Arrowhead Research
CORPORATION

# Analyst and Investor Luncheon
# ARC-520 Hepatitis B Clinical Candidate
# Arrowhead Research Corporation
# March 25, 2013

# Safe Harbor Statement

This presentation contains forward-looking statements within the meaning of the "safe harbor" provisions of the Private Securities Litigation Reform Act of 1995. These statements are based upon our current expectations and speak only as of the date hereof. Our actual results may differ materially and adversely from those expressed in any forward-looking statements as a result of various factors and uncertainties, including, without limitation, our developmental stage and limited operating history, our ability to successfully and timely develop products, enter into collaborations and achieve other projected milestones, rapid technological change in our markets, demand for our future products, legislative, regulatory and competitive developments and general economic conditions. Our Annual Report on Form 10-K, recent and forthcoming Quarterly Reports on Form 10-Q, recent Current Reports on Forms 8-K, and other SEC filings discuss some of the important risk factors that may affect our ability to achieve the anticipated results, as well as our business, results of operations and financial condition. Readers are cautioned not to place undue reliance on these forward-looking statements. Additionally, Arrowhead disclaims any intent to update these forward-looking statements to reflect subsequent developments.

# Panel Members

- Dr. Robert Gish

Dr. Gish joined the full time faculty at University of California, San Diego in December 2010 as Clinical Professor, Section Chief of Hepatology, and Medical Director for the Center of Excellence for Hepatobiliary Disease and Abdominal Transplantation (CHAT). Since that date, research efforts have expanded to include Point of Care testing, public policy for viral hepatitis, collaboration with co-investigators to study NASH, metabolism of intralipid infusions and clinical trials for HCV and HBV.

Dr. Gish is actively involved in numerous professional societies, including the American Association for the Advancement of Science and the American Society of Transplant Physicians. He is a fellow of both the American College of Physicians and the American Association for the Study of Liver Disease. He is also the Chairman of Arrowhead's hepatitis B Clinical Advisory Board

# Panel Members

- Joan Block, Executive Director and Co-Founder Hepatitis B Foundation

Ms. Block has provided leadership for the Hepatitis B Foundation's comprehensive outreach, public health and national advocacy programs for the past 22 years. The Foundation is the only national nonprofit organization solely focused on providing information and support to those living with chronic hepatitis B infections.

Ms. Block serves on the Board of the Hepatitis B Foundation, Institute for Hepatitis and Virus Research, and Medical Advisory Board of PKIDS. She spearheaded and serves as Co-Chair of the Hep B United national campaign that includes community, federal and corporate partners. Ms. Block is a Member of the National Task Force on Hepatitis B, the Hepatitis B Expert Panel of the U.S. Office of Minority Health, and has been invited to provide expert testimony before Congress and the Institute of Medicine.

# Agenda

- Dr. Robert Gish
  - Hepatitis B – State of the art
- Dr. Bruce Given
  - ARC-520
- Dr. Chris Anzalone
  - Conclusions
- Q&A
  - Robert Gish, Joan Block, Bruce Given, Chris Anzalone

# Dr. Robert Gish

# Current Therapy of Hepatitis B Planning for 2013 and Beyond

Investor Meeting NYC

March 2013

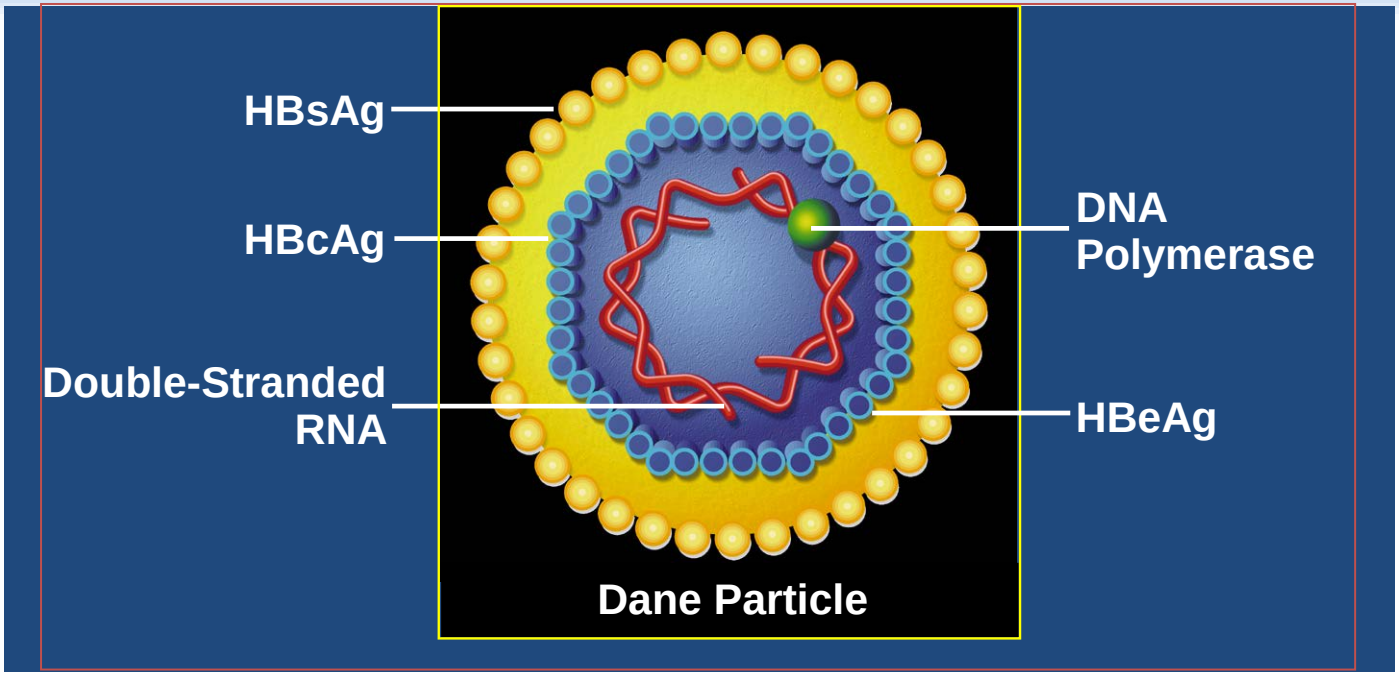
***Robert Gish***, Professor of Medicine
UCSD

# Disclosures

- Grants, research, consulting fees from BMS, Gilead and Roche/Genentech are donated to UCSD research and education

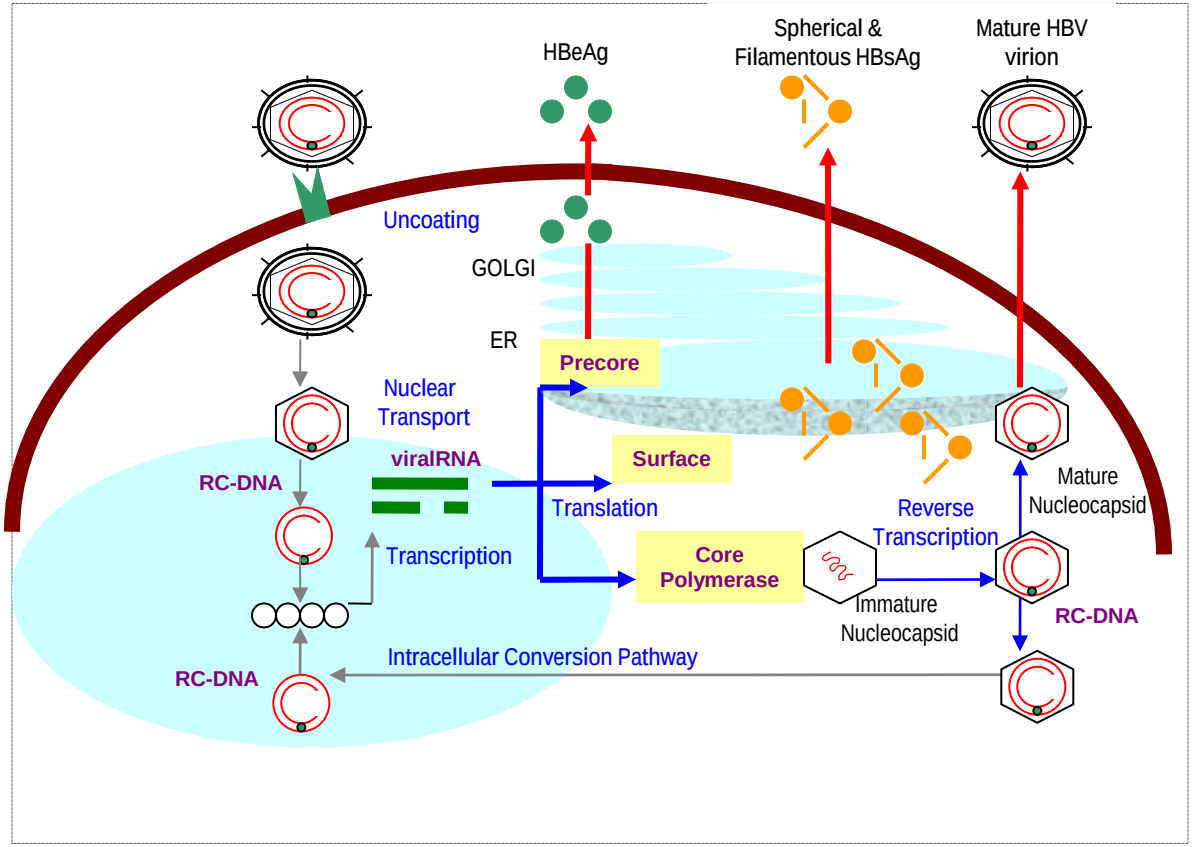
- Chair CAB: Arrowhead

# Hepatitis B Virus



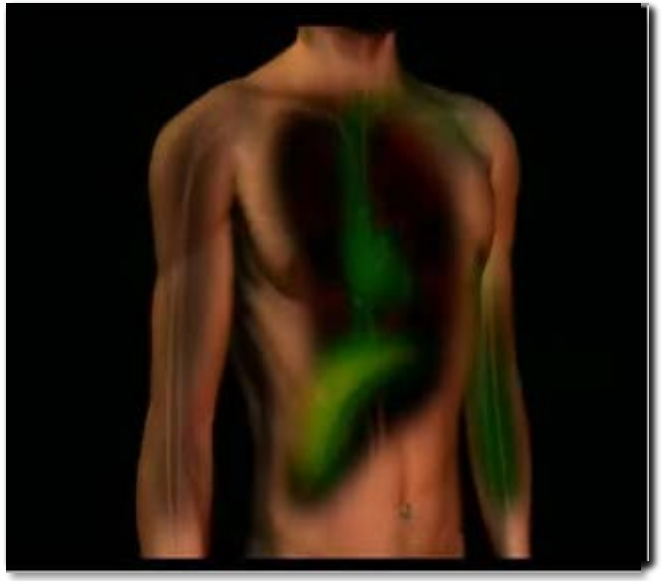

- Partially double-stranded circular DNA virus
- A member of the Hepadnaviridae
- Central Core nucleocapsid containing viral DNA; surrounding envelope with surface protein or antigen

# HBV Replication: cccDNA Pathway



# Hepatitis B: The Facts

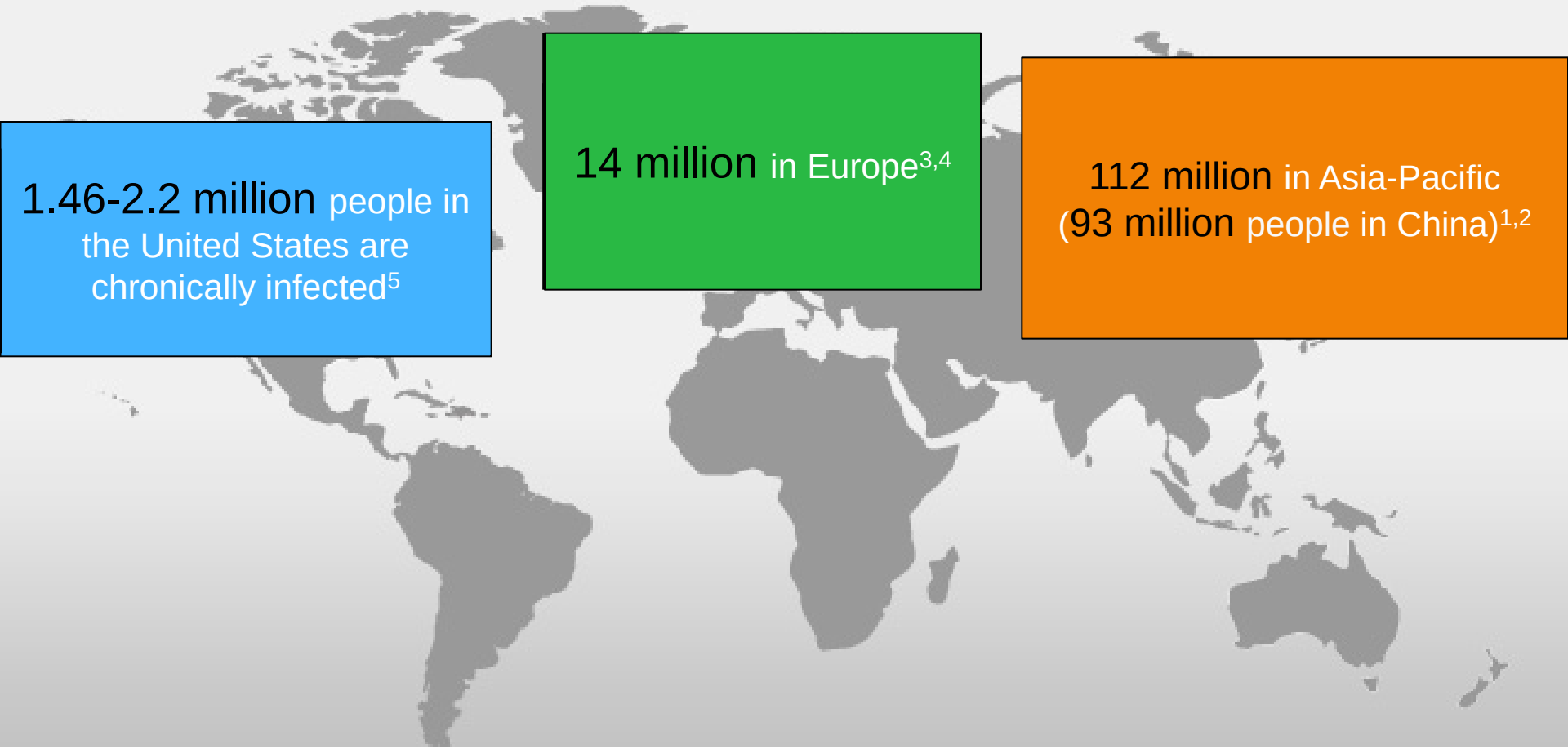
- Hepatitis B is the world's most common serious liver infection[1] and is a widespread global health issue
  - HBV is 100 times more infectious than HIV (human immunodeficiency virus)[2]
  - 10 times more infectious than hepatitis C[3]
- The virus is transmitted via the blood and bodily fluids[1]
  - Hepatitis B progresses slowly over time
  - Complications generally involve vague symptoms or none at all, and are often undetected for many years



