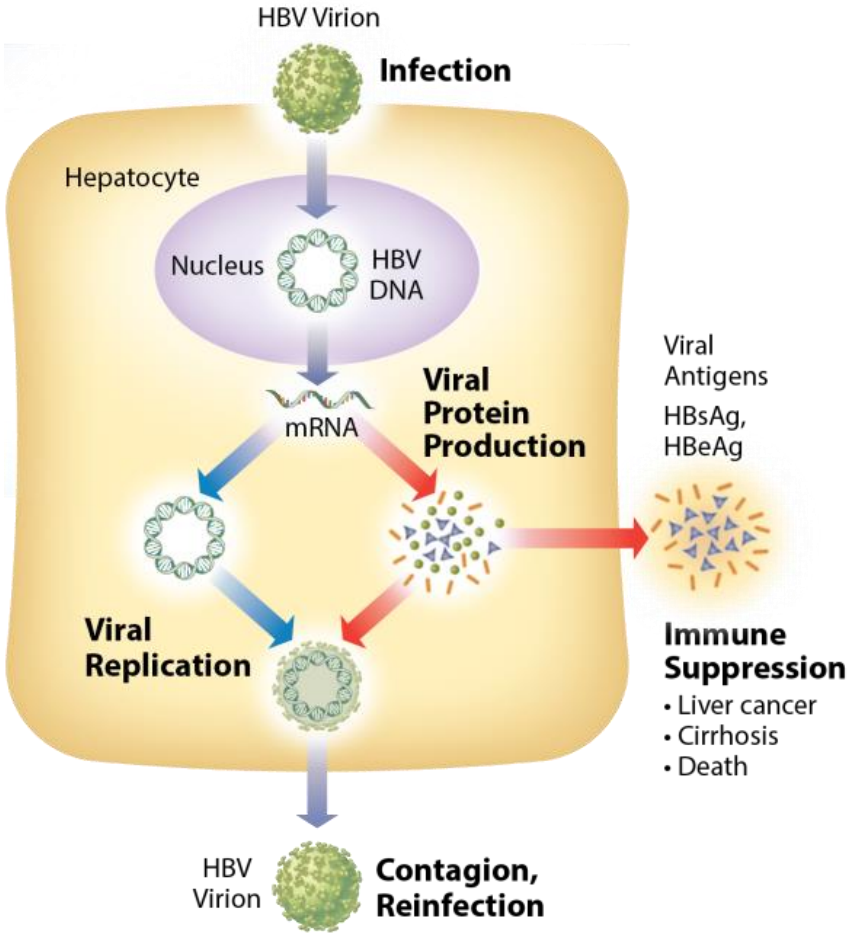
ARC-520 for Treatment of Chronic HBV

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 and chronic infection with levels of viral proteins playing an integral role (“T-cell exhaustion”)
  - Loss of surface antigen (HBsAg) or seroconversion ≈ functional cure