1. Hepatitis Australia. Available at http://www.hepatitisaustralia.com/about_hepatitis/hep_b.html . Accessed April 2009. 2. World Health Organization. Hepatitis B Fact Sheet. Available at http://www.who.int/mediacentre/factsheets/fs204/en/. Accessed April 2009. 3. Ulmer T. European orientation towards the Better Management of Hepatitis B in Europe: Recommendations of the Hepatitis B expert group. Hepatitis B Expert Meeting at the European Parliament, 3 July 2007

# Hepatitis B: By The Numbers

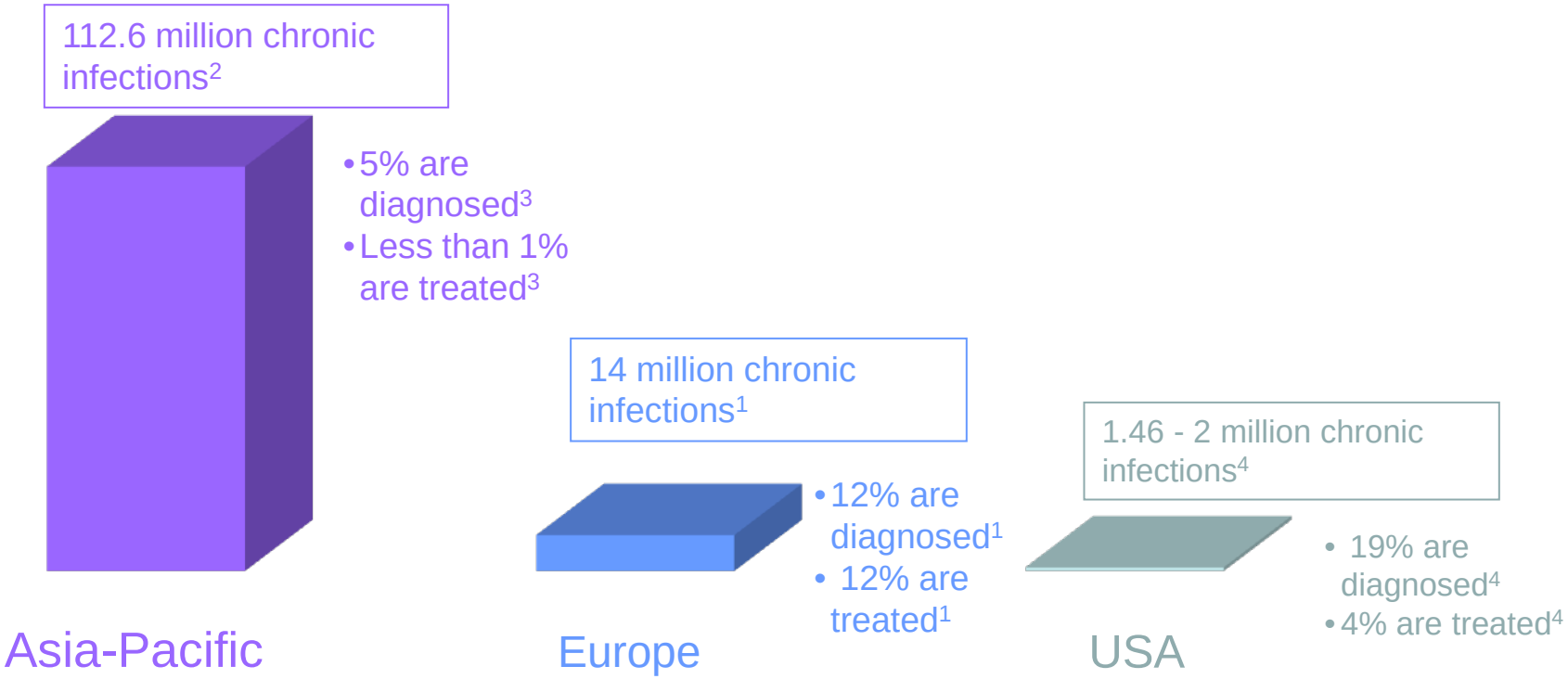
More than 350 million or 1 in 20 people worldwide have chronic hepatitis B infection[1]
(Compared with the 33 million living with HIV[2])




1 WHO. Available at: www.who.int/csr/disease/hepatitis/en/. 2 Ferlay et al. Globocan 2002, Cancer incidence, mortality and prevalence worldwide, IARC Press, Lyon 2004. 3 Records of the thematic press conference of the Ministry of Health of the PRC at April 21, 2008, from the website of the Ministry of Health of the People's Republic of China; 4 Ulmer, T et al. (2007). European orientation towards the better management of hepatitis B in Europe; 5. CDC. Hepatitis B FAQs for Health Professionals. Available at http://www.cdc.gov/hepatitis/HBV/HBVfaq.htm#overview.

# An Unmet Medical Need

- Worldwide, hepatitis B is significantly:
  - Under-diagnosed and
  - Under-treated[1]






1.BMS Market Research. Information available upon request from Bristol-Myers Squibb; 2. Mohamed R, et al. *J Gastroenterol Hepatol* 2004; 19: 958-69; 3. D*ecision Resources*. Hepatitis B virus in China – Emerging markets study #5; 4. BMS Market Research.

# Hepatitis B: By The Numbers

- 2 M people in the US are HBV infected in 2012
- If it is not treated, in 1/3 of patients, hepatitis B can cause liver damage leading to cirrhosis and liver cancer[1]

- Hepatitis B is responsible for 80% of primary liver cancer globally, which is almost always fatal[2]

  - Liver cancer is the 3rd highest cause of death by cancer in men[3]
  - Without appropriate treatment or monitoring, 1 in 4 person with chronic hepatitis B will die of liver cancer or liver disease
  - #1 cause of cancer death in Viet Namese Men

- **1 M people die from HBV related disease each year**

1 WHO. Available at: www.who.int/csr/disease/hepatitis/en/. 2 Hepatitis B Foundation. Hepatitis B and Primary Liver Cancer. Available at http://www.hepb.org/professionals/hepb_and_liver_cancer.htm. Accessed 4 February 20103. World Health Oragnisation. Cancer Fact Sheet. Available at http://www.who.int/mediacentre/factsheets/fs297/en/index.html.

# HBV Core Tests: Phase I

- HBsAg = infection
- Anti-HBc = exposure
- Anti-HBs = immunity

# HBV Core Tests: Phase II

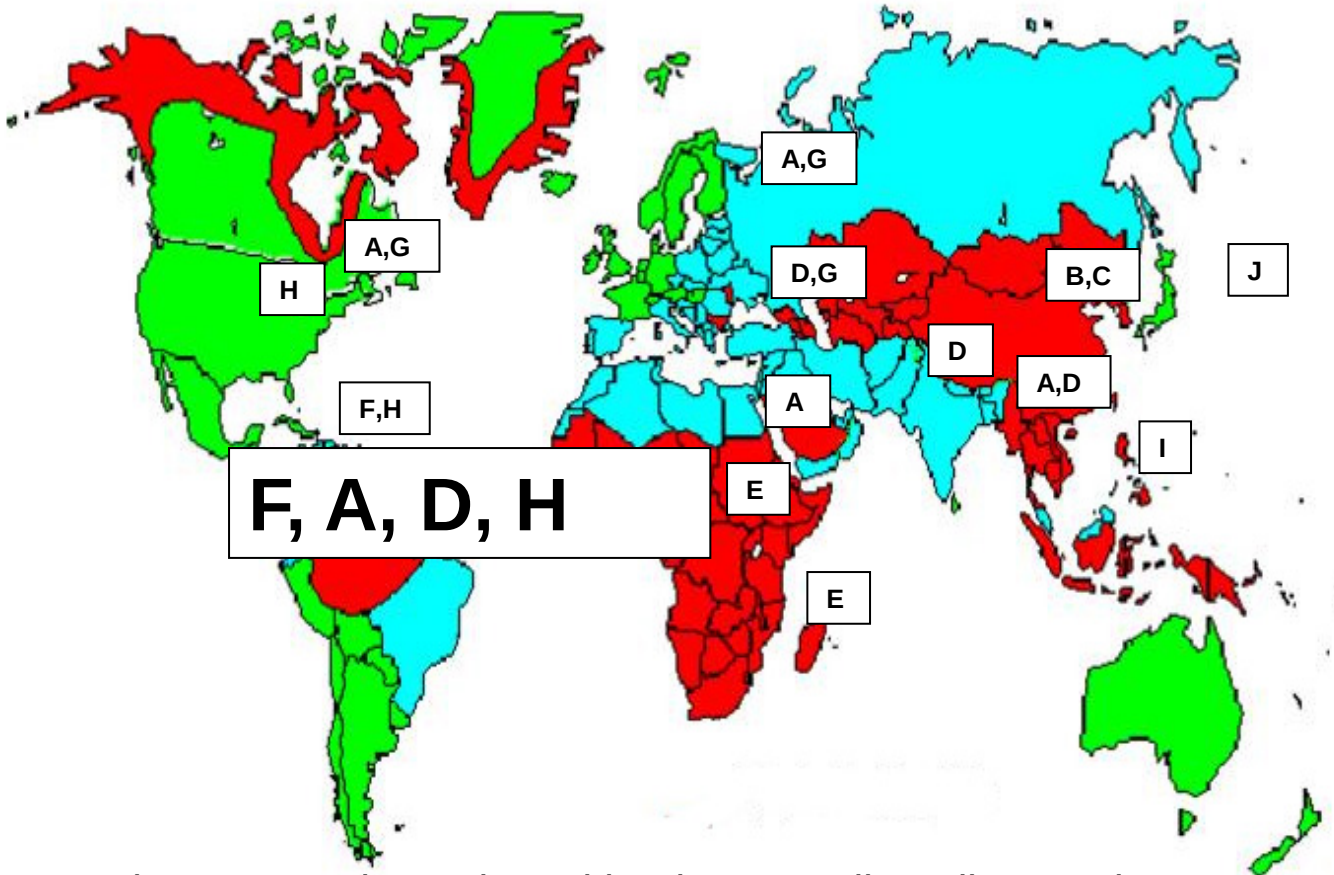
- HBsAg + = infection
  - HBV DNA quant
  - HBeAg
  - Anti-HBe
  - HIV
  - HDV
  - HCV
  - HAV
  - AFP
  - Ultrasound

# HBV Core Tests: Phase III

- HBV DNA Quant +  = active infection/replication
  - HBV
    - Genotype
    - Core and preCore mutations
  - If on an oral HBV therapy
    Resistance testing
  - If AFP elevated
    - 4 phase MR of liver

# HBV Genotypes

**HBV classified into 10 genotypes (A - J)**



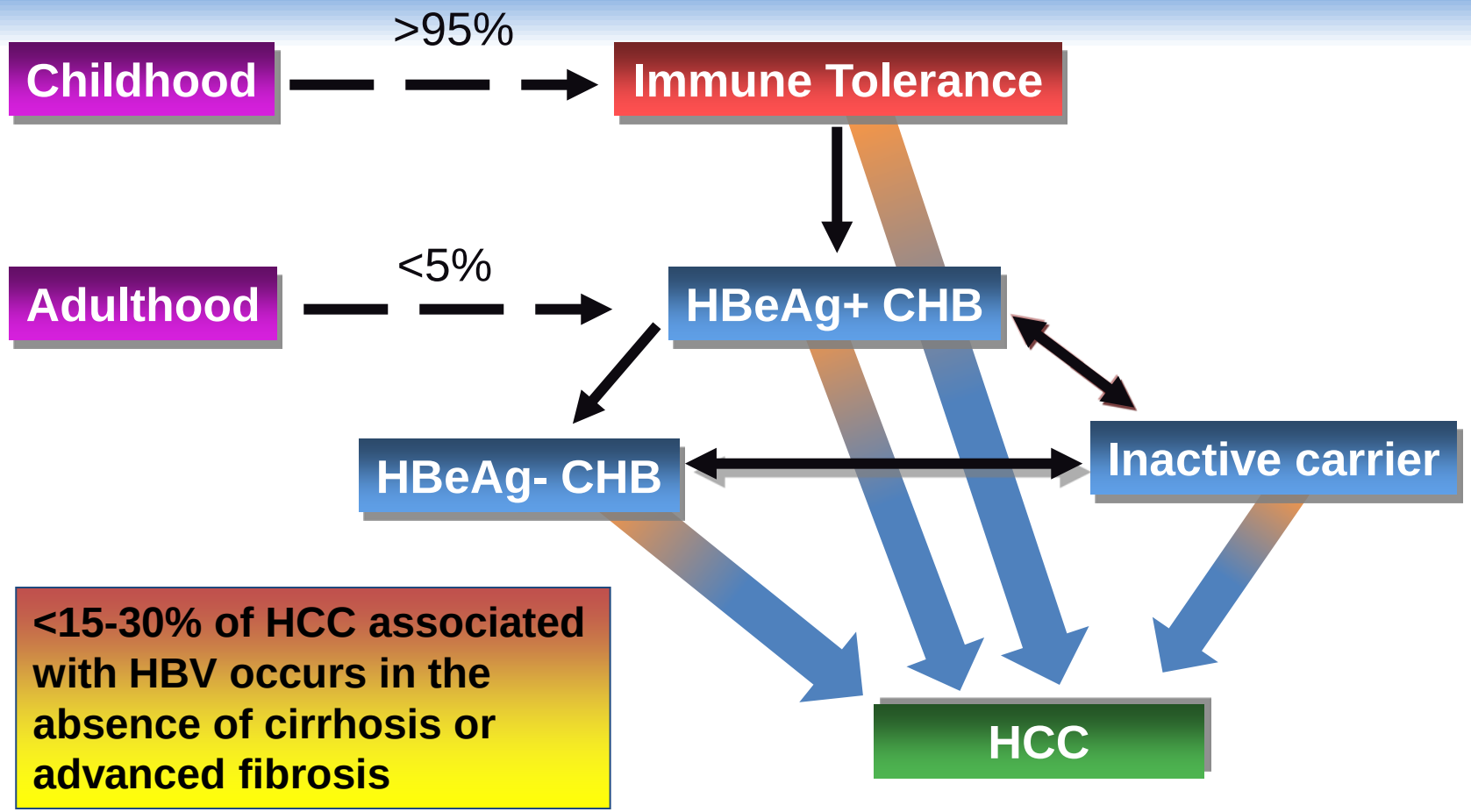

- C may be more pathogenic and lead to more liver disease than B; B may be associated with less active disease
- A and B respond better to interferon than C and D

Adapted from Keeffe EB et al. *Clin Gastroenterol Hepatol*. 2004;2:87-106.

# Genotype Comments

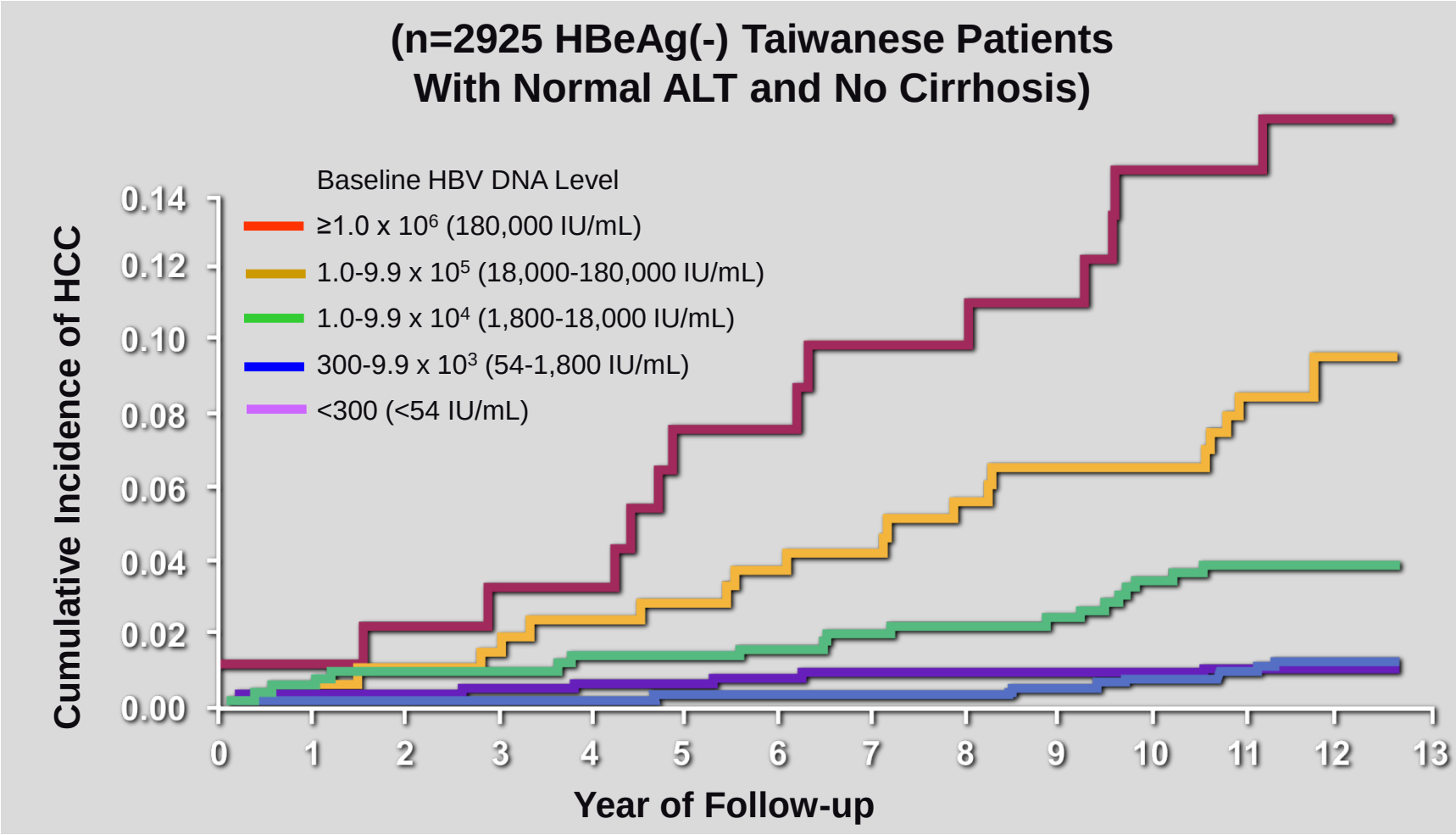
- Genotype A
  - Greater chance of seroconversion
    - Spontaneous
    - With interferon
- Genotype B
  - Better INF response
  - Less cirrhosis
  - Less HCC
- Genotype C
  - Higher risk of HCC and cirrhosis
  - Lower INF response
- Genotype D
  - Very common CHB with eAg(-) disease
  - Poorly responsive to interferon therapy
- Genotype E
  - Good response to interferon and nuc therapy
- Genotype F
  - FHF, more active disease and higher rates of progression to ESLD
- Genotype G
  - All have precore and core stop codons
  - All have coinfection with genotype A to explain HBeAg+
- Genotype H
  - Good response to interferon therapy

# Natural History of HBV Infection




Pungpapong S et al. *Mayo Clin Proc*. 2007;82:967-975.
Chen DS. *J Gastroenterol Hepatol*. 1993;8:470-475.
Seeff LB et al. *N Engl J Med.* 1987;316:965-970.
Lok ASF, McMahon BJ. *Hepatology.* 2009;50:1-36.

# Cumulative Incidence of HCC by Serum HBV DNA Level at Study Entry

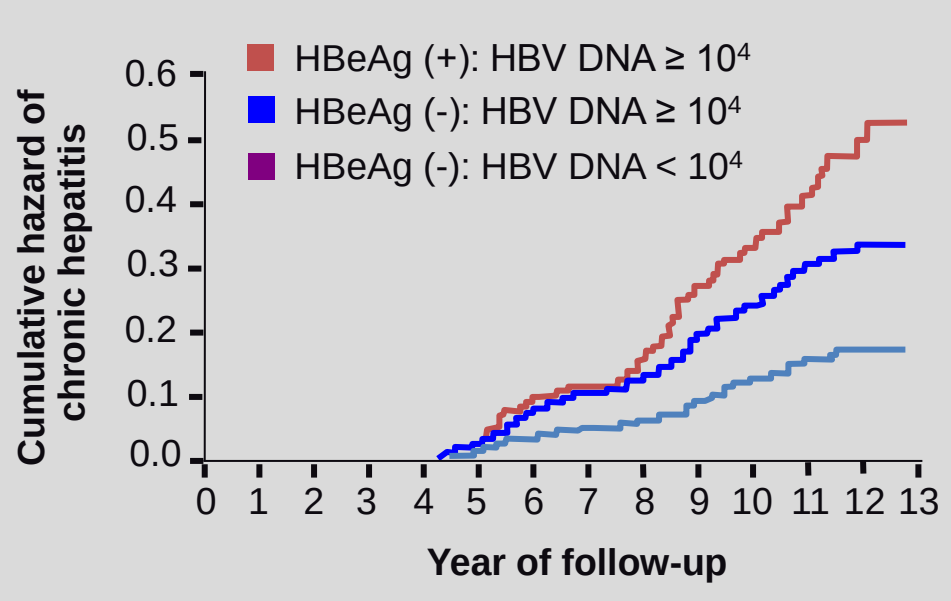





Chen C-J et al. *JAMA.* 2006;295:65-73.

# REVEAL Subset Analysis: HBV DNA and Outcomes in Normal ALT Patients

- Subset of REVEAL (n = 2097)
  - Persistently normal serum ALT levels for the first year
  - Free from cirrhosis and HCC
- 27,282 person-years of follow-up
  - Mean duration: 12.5 years using death as the final outcome



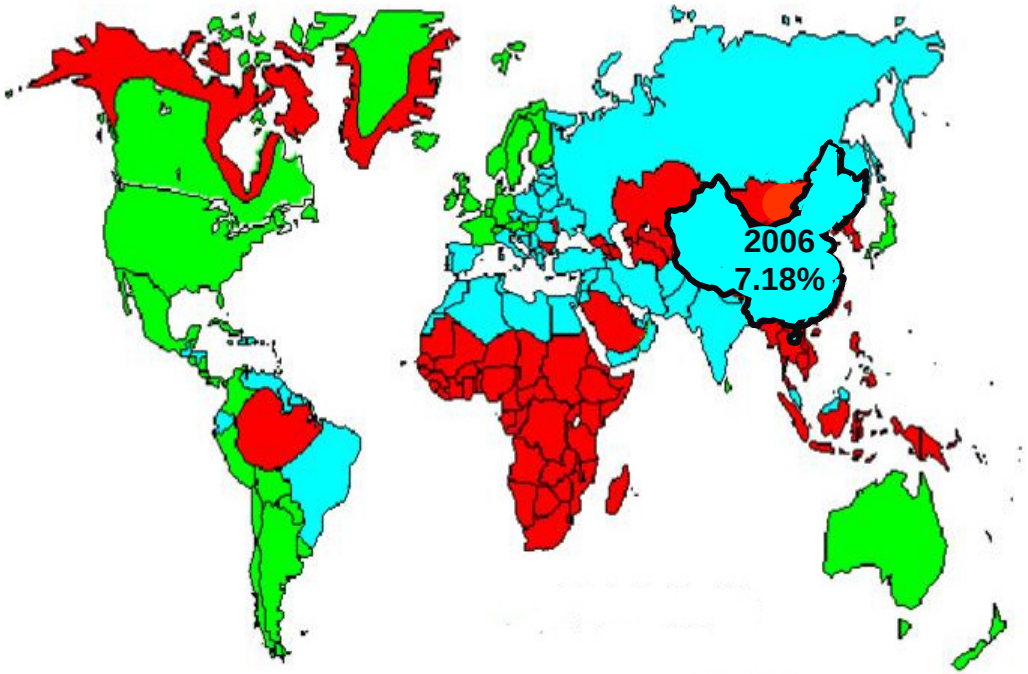

Sequential incidence rates (per 100,000 person-years) from asymptomatic carriers

| | CHB | Cirrhosis | HCC | Liver-related death |
|---|---|---|---|---|
| HBeAg+ (≥$10^4$ c/mL) | 3089 | 3265 | 3005 | 24,933 |
| HBeAg- (≥$10^4$ c/mL) | 2017 | 2728 | 3928 | 14,548 |
| HBeAg- (< $10^4$ c/mL) | 842 | 912 | 2826 | 5932 |



Chen DJ, et al. 2008 EASL. Abstract . 644

# Geographic Pattern of Hepatitis B Prevalence: What Happens with Institution of a Broad-Based Vaccine Program




**HBsAg Prevalence**

- >8% High
- 2-8% Intermediate
- <2% Low

WHO. Available at: http://www.who.int/csr/disease/hepatitis/HepatitisB_whocdscsrlyo2002_2.pdf. Accessed 02/03/11.
Sun Z et. al. *J Med Virol* 2002, 67:447-450.

# Aims of Antiviral Therapy

To achieve elimination or sustained suppression of HBV replication*

To prevent cirrhosis and liver failure

To prevent hepatocellular carcinoma

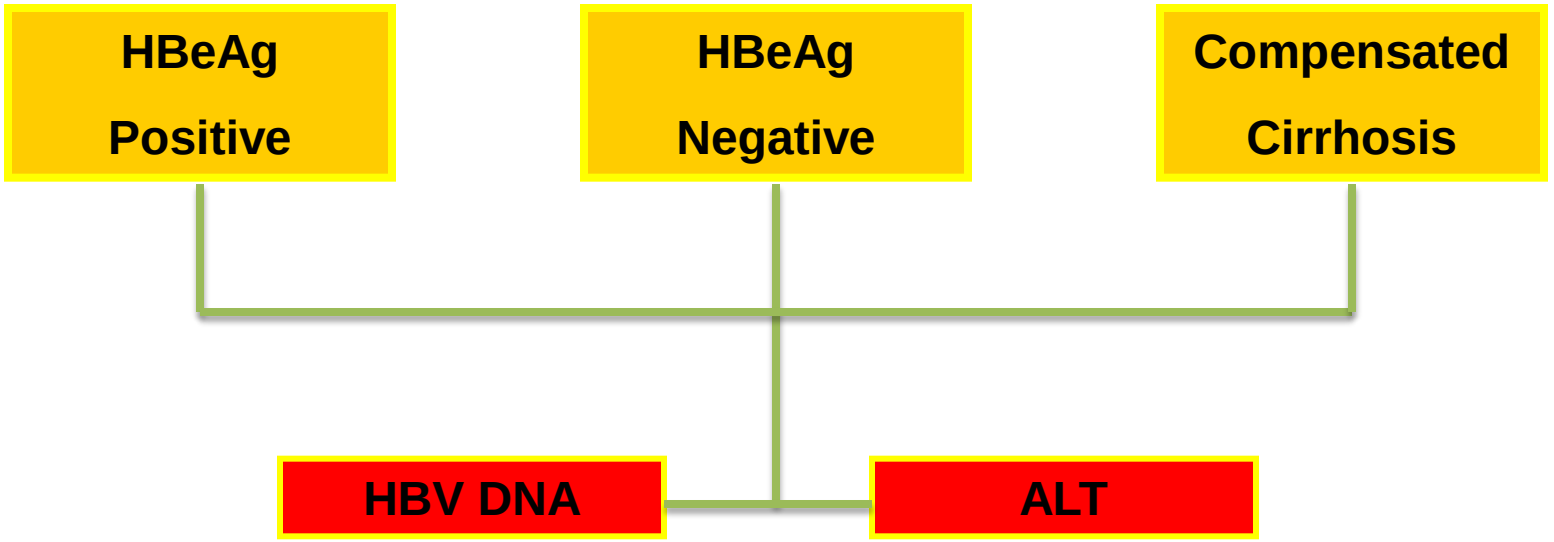
*There is no cure  of HBV

# Next Steps in HBV Management

- **Stop indefinite (forever) oral (Nuc) therapy**
- Maximize Interferon therapy
- Permanent clearance of HBV
  - HBsAg clearance
    - cccDNA clearance
      - CURE?