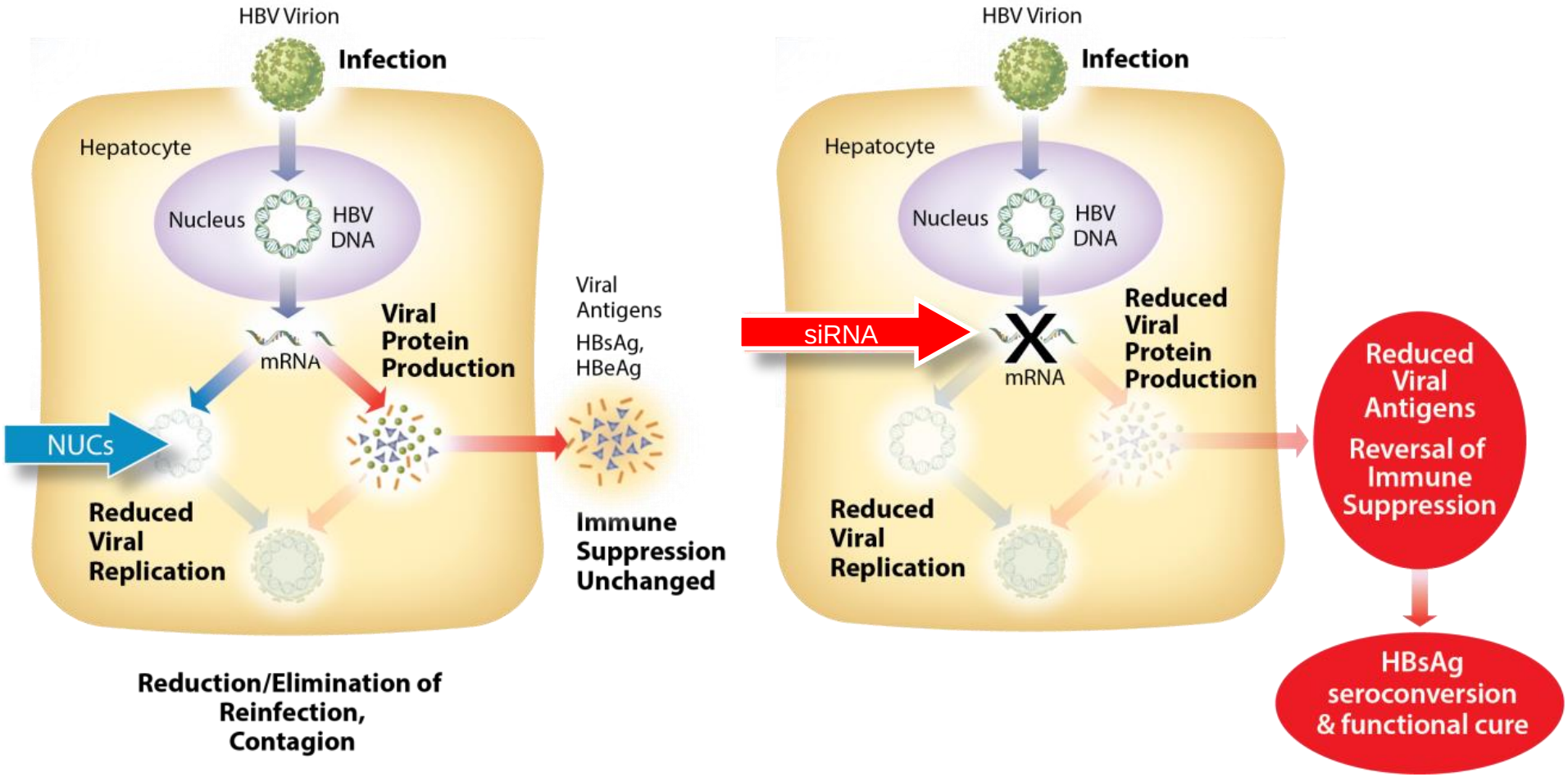
- Existing drugs (reverse transcriptase inhibitors, PEG-Interferon) are unsatisfactory
    - RT inhibitors “Nucs” (eg. tenofovir, entecavir, lamivudine)
      - Can improve patient outcomes
      - Do not significantly decrease HBsAg levels nor result in HBsAg seroconversion → life-long treatment required
    - PEG-Interferon (PEGASYS)
      - 48 week course
      - 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 suppression of the immune response



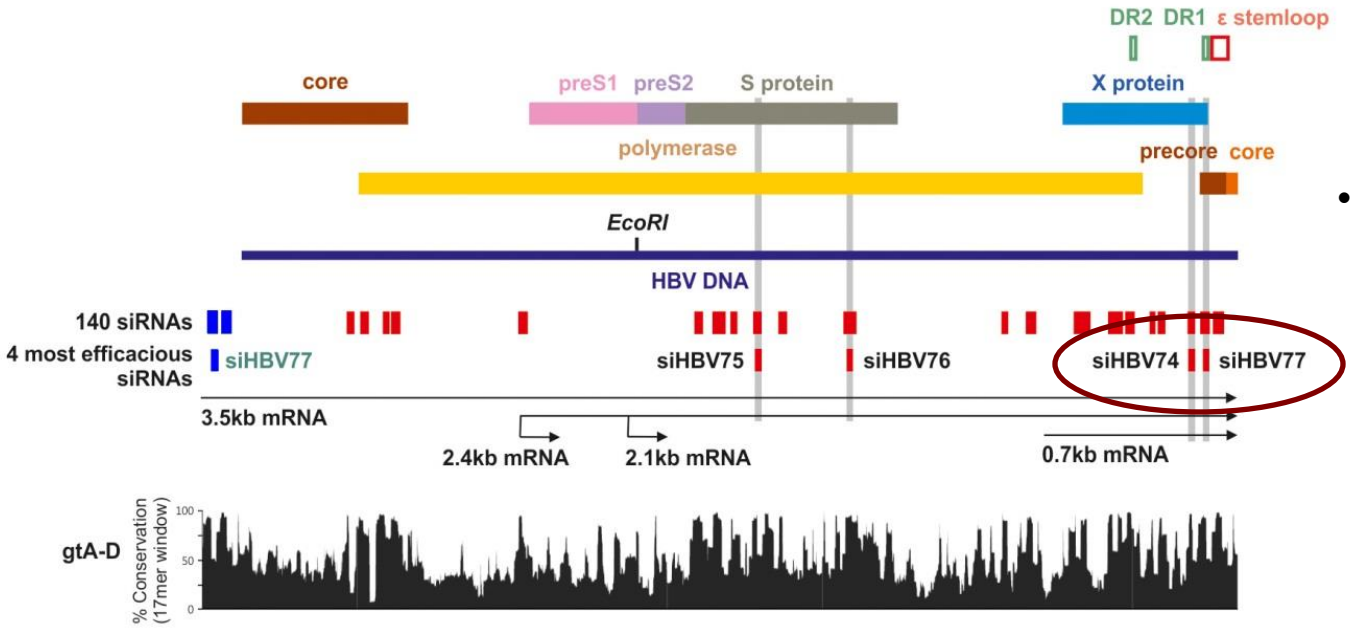
Sustained, high levels of viral protein production (esp. HBsAg) leads to immune suppression and chronicity.