# Indications for Antiviral Therapy

Based on biochemical, serological, virological, and histological status

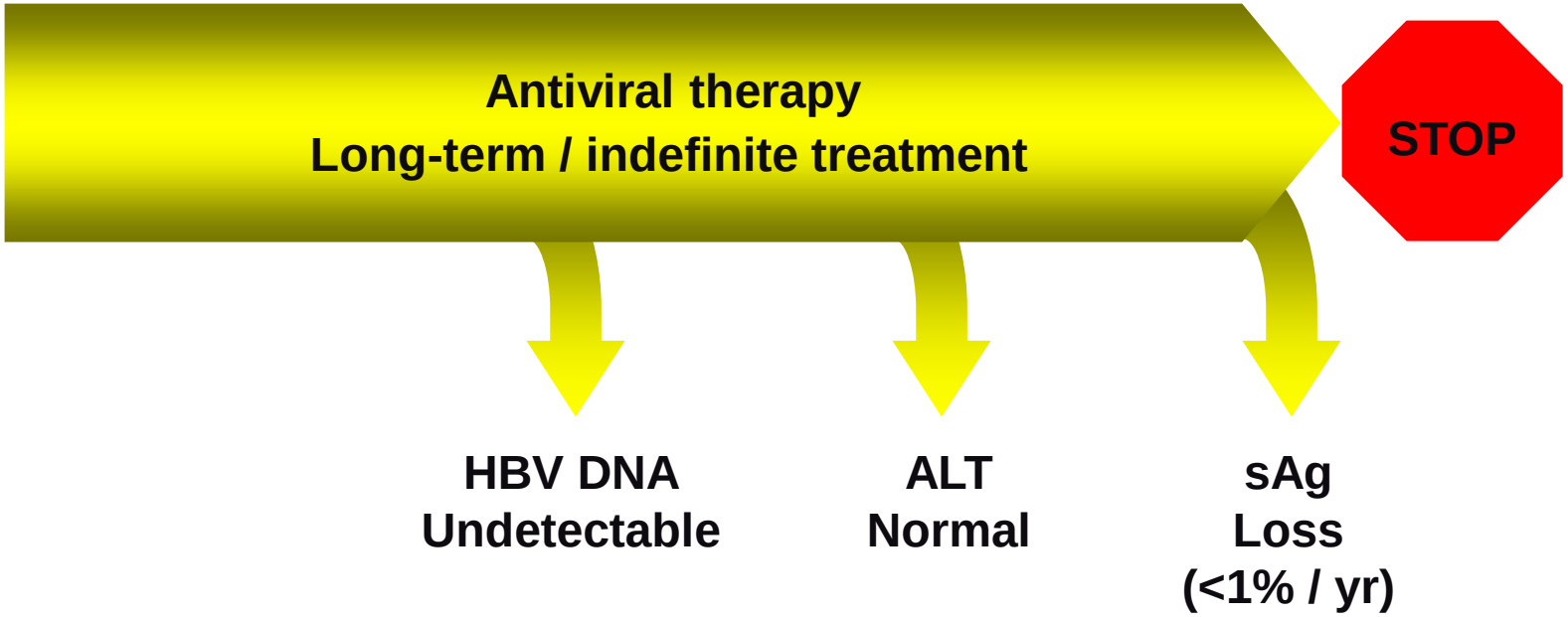


# Contrasting Treatment Guidelines for Chronic Hepatitis B

| Guideline | HBeAg+ | | HBeAg- | |
|---|---|---|---|---|
| | HBV DNA IU/mL | ALT U/L | HBV DNA IU/mL | ALT U/L |
| EASL 2012[1] | >2,000 | >ULN Or if active LD | >2,000 | ➢ ULN ➢ Or if active LD |
| US Algorithm 2008[2] | ≥20,000 | >ULN or (+) biopsy | ≥2,000 | >ULN or (+) biopsy |
| APASL 2008[3] | ≥20,000 | >2x ULN | ≥2,000 | >2x ULN |
| AASLD 2009[4] | >20,000 | >2x ULN or (+) biopsy | >20,000 or >2,000 | ≥2x ULN or (+) biopsy |


1. European Association for the Study of the Liver. *J Hepatol.* 2012.
2. Keeffe EB et al. *Clin Gastroenterol Hepatol.* 2008;6:1315-1341.
3. Liaw Y-F et al. *Hepatol Int.* 2008;2:263-283.
4. Lok ASF, McMahon BJ. *Hepatology.* 2009;50:1-36.

# Endpoints of Antiviral Therapy HBeAg Negative CHB



# Endpoints of Antiviral Therapy Compensated Cirrhosis

Clinical endpoints are similar to those for HBeAg-positive and HBeAg-negative CHB patients.

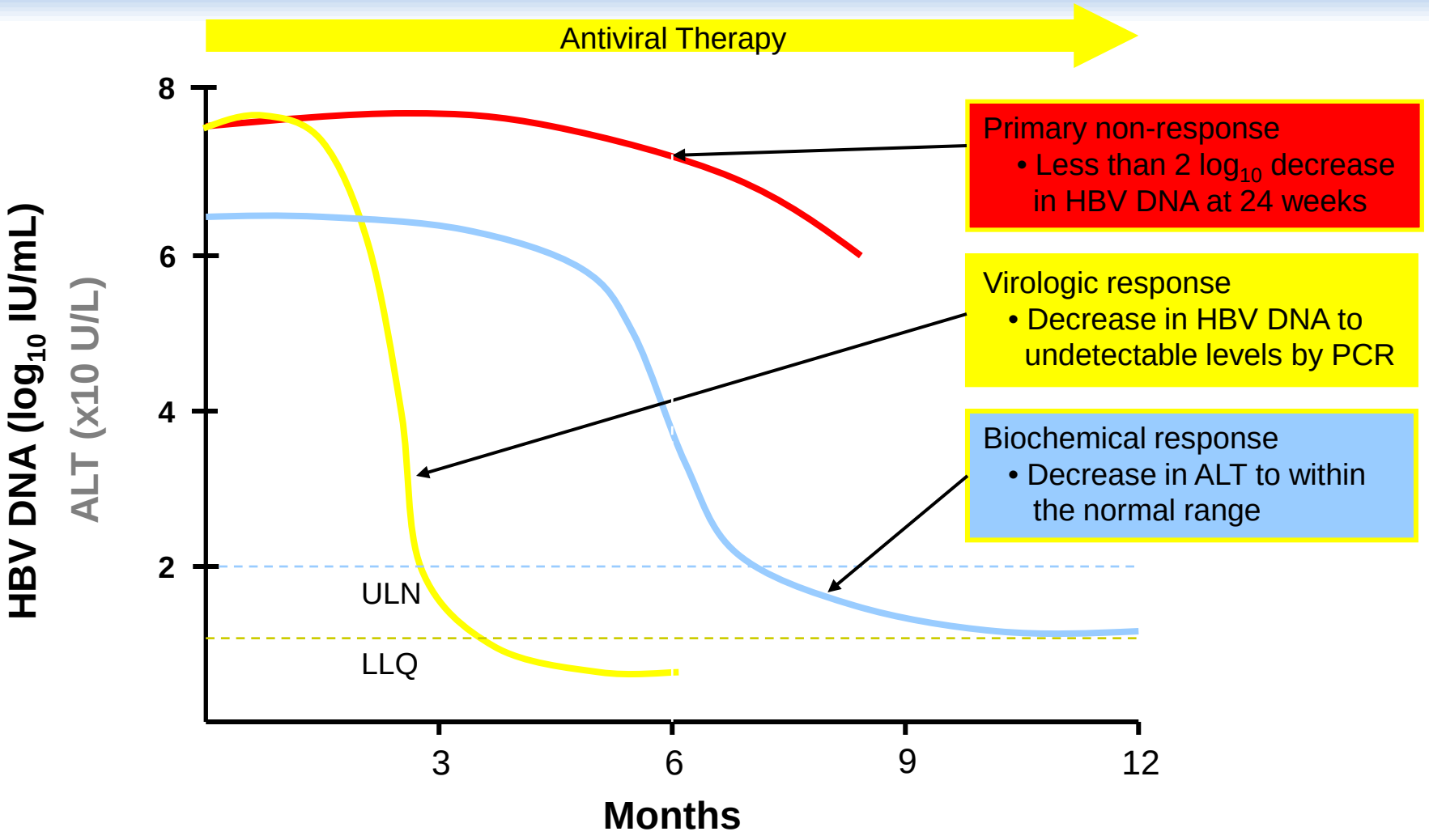
No liver failure

No HCC

No liver transplant

No death

# On-Treatment Assessment of Response

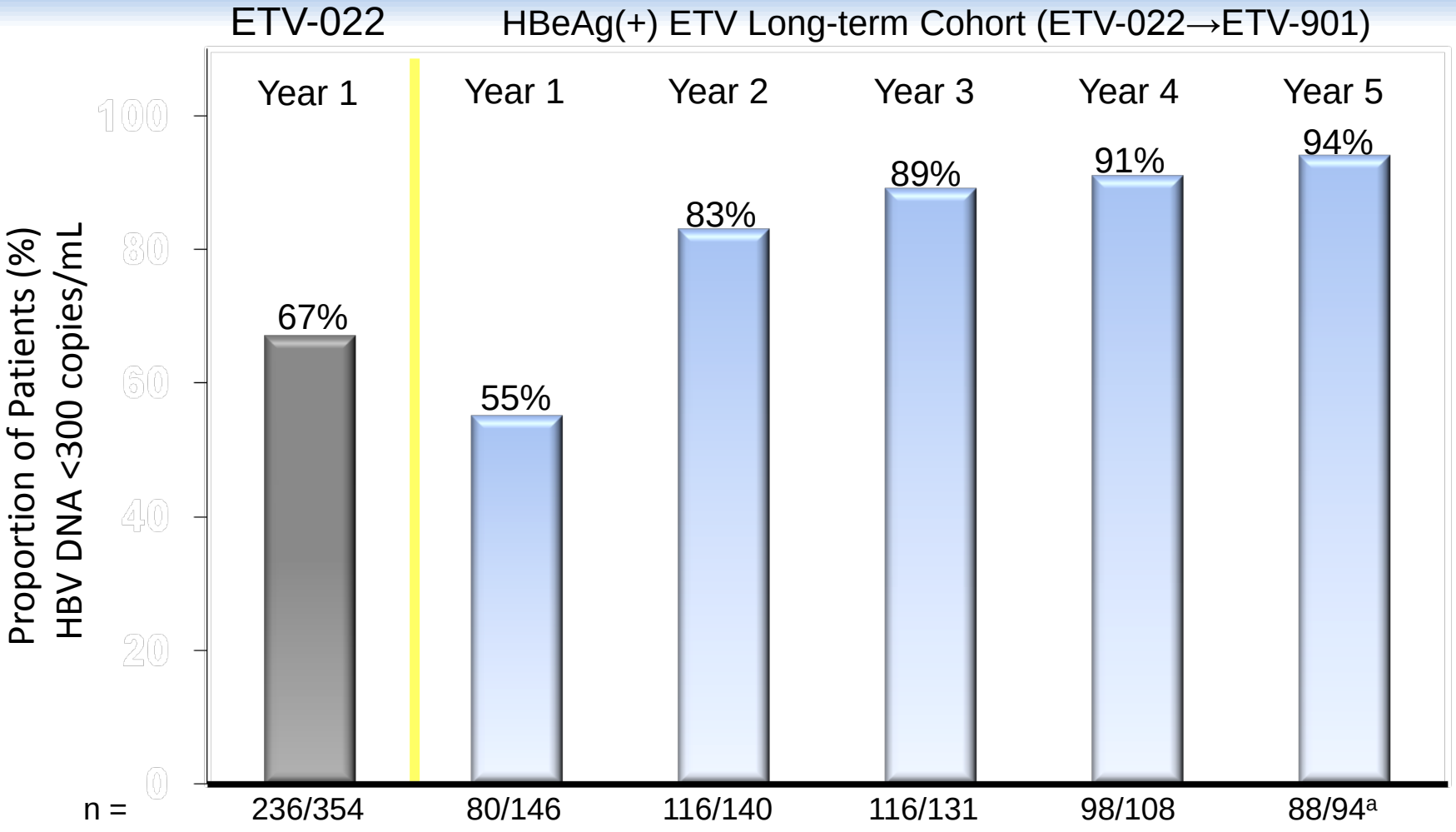


# Approved Therapies for CHB

| Nucleosides/Nucleotides | | | |
|---|---|---|---|
| Tenofovir | VIREAD® | Gilead Sciences | 2008 |
| Telbivudine | TYZEKA™ | Idenix / Novartis | 2006 |
| Entecavir | BARACLUDE™ | Bristol-Myers Squibb | 2005 |
| Adefovir dipivoxil | HEPSERA™ | Gilead Sciences | 2002 |
| Lamivudine | EPIVIR-HBV® | GlaxoSmithKline | 1998 |
| **Interferons** | | | |
| Peginterferon alfa-2a | PEGASYS® | Roche Laboratories | 2005 |
| Interferon alfa-2b, recombinant | INTRON® A | Schering / Merck | 1992 |

Preferred therapies – AASLD Guidelines

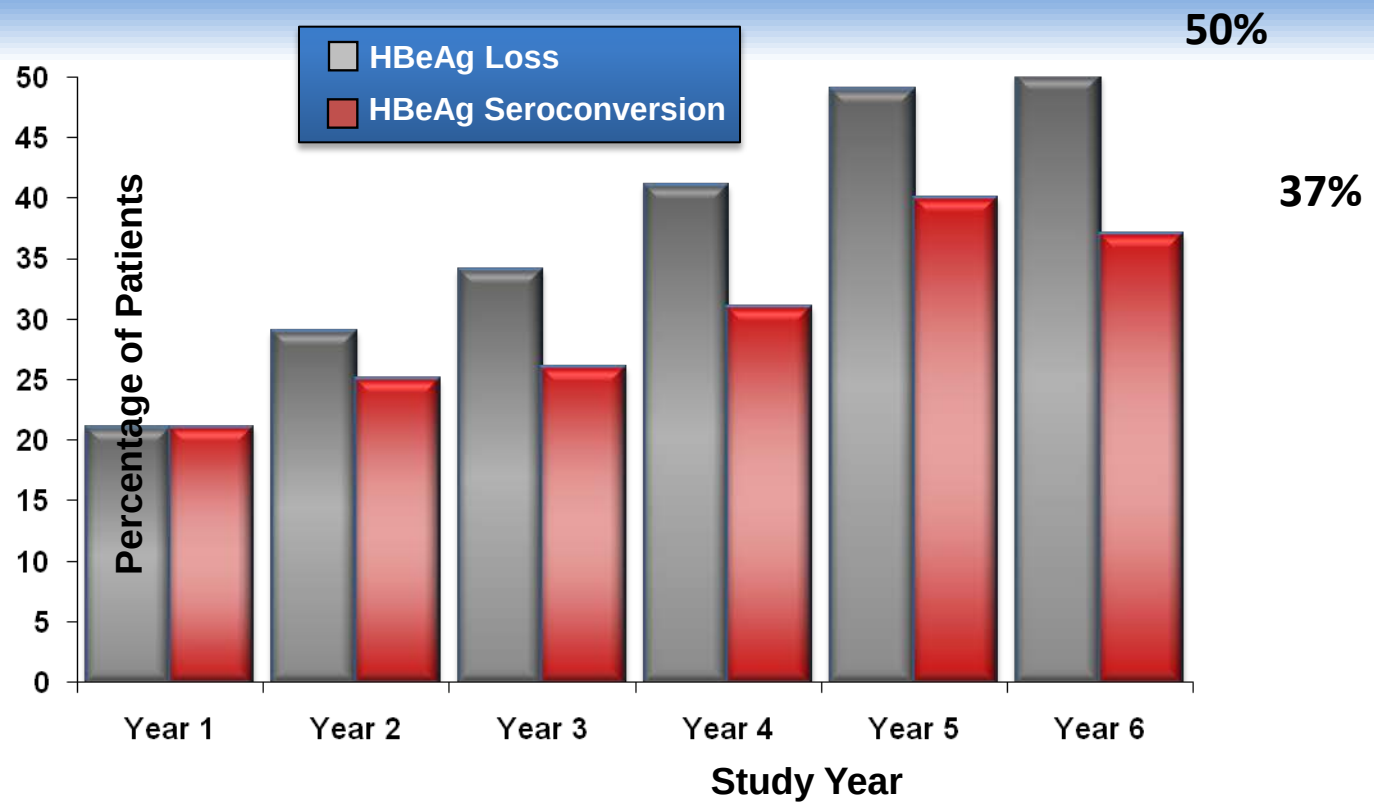
# Proportion of Patients Achieving HBV DNA <300 copies/mL Through 5 Years




[a] 5 patients who remained on treatment at the Year 5 visit had missing PCR values (NC=M)

Han S, et al
Poster 893

# eAg and sAg Serologic Responses on TDF Through 6 Years*




- **Overall, HBsAg loss: n=24 (11%) †; HBsAg seroconversion: n=18 (8%)†**
- **1 HBeAg- patient in Study 102 had confirmed HBsAg loss (Week 240)**

*On treatment (observed) analysis, missing = excluded / addition of FTC = included; † KM-ITT (%)



Marcellin et al. AASLD 2012. Poster 374.

# Peg-Interferon

## Pros

Finite treatment duration: 1 year

No resistance

Potentially best chance of achieving sAg loss

Good durability of response with increasing clinical benefits following cessation of therapy

17% of patients have durable HBDDNA suppresssoin

~10% have sAg loss

## Cons

Significant side effect profile

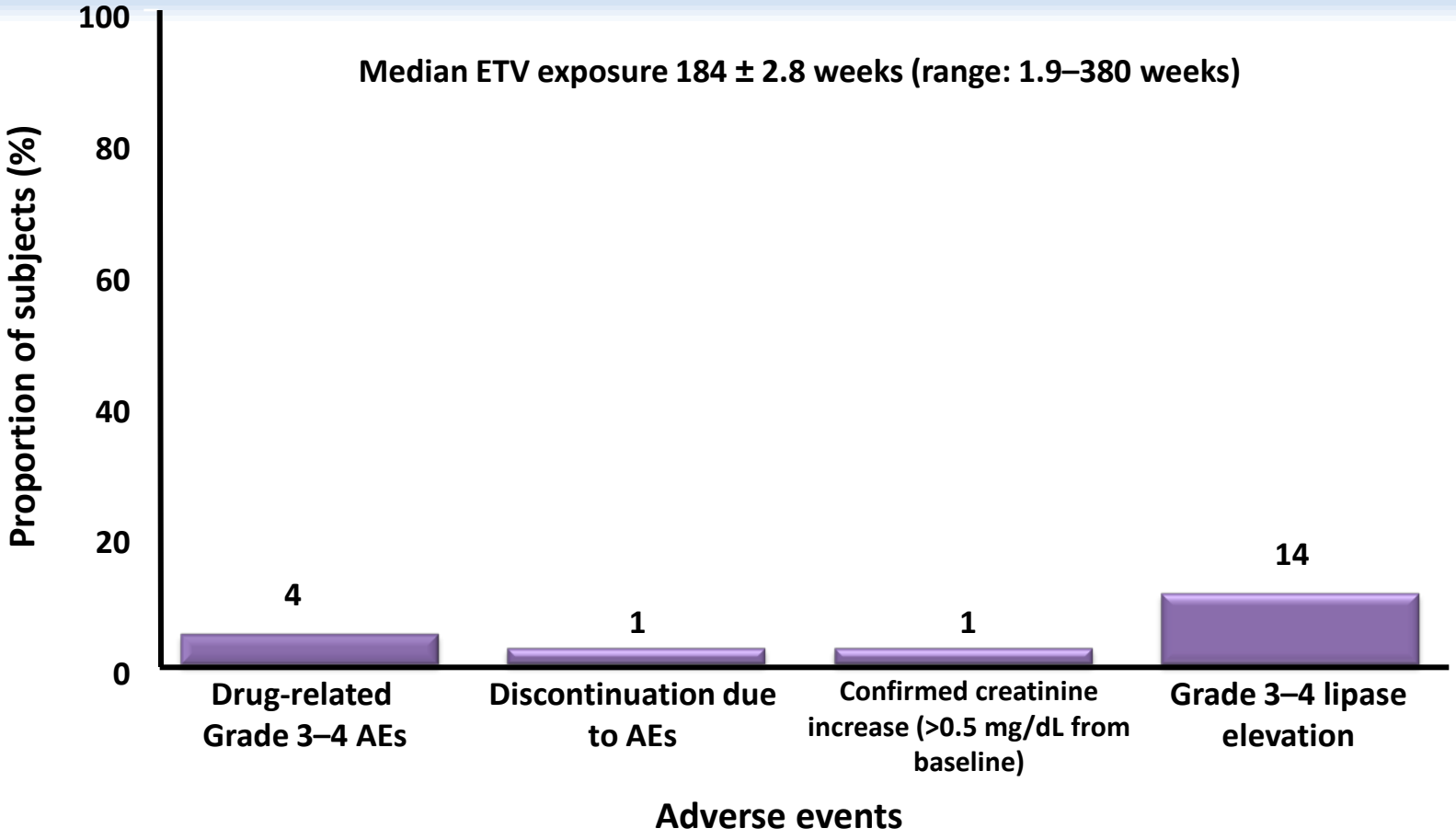
Parenteral administration

Most expensive annual cost

Less efficacious in Asian patients

# LONG-TERM SAFETY CONCERNS

# ETV Has a Generally Favorable Open-Label Safety Profile up to 380 Weeks* (n=1051)




*49% patients enrolled in ETV-901 had >5 years total ETV treatment (including treatment time in parent protocols). Patients in the ETV-901 rollover study received 1-mg ETV.



1. Adapted from Manns M, et al. 45th EASL, 2010. Poster 1016. http://www.natap.org/2010/EASL/EASL_39.htm [Accessed Mar. 2011].

# Detection of Proximal Tubular Damage

**Increasing proximal tubule dysfunction**

## Signs of early damage

urine

- Glycosuria[1,6]
- Hyperaminoaciduria[1]
- Phosphaturia[1]
- β2-microglobulin/amino-aciduria[1,5,6]
- Fractional tubular resorption of phosphorus[1]
- Fractional excretion of uric acid[1]
- Additional markers: TmPO4/GFR[4]

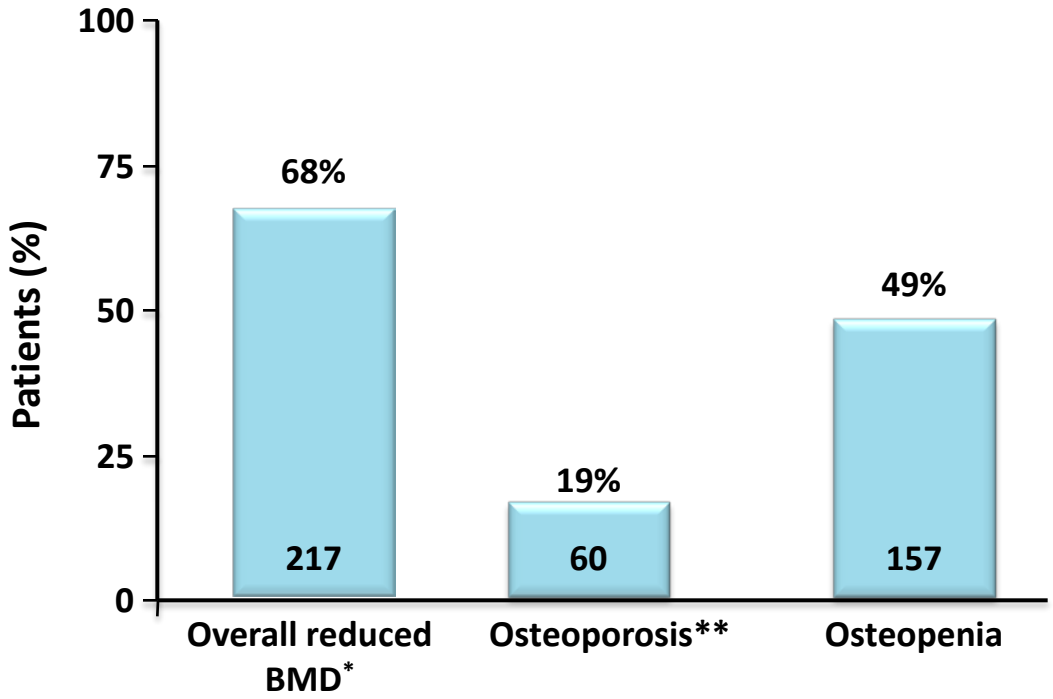
## Signs of later damage

- Decrease in GFR[2,4]
- Proteinuria[5,6]
- Hypophosphatemia[4]
- Bone disease[4]
- Osteomalacia[3]

**Clinically meaningful tubular damage is defined when two or more of these markers are present[1]**

1. Labarga P, et al. *AIDS*. 2009:23:689–96. 2. Post FA, et al. *Curr Opin HIV AIDS* 2010;5(6):524–30 . 3. Woodward CLN, et al. *HIV Medicine.* 2009;10:482–7. 4. Essig M, et al. *JAIDS* 2007; 46: 256–8. 5. D'Amico G, et al. *Curr Opin Nephrol Hypertension* 2003; 12: 639–43. 6. Ludwig M, et al. *Int Urol Nephrol* 2011; DOI 10.1007/s11255-011-9914-0

# Nucleos(t)ide Cohort: More Than Two Thirds of Patients with CHB Had Reduced Bone Mineral Density (BMD)



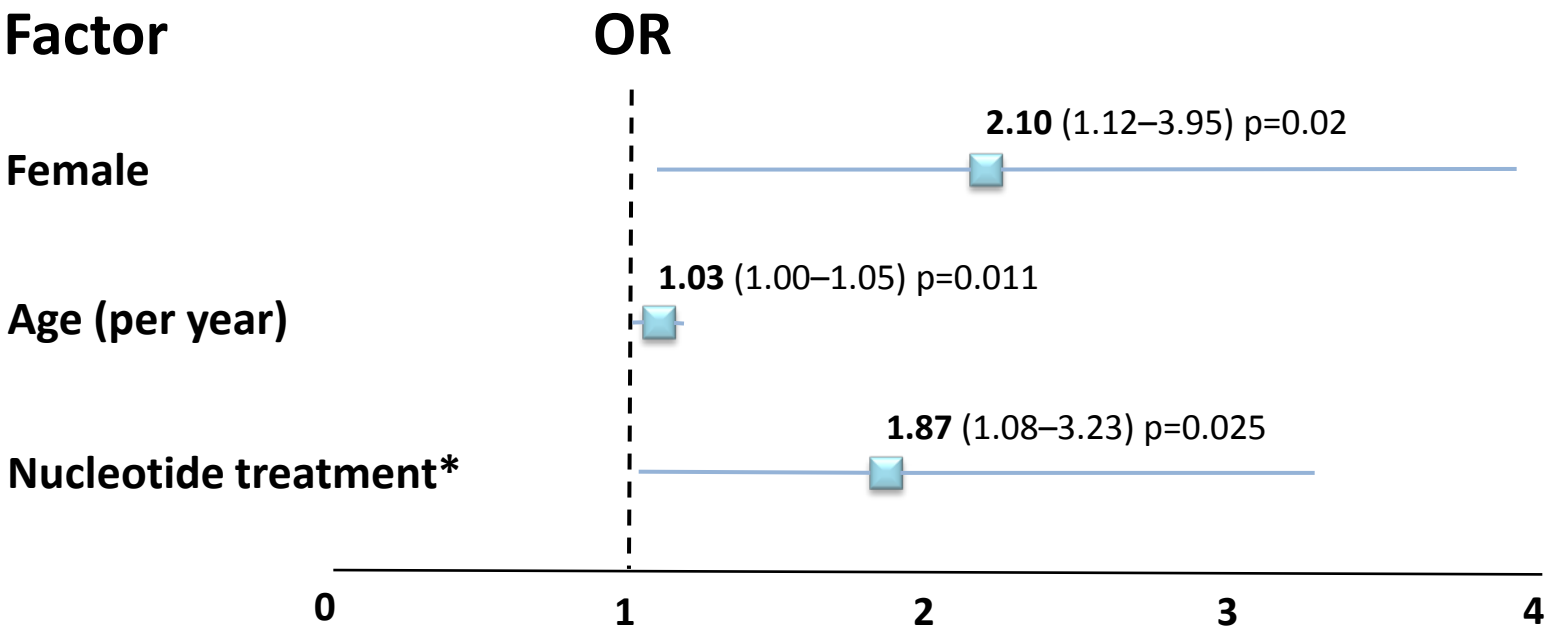

Median months on treatment: 36 (6–183)
*Defined as the presence of osteopenia or osteoporosis;
**At lumbar spine (48%); femoral neck (17%) or both (35%).

Treatment regimen, n (%): ADV+LMV 194 (61%); TDF+LMV 36 (11%); ETV 61 (19%); LMV 21 (7%); TDF 7 (2%)



Adapted from Viganò M, et al. AASLD 2010. Poster 414.

# Treatment with Nucleotide Analogues (with or without LMV coadministration) is an Independent Risk Factor for Reduced Bone Density**




*ADV (n = 194) or TDF (n =43) in combination with LMV, except 7 patients who received TDF monotherapy.
**Included were variables with p value <0.1 at univariate analysis

Adapted from Viganò M, et al. AASLD 2010. Poster 414.