RNAi therapeutics vs. RT inhibitors for treatment of chronic Hepatitis B

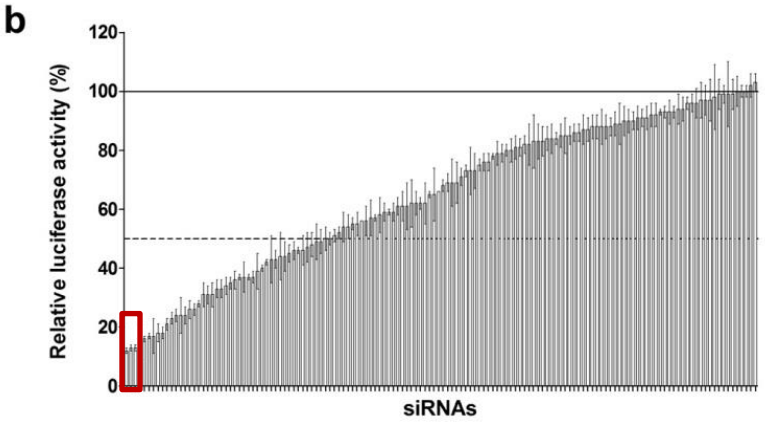


RNAi for treatment of chronic Hepatitis B

siRNA design and in vitro screening



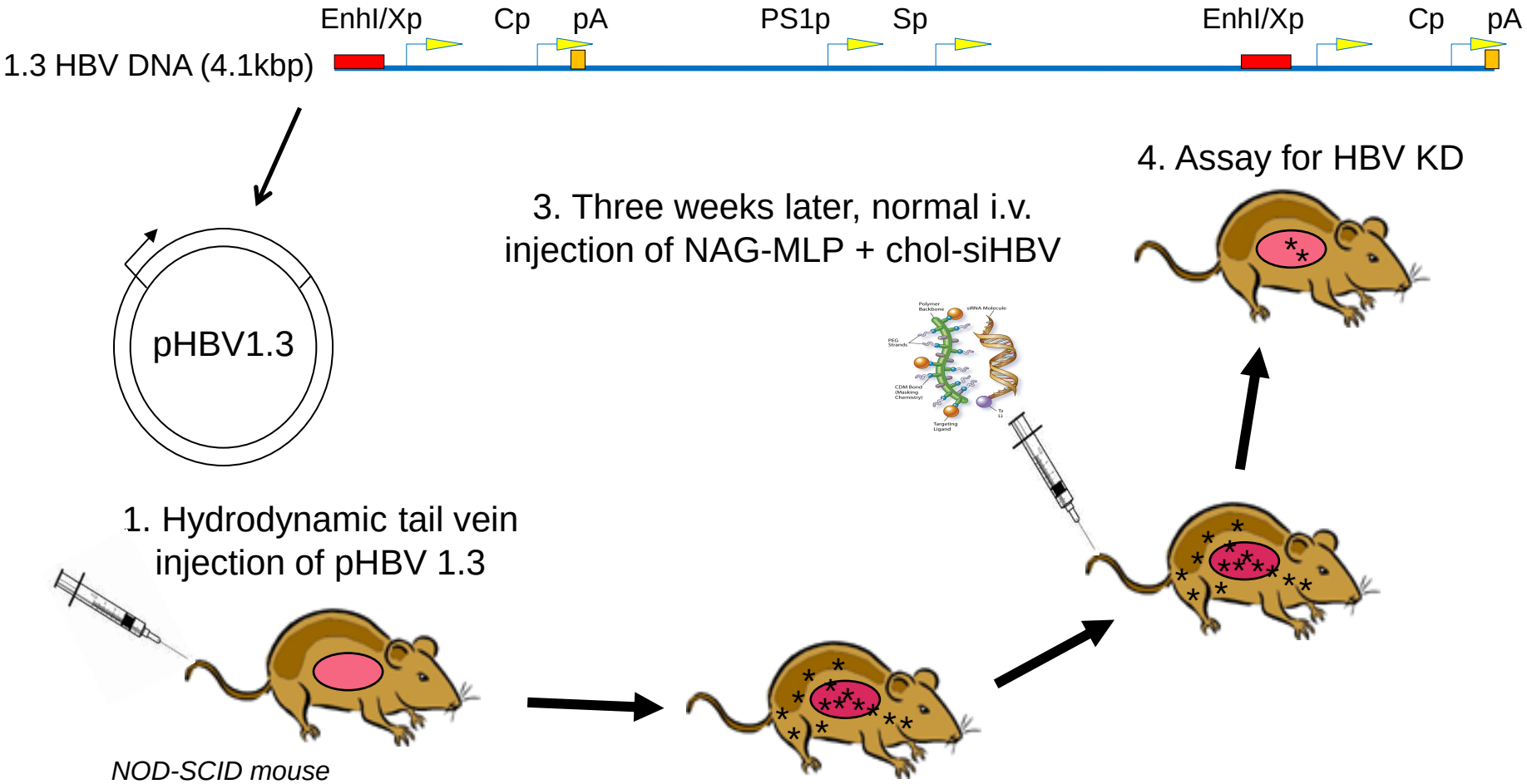
- Designed 140 siRNAs targeting conserved regions in GenBank HBV sequences (2,754)



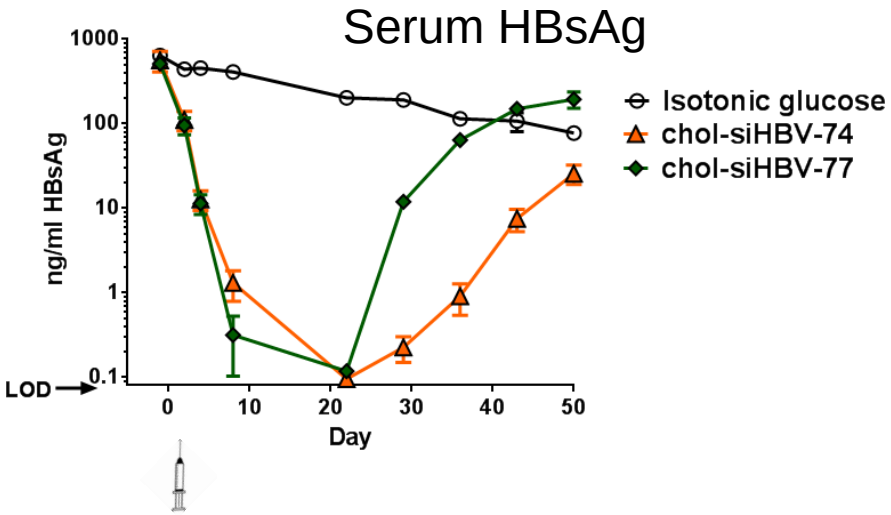
- Screened candidate siRNAs in a cell culture system
- 4 highly potent siRNAs chosen for further testing in animal models
- siHBV-74 and siHBV-77 chosen as leads

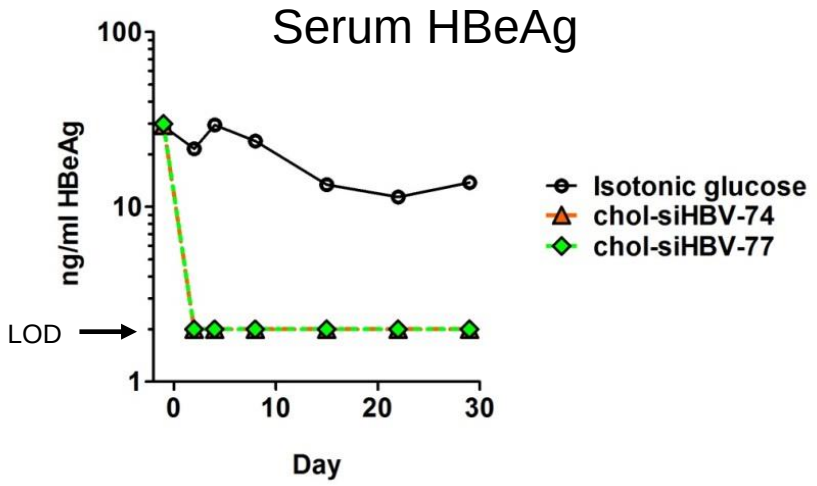
Roche-Kulmbach (Axolabs GmbH)