# RESISTANCE

# US Treatment Algorithm – Resistance Profile: First Line Treatment Options Have The Lowest Resistance Rates


Tenofovir

Adefovir

Lamivudine

Entecavir

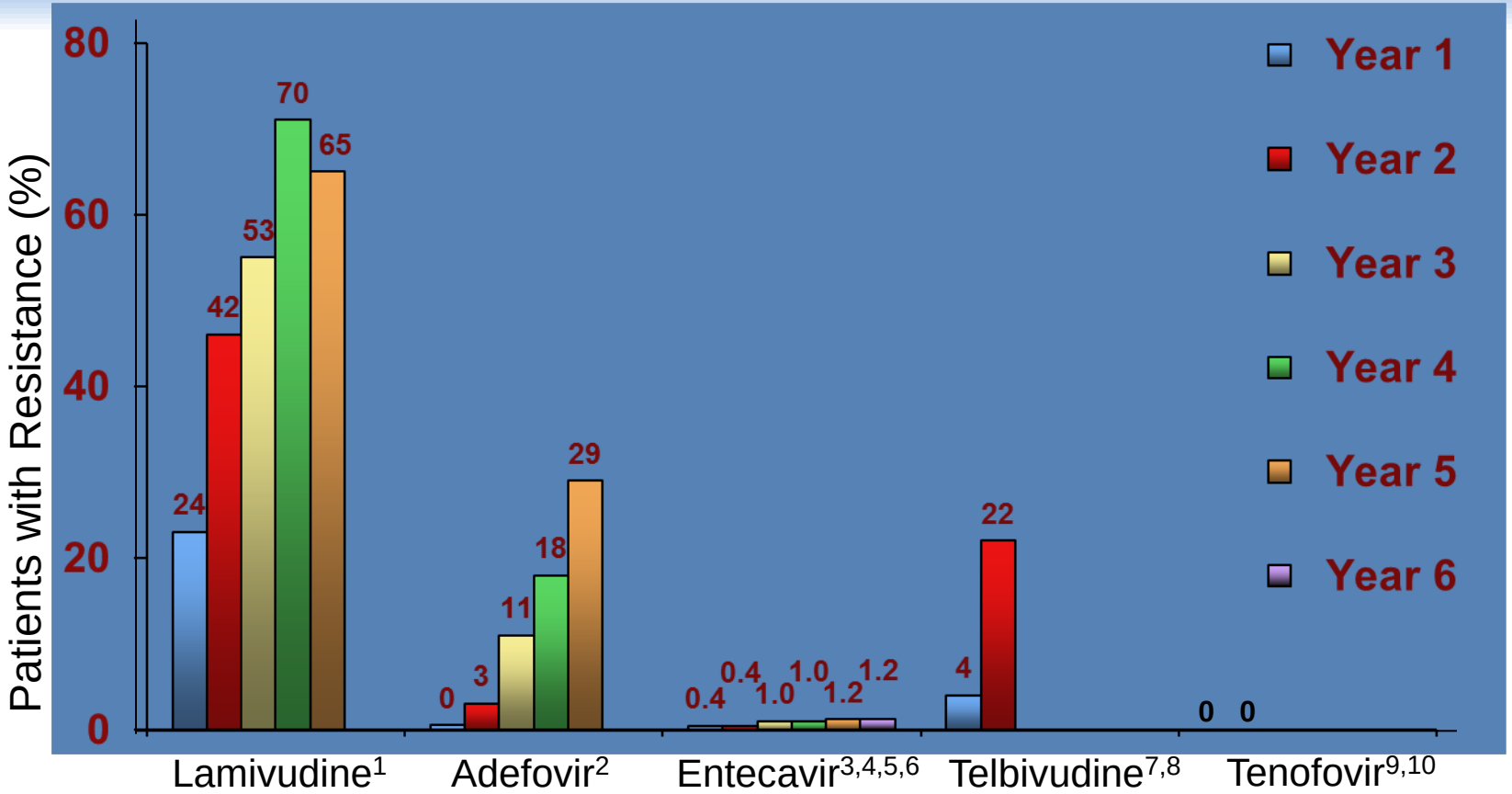
Telbivudine

Resistance does not appear to emerge during treatment with IFN $\alpha$-2b or PegIFN $\alpha$-2a

Adapted from Keeffe EB et al. *Clin Gastroenterol Hepatol.* 2008;6:1315-1341.

# Differences in Development of Resistance with Long-Term Treatment in Nucleoside-naïve Patients

***Not Head to Head Trials, Different Patient Populations and Trial Designs***



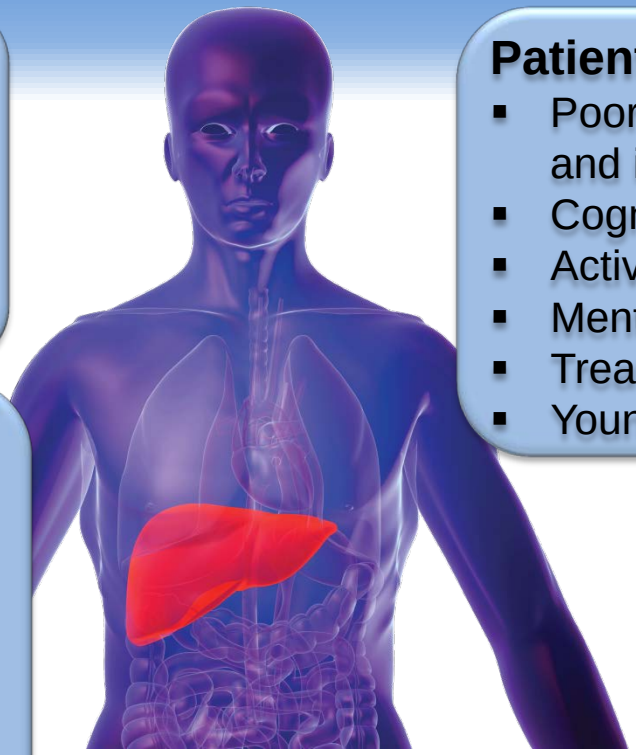


1. Lok ASF et al. *Gastroenterology* 2003;125:1714-1722.
2. Hadziyannis SJ et al. *Gastroenterology* 2006;131:1743-1752.
3. Colonno RJ et al. *Hepatology* 2006;44:1656-1665.
4. Colonno et al, *Hepatology* 2006, 44 (Suppl 1):229A
5. Colonno RJ et al. *J Hepatol.* 2007;46(Suppl 1):S294.
6. Tenney DJ et al. *Gastroenterology* 2009;136(Suppl 1):A-865.
7. Telbivudine (Tyzeka®) prescribing information; May 2009; Novartis Pharmaceuticals, East Hanover, NJ.
8. Lai CL, *Hepatology* 2006;44(Suppl 1):222A.
9. Tenofovir (Viread®) prescribing information; May. 2009; Gilead Sciences, Foster City, CA.
10. Snow-Lampart A et al. *Hepatology* 2008;48(Suppl 1):745A.

# What Factors Influence Patient Adherence?

**Situational conditions**
- Stigmatisation and lack of social support or social isolation
- Access to healthcare system
- Childcare responsibilities

**Patient characteristics**
- Poor understanding of the disease and importance of adherence
- Cognitive impairment and literacy
- Active substance abuse
- Mental illness
- Treatment fatigue
- Younger age



**Drug factors**
- **Adverse events/tolerability**
- **Monitoring frequency**
- Dosing frequency
- Pill burden
- Dietary requirements
- Treatment duration

- When selecting an antiviral drug, protecting your patient is key
- Using a drug with a good safety profile and low monitoring burden may help improve patient adherence



1. Trotta P, et al. *JAIDS* 2002;31:S128–31; 2. Ammassari A, et al. *JAIDS* 2002;31:S123–27.
3. Kalichman S, et al. *J Gen Intern Med* 1999;14:267–73.

# Non-Adherence is Associated With HBV Drug Resistance

**Up to 30% of virologic breakthrough in CHB observed in clinical trials is related to medication non-compliance thus, compliance should be ascertained before testing for genotypic resistance**

AASLD PRACTICE GUIDELINES

Chronic Hepatitis B: Update 2009

Anna S. F. Lok[1] and Brian J. McMahon[2]

Preamble

Introduction

Screening High Risk Populations to Identify HBV-Infected Persons

Lok ASF & McMahon BJ. AASLD practice guidelines. Chronic hepatitis B: Update 2009. Available at http://www.aasld.org/practiceguidelines/Pages/NewUpdatedGuidelines.aspx. [Accessed Mar. 2011].

# Strategies to Improve Adherence

- Toxicities / side effects
- Monitoring burden
- Doctor / patient relationship
- Patient / physician education
- Increased demand on patient time
- Patient acceptance of long-term treatment
- Language / lifestyle / cultural / economic barriers
- Short term or one time dosing

Jin J, et al. *Ther Clin Risk Manag* 2008;4:269–86.

# What Tests and Factors Need to be Performed to Evaluate Patients who have the Diagnosis of CHB

- Most important tests
  - **HBV DNA quant**
  - FH of HCC ?
  - Cirrhosis: yes no
  - Elevated AFP >20
  - Age/duration of disease
  - Co-infection: HDV, HCV, HIV
  - **ALT, duration of ALT elevation**

- Intermediate importance testing
  - Alcohol
  - Fatty liver, DM, MS
  - eAg status
  - {sAg quant}
  - patient motivation, compliance, adherence

## HBV: Disease Evaluation

- Tertiary considerations
  - If HBV DNA +
    - Blood tests for
      - Precore
      - Core
      - Genotype
  - Smoking
  - Cost of testing and treatment/insurance
  - Comorbidities, renal, bone
  - Patient motivation
  - On treatment?: resistance testing

# What is Next ?

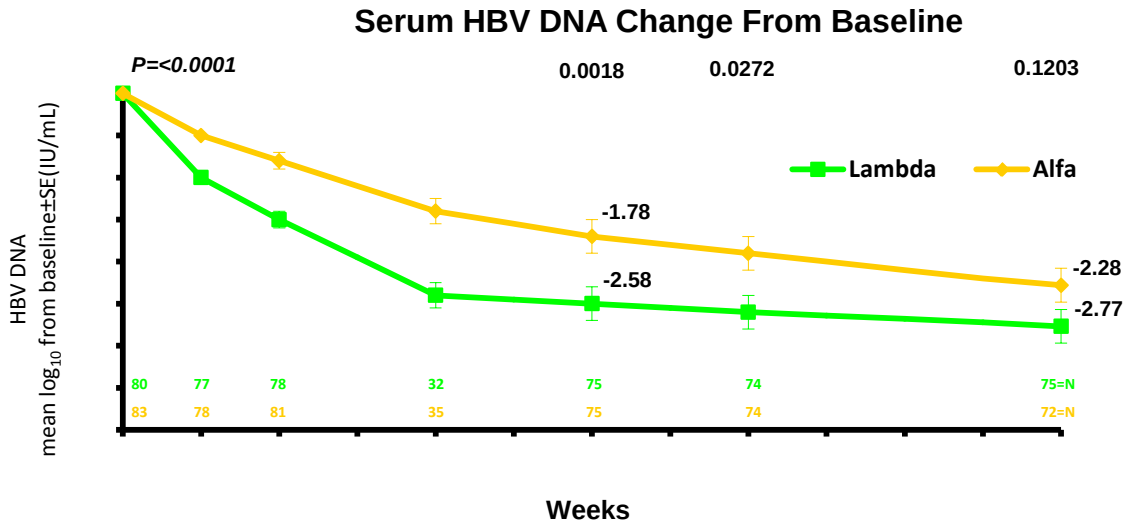
"Functional Cure" defined as:

Persistent HBV DNA and sAg negativity with normal liver tests and normal histology

Normal life span no increased  risk of HCC or cirrhosis

# PEG-IFN Lambda for HBV is More Effective Than PEG-IFN 2a

- Phase 2B study of PEG-IFN Lambda vs. PEG-IFN alfa-2a n HBeAg-positive CHB patients
  - Baseline: IFN naïve, HBV-DNA > $10^5$ copies/ml
  - 75% males, 90% Asians, 5% cirrhotics, 85% genotype B or C
- 24 week interim data of planned 48 week treatment course
  - HBV-DNA dropped more at 12 and 24 weeks with Lambda
  - HBsAg dropped more with Lambda
  - HBeAg loss was equivalent
  - ALT normalized more with alfa-2a





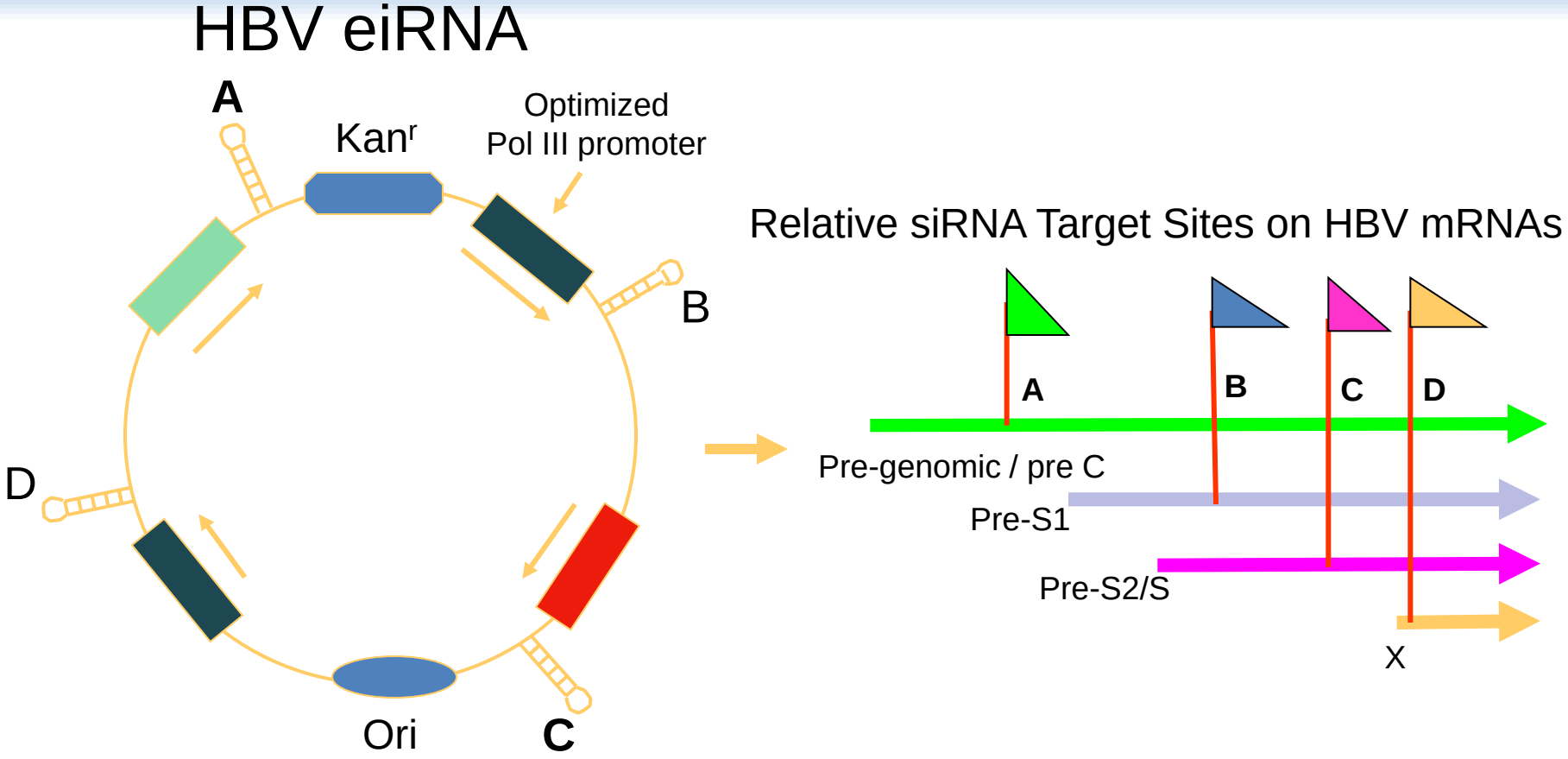

Chan HLY, et al. 63rd AASLD; Boston, MA; November 9-13, 2012; Abst. LB-14.