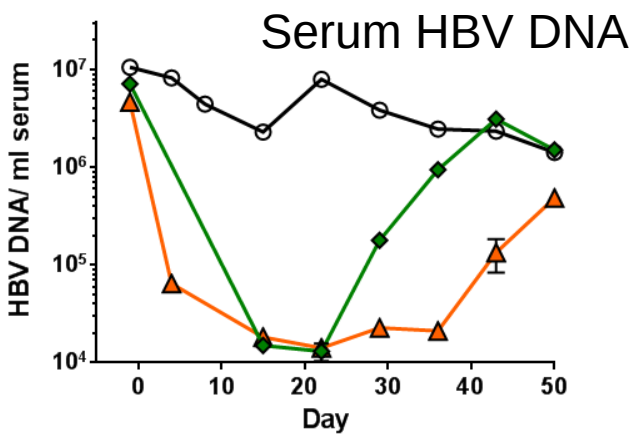
Mouse model for HBV infection



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

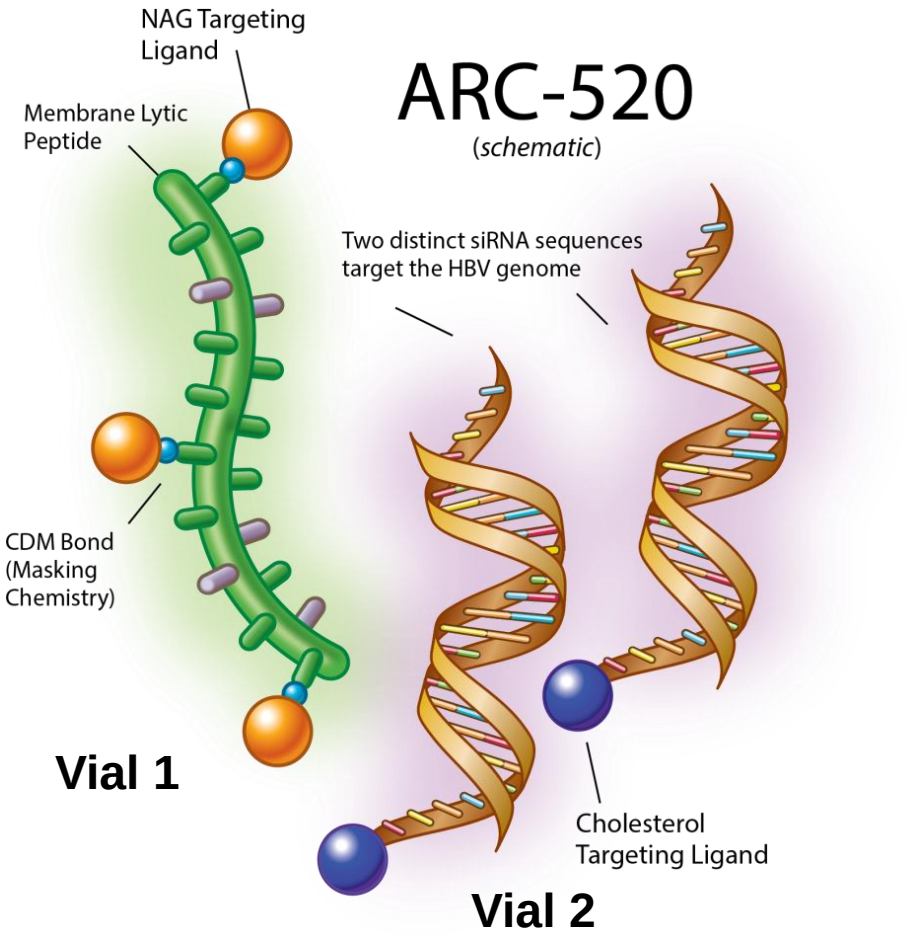
Decreased HBV DNA

– ~ 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 is a two vial drug

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

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

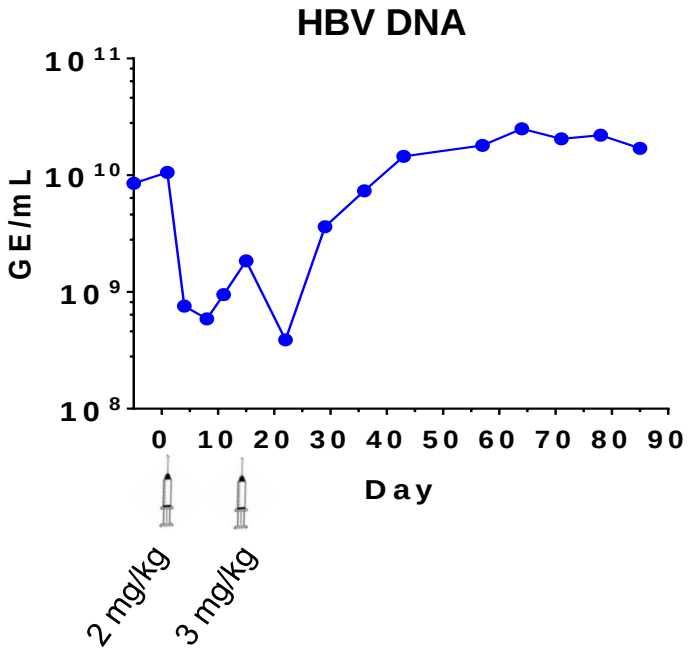
- Chimpanzee key historical attributes
  - 36 year old female, weight 113 pounds (51 kg)
  - Chronically infected since 1979
  - Liver biopsy shows near 100% staining for HBV
  - Exceptionally high titers of circulating HBV DNA (10^{10} vs. 10^{7} in average patient) and HBsAg

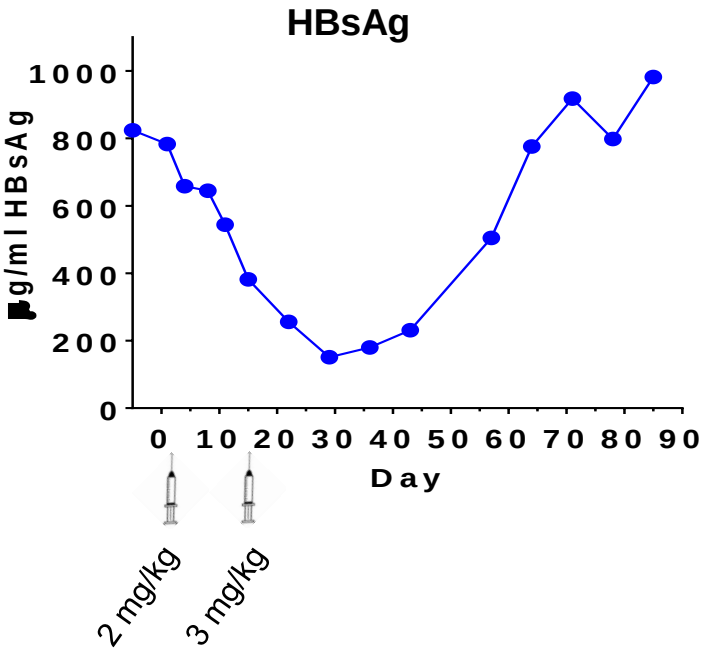
- Study design
  - Goals:
    1. Demonstrate KD by monitoring HBV markers
    2. Look for signs of immune system reactivation
  - Treatment:

    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





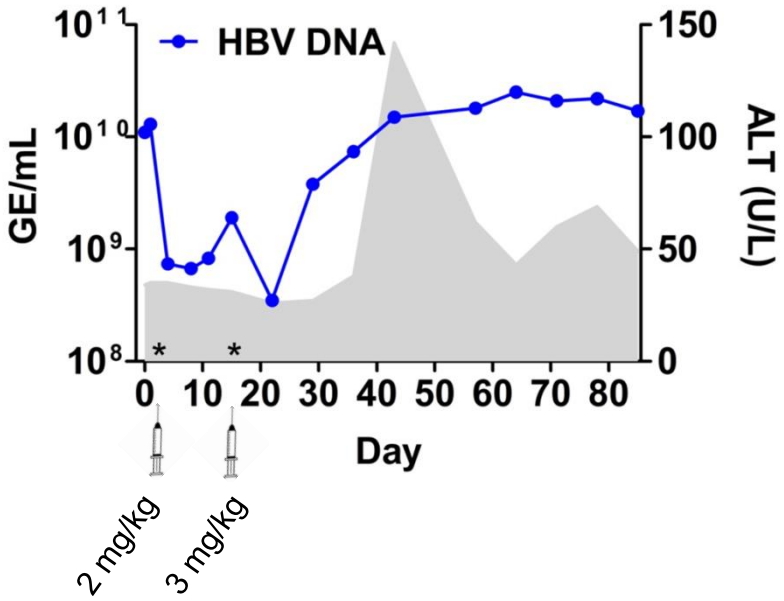
HBV DNA

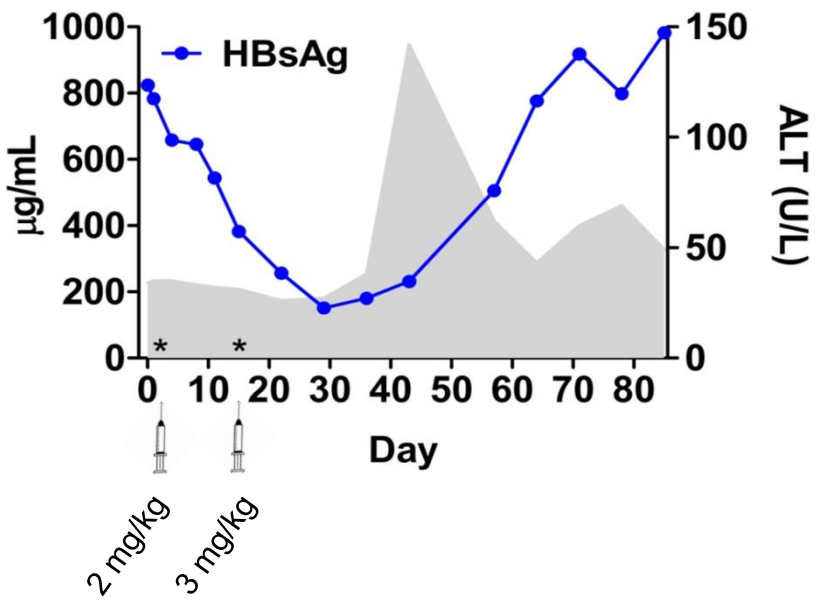
- 17-fold reduction on Day 4
- 36-fold reduction following second dose