| | | |
|---|---|---|
| **Nucleos(t)ide analogues (NA)** inhibit viral DNA polymerase | | |
| Pradefovir | Inhibits viral DNA polymerase | Phase II |
| Elvucitabine | Inhibits viral DNA polymerase | Phase II Central and Eastern Europe |
| Valtorcitabine | Inhibits viral DNA polymerase | Phase II |
| Amdoxovir | Inhibits viral DNA polymerase | Phase II |
| LB 80380 (ANA 380) | Inhibits viral DNA polymerase | Phase II |
| Racivir | Inhibits viral DNA polymerase | Phase II |
| Pentacept (L-3'-FD4C) | Inhibits viral DNA polymerase | Preclinical |
| **Other small molecule (other than NA) antivirals** interfere with proteins involved in viral reproduction at various points in life cycle | | |
| NOV-205 | Small molecule | Phase II/III China/approved in Russia |
| *HepeX-B* | Human monoclonal antibodies | Phase II Israel and US (orphan drug approval in US for liver transplants) |
| UT 231-B | Small molecule | Preclinical HBV (phase II HCV) |
| Bay 41-4109 | Inhibits viral nucleocapsid | Preclinical |
| **Noninterferon immune enhancers** boost T-cell infection-fighting immune cells and natural interferon production | | |
| EHT899 | Oral viral protein | Phase II Israel |
| Thymosin alpha-1 | Immune stimulator | Phase II with lamivudine/orphan drug approval in US for liver cancer |
| EP-HBS | Immune stimulator (HBV therapeutic vaccine) | Phase I |
| HBV core antigen vaccine | Immune stimulator | Phase I |
| SpecifEx-HepB | Immunological cell transfer | Preclinical/phase I |
| HepX | Expressed interfering RNA | Preclinical |

**AGENTS IN EARLY CLINICAL DEVELOPMENT OR PRECLINICAL STUDY**

**Marcellin P, Clinical Care Options, 2006**

# Design of Nucleonics' Multi-Targeting Anti-HBV Drug Substance NUC 500-001



# Preclinical Studies on Myrcludex B the First in Class Entry Inhibitor of HBV and Hepatitis Delta Virus (HDV).


- The GMP synthesis of 100 g Myrcludex B (API) is finished.
- A formulation for s.c. application has been developed.
- 9000 vials for clinical studies have been filled.
- Myrcludex B has been characterized for purity, stability etc.

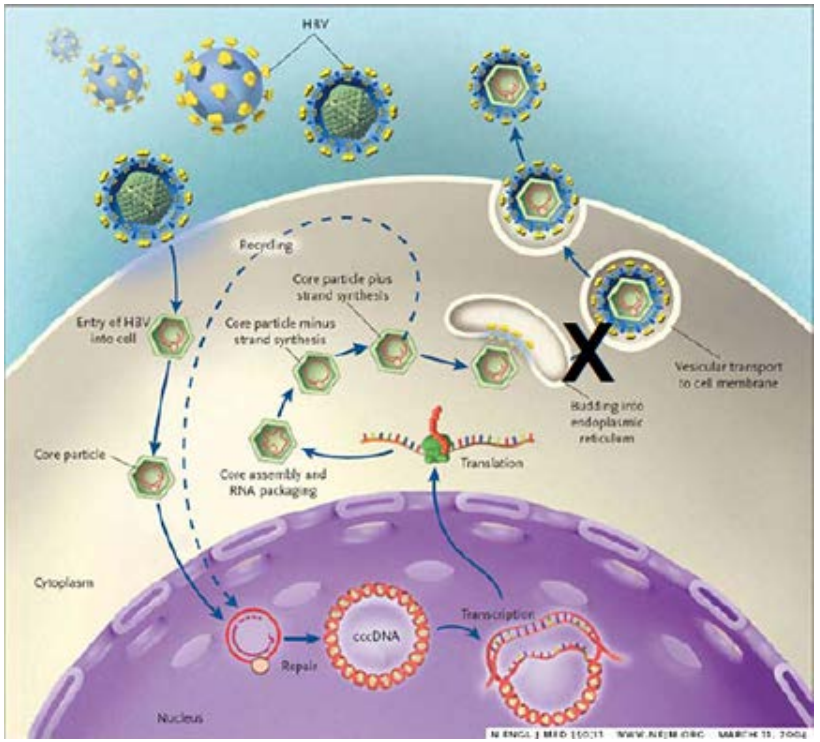
# Secretion / Anti-Antigenemia Inhibitors

HBF sponsored:

- *Gluc inhibitors (Cellgosivir)*
- *Secretion inhibitors*

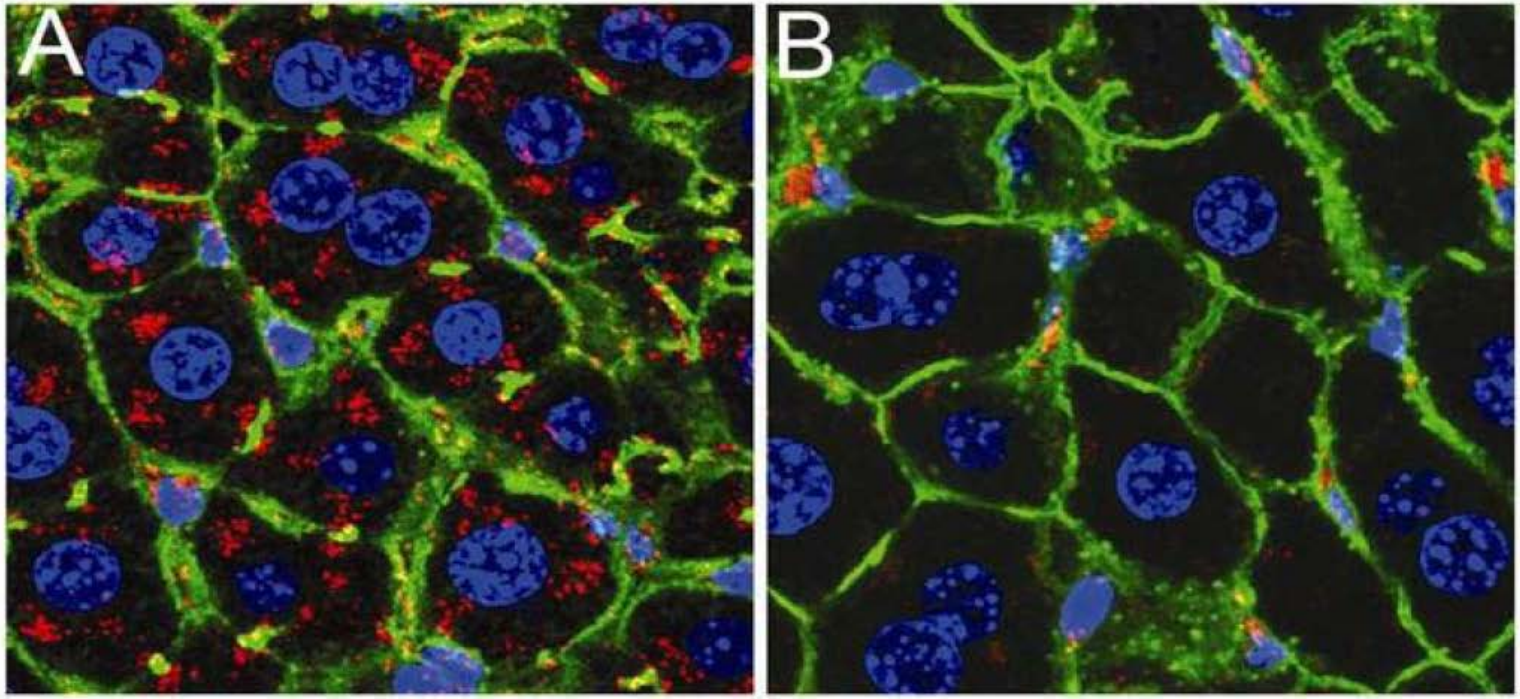
Bayer:

- *Bay 4109 Capsid inhibitors*




Graphic from D.Ganem, NEJM 35;11 (Mar. 11,2004)

# Ligand-Mediated Targeting of DPCs to Hepatocytes in Liver

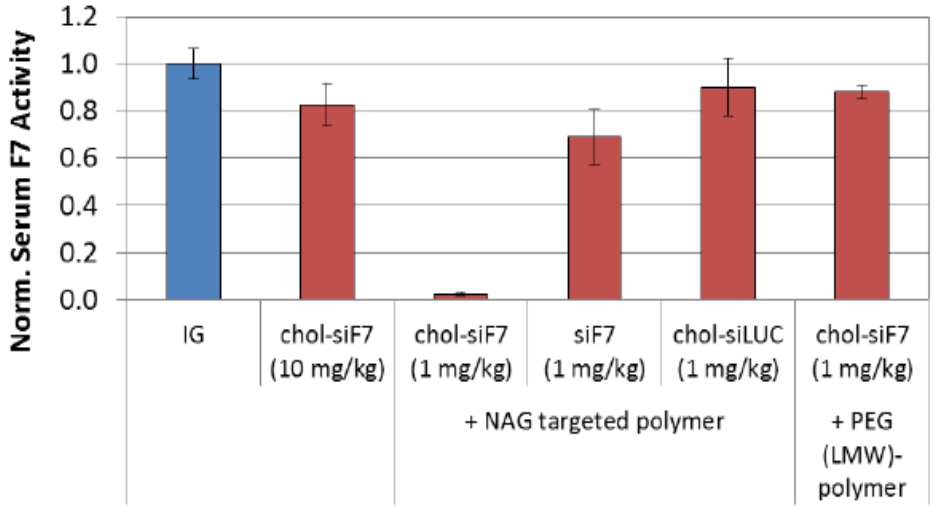



NAG* ligand
(hepatocyte targeted)

glucose ligand
(non-targeted)

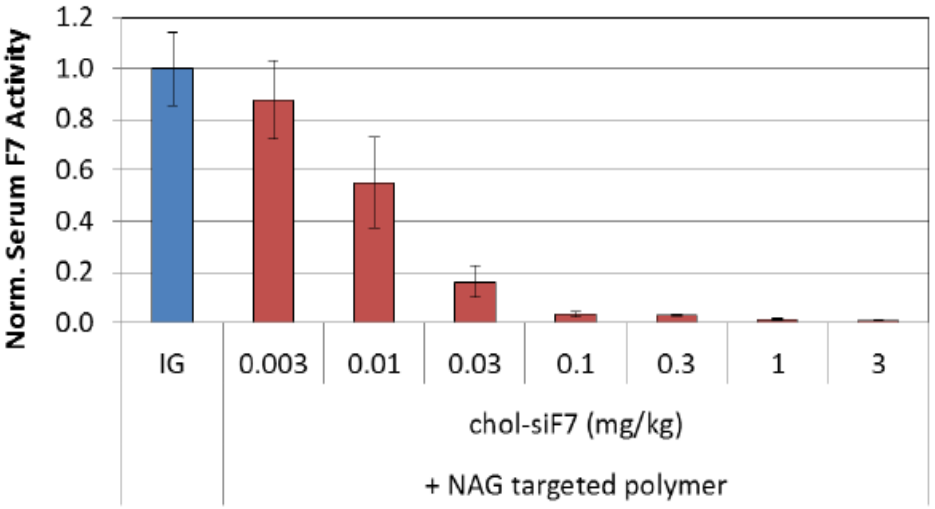
* N-acetyl galactosamine ligand is targeted to asialoglycoprotein receptor on hepatocytes

*Rozema, Lewis et al. PNAS, 2007*




**Target gene knockdown requires:**

Liver-tropic siRNA (cholesterol-siRNA) *plus* targeted MLP-(CDM-NAG)




**Chol-siRNA delivery using DPC 2.0 is highly efficient**

- $ED_{80}$ = 0.03 mg/kg chol-siF7
- $ED_{50}$ = 0.01 mg/kg chol-siF7

>5000-fold more efficacious than chol-siRNA alone (Soutschek et al. Nature 2004)

# Concluding Points

- The primary aim of antiviral therapy for CHB is to suppress viral replication in order to prevent cirrhosis, liver failure, and hepatocellular carcinoma.

- The primary indications for initiating antiviral therapy in patients with CHB include persistently elevated serum HBV DNA and persistently elevated serum ALT levels (“two green lights”).

# Concluding Points

- Recommended endpoints for antiviral therapy currently include HBeAg seroconversion and HBsAg loss in HBeAg-positive CHB patients and HBsAg loss in HBeAg-negative CHB patients.

- During antiviral therapy, regular follow-up is important to gauge response to therapy and to monitor for development of antiviral drug resistance.

# Concluding Points

- There are currently 7 approved therapies for CHB and determination of which therapy to use includes careful consideration of drug efficacy, side effects, and potential for antiviral resistance with the nucleos(t)ide analogs.

- We need newer therapies to allow us to stop treatment of HBV with our current foundation of oral therapies.