HBsAg

- Gradual reduction
- >80% reduction by Day 29

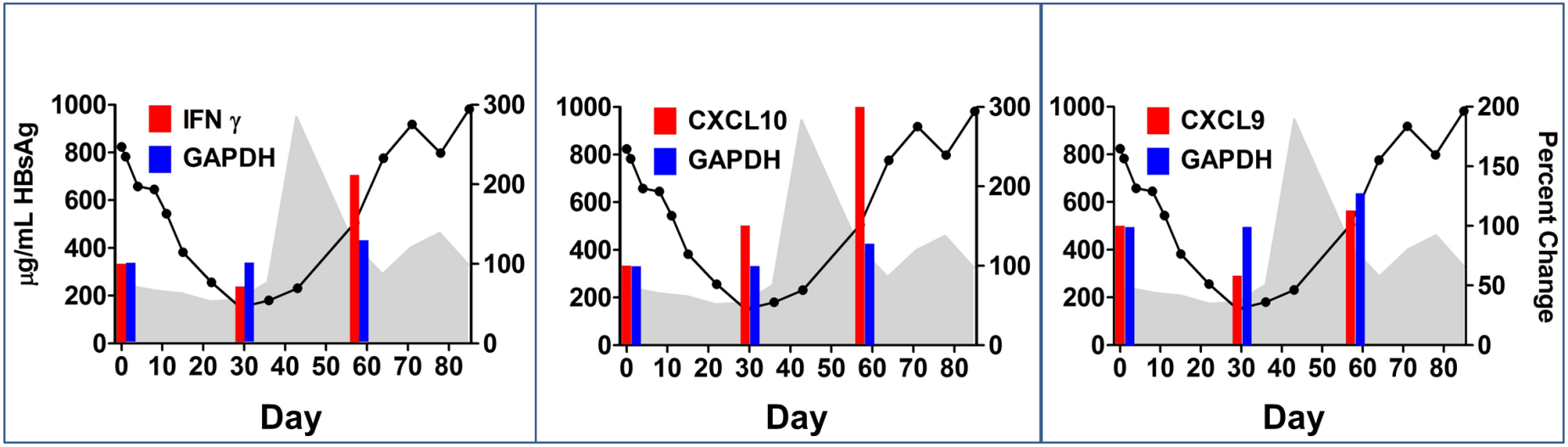
Elevated liver enzymes observed 4 weeks post-last dose





- 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 reactivation?

Intrahepatic cytokine/chemokine mRNA



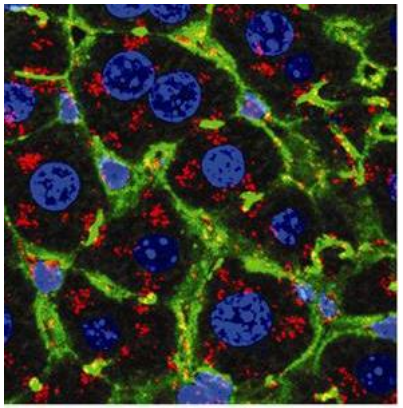
- Liver core biopsies taken on Days -6, 29 and 57
- RT-qPCR performed to determine mRNA levels of IFNγ and the IFNγ-inducible genes CXCL10 (IP10) and CXCL9 (Mig)

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

Induction of IFNγ and downstream genes characteristic of increased T-cell function

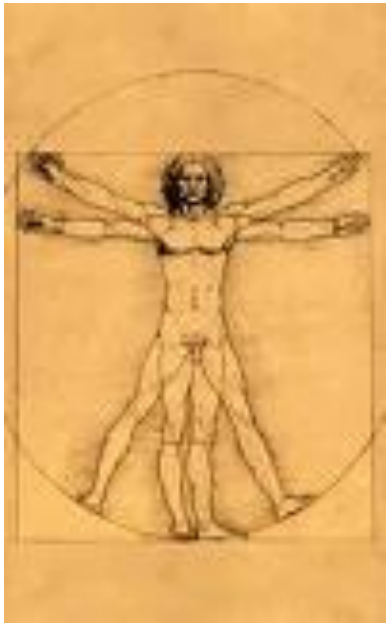
Summary: ARC-520 pre-clinical data

- IV injectable drug containing NAG-MLP and 2 chol-siHBVs:
  - Two chol-siHBVs provide broad genotype coverage (99.6% of all known HBV sequences)
  - NAG-MLP targeted to hepatocytes enables efficient endosomal release of chol-siRNA
  - Multi-log reduction of HBV mRNAs, proteins, and DNA with long DoE (~1 month) after single injection in mouse models
- Treatment of a chimpanzee chronically infected with human HBV reveals:
  - Significant, rapid reductions in viral load and viral antigens including HBsAg
  - 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 event



ARC-520 Phase I FIH clinical trial – Heparc-1001

- Phase I trial design
  - Normal healthy volunteers
  - Randomized, double-blind, placebo controlled, single IV dose escalation
  - Six cohorts: 0.01, 0.1, 0.3, 0.6, 1.2, 2 mg/kg
  - 6 subjects/cohort (2 placebo, 4 drug)
  - Assessment of safety and tolerability, PK



Heparc-1001: Results

- Enrollment completed for all 6 cohorts. All subjects received full assigned dose without discontinuation.

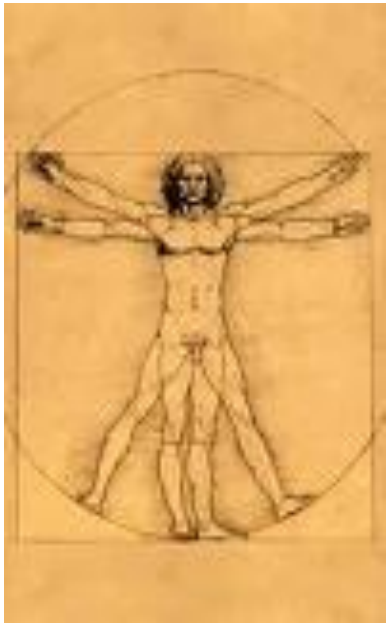
- No differences relative to Placebo and no abnormal findings rated clinically significant on:
  - Vital Signs, Physical Exams, Clinical Labs (including liver, kidney or muscle (heart, skeletal)) in any subject

- Adverse events (all attributions) reported in 75% of placebo and 75% of ARC-520 subjects (headache, lightheadedness, URIs, lethargy, muscle ache). No SAEs.
  - Placebo (Saline): Mild (64%), Moderate (36%)
  - ARC-520: Mild (63%), Moderate (37%)
    - One subject receiving ARC-520 in cohort 3 (0.3 mg/kg) was noted to have sinus pause with non-conducted beats on telemetry while sleeping.
      - Pre-dosing telemetry (t = -1 hr) review demonstrated previously un-observed Wenckebach rhythm. Patient history and follow-up suggests pre-existing hypervagal syndrome.
    - One subject receiving ARC-520 in cohort 6 (2.0 mg/kg) developed a localized urticarial rash – resolved shortly after appearance.

→ ARC-520 at doses as high as 2.0 mg/kg appears to be safe and well tolerated.

Phase IIa clinical plan for ARC-520

- Chronic HBV patients
  - Double blinded, placebo-controlled single dose study in chronic HBV patients on the RTI Baraclude (entecavir)
  - 1.0 and 2.0 mg/kg ARC-520
  - Single cohort with 8 patients for each dose level (2 placebo, 6 ARC-520)
  - Dosing initiated 3/24/2014 in Hong Kong (Queen Mary and Prince of Wales Hospitals), first cohort completed
  - Primary objective
    - Evaluate depth and duration of HBsAg decline in combination with entecavir
  - Secondary objectives
    - Assess safety/PK in chronic HBV patients
    - Evaluate effect on Abs to HBsAg
    - Assess signs of immune reactivation (key cytokines)
    - Evaluate effects on HBV DNA serum titers
  - Patients to be monitored for 12 weeks
  - Topline results expected to be released Q3 2014



Contributors

Biology
Chris Wooddell
So Wong
Qili Chu
Holly Hamilton
Stephanie Bertin
Jacob Griffin
Jessica Montez
Julia Hegge
Tracie Milarch

Chemistry
Dave Rozema
Jason Klein
Darren Wakefield
Vladimir Trubetskoy
Collin Hagen
Anthony Perillo-Nicholas
Andrei Blokhin
Jeff Carlson
Jonathon Benson

Axolabs GmbH
Hans-Peter Vornlocher
Ingo Roehl
Philipp Hadwiger
Markus Hossbach
Mathias John
Jochen Deckert
Kerstin Jahn-Hofmann

Clinical
Chris Anazlone, CEO
Bruce Given, COO
Lynn Kalinoski
Thomas Schluep
Jeremy Heidel

LAR
Julia Hegge
Tracie Milarch
Sheryl Ferger
Rachel Schmidt
Linda Goth

Texas Biomedical Research Institute
Robert Lanford
Debbie Chavez

Jason Lickliter – Nucleus Network Ltd. (Phase I Unit CMO)
Robert Gish, MD – Consultant, CAB Chair


Arrowhead Research
CORPORATION