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# Dr. Bruce Given

Arrowhead Research
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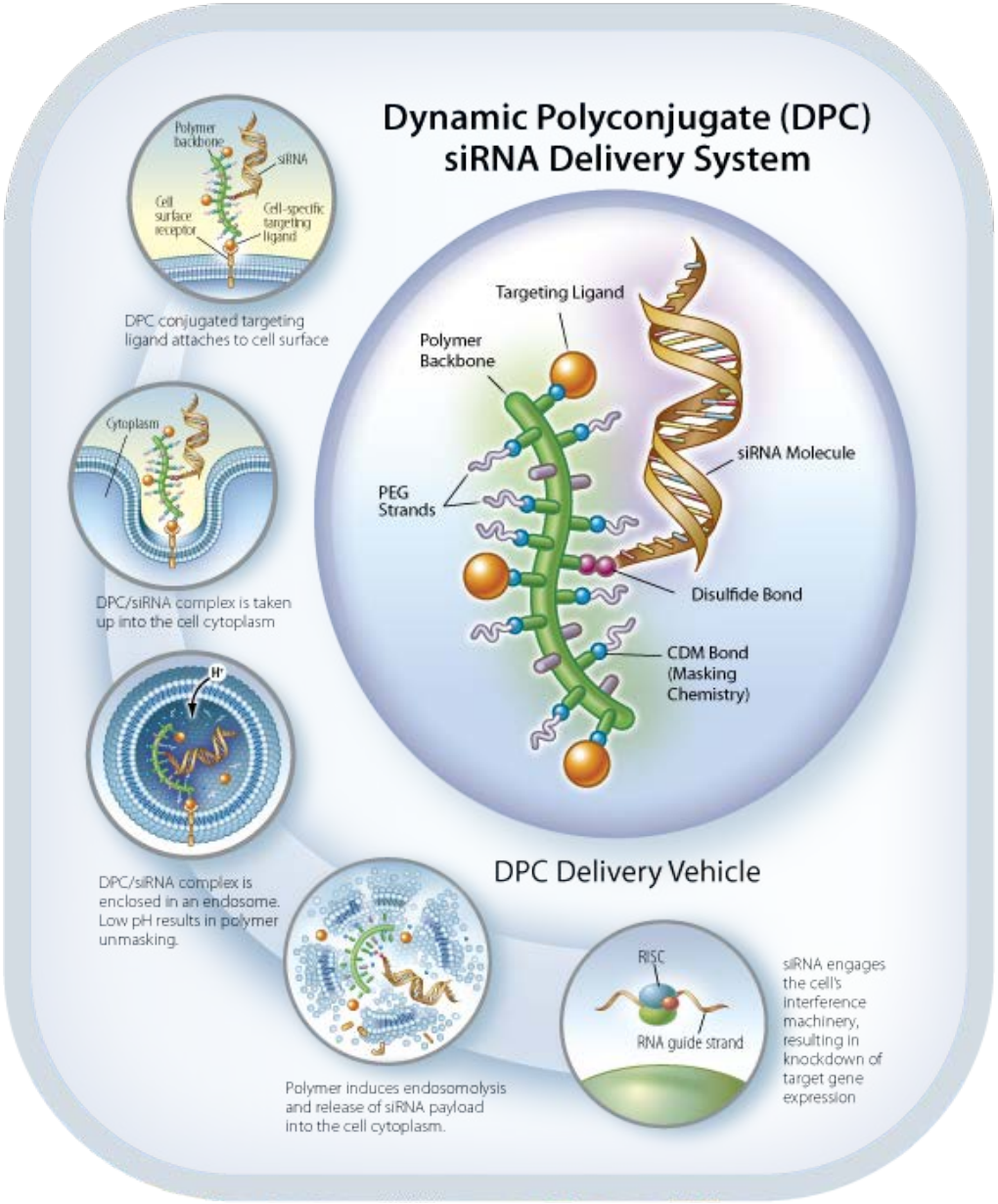
# Development of ARC-520 for Treatment of Chronic Hepatitis B Virus Infection

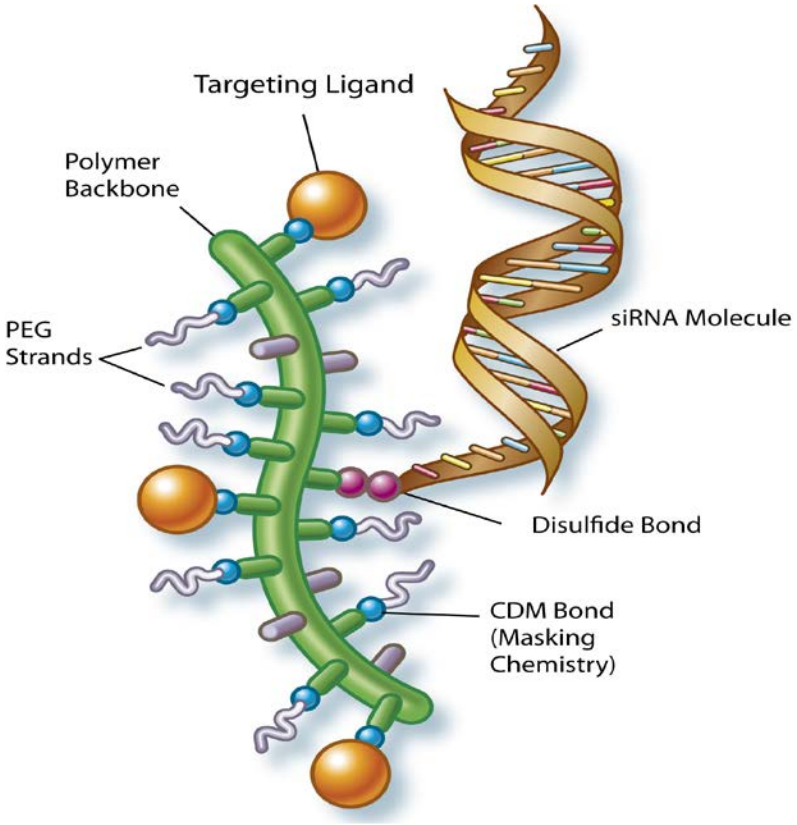
Bruce D. Given, MD
COO, Arrowhead Research Corporation

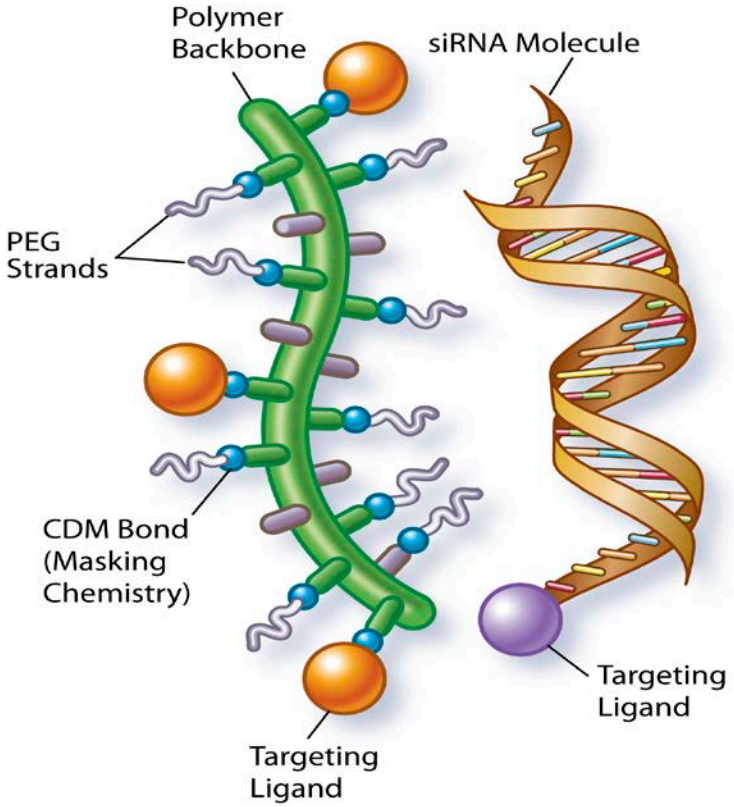
# Dynamic Polyconjugate (DPC) technology for siRNA delivery *in vivo*




- **DPCs**
  - Endosomolytic polymer (amphipathic)
  - Polymeric amines "masked" with CDM derivatives containing PEG and targeting ligand
  - siRNA is attached through disulfide linker
  - 5-15 nm in size
  - Slightly negatively charged
- **Cellular uptake is ligand-driven (N-acetyl galactosamine (NAG) for hepatocytes)**
- **↓ pH in maturing endosomes drives polymer unmasking**
- **Unmasked polymer disrupts endosomal membrane**
- **siRNA released to cytoplasm**

# Separating the siRNA and the DPC polymer



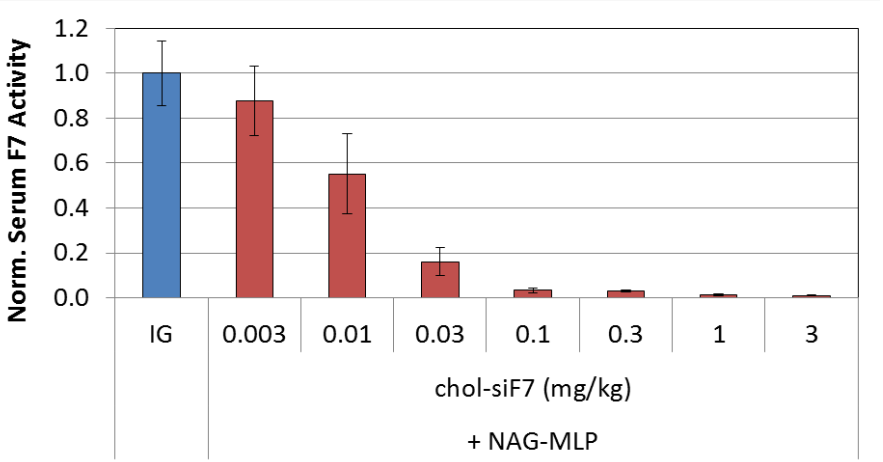




***Prototypical DPC***
Covalent attachment of siRNA to masked endosomolytic polymer

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

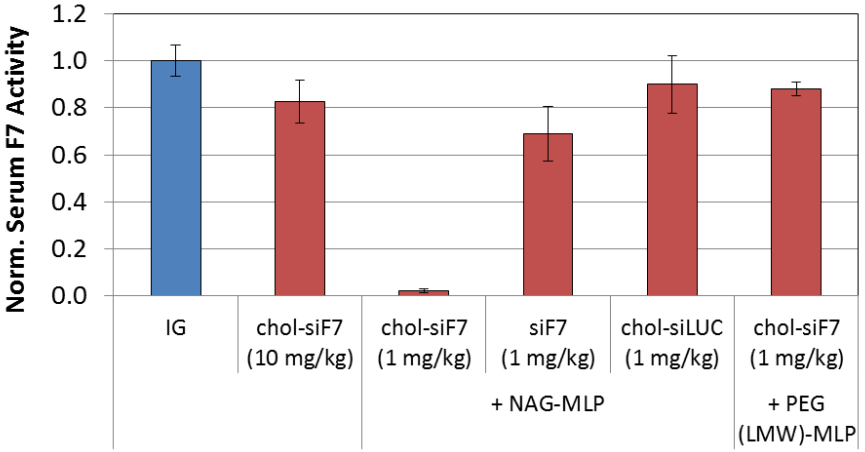
# Co-injection of NAG-MLP (L) and chol-siRNA

*Requirements for target gene KD in liver (Factor 7 target)*




Co-injection of NAG-MLP with chol-siF7 is highly effective

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




Target gene knockdown requires:

Liver-tropic siRNA (cholesterol-siRNA) ***and*** hepatocyte-targeted DPC peptide (NAG-MLP)

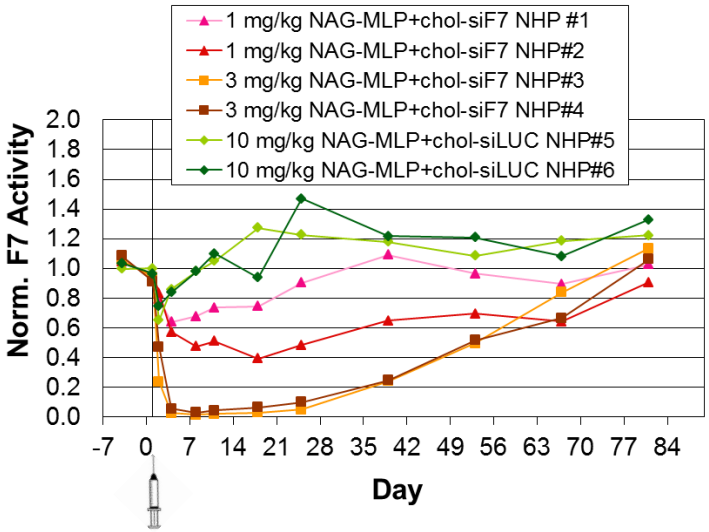
*mice, 6 mg/kg NAG-MLP, 48 hr timepoint* Wooddell, et al *Mol Ther* (Feb 26, 2013 Epub ahead of print)

# Efficacy in non-human primates

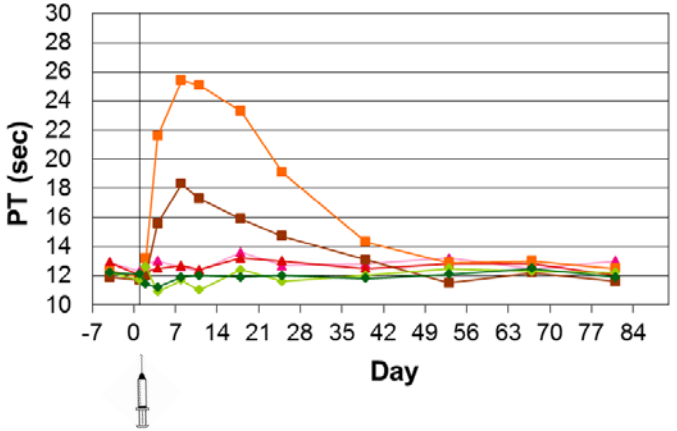
*NAG-MLP dose titration + chol-siRNA, single iv dose*

*Target: Coagulation Factor 7*

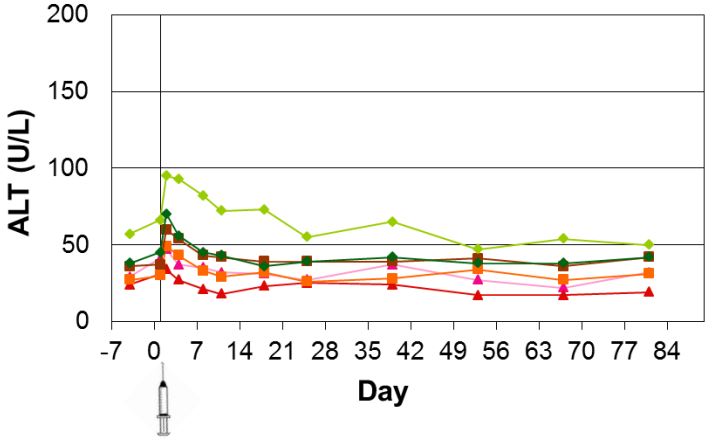
- **Highly efficacious**
  - *$ED_{50\ NAG\text{-}MLP}$ = 1 mg/kg*
  - *>99% KD at 3 mg/kg NAG-MLP*
  - *>80% KD for 5 weeks*
  - *↑ PT with F7 KD*
  - *No change using chol-siLuc control*

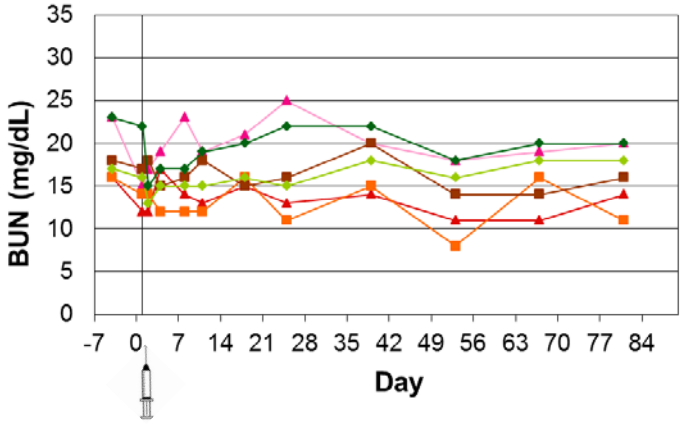



*Increased clotting time due to F7 knockdown*




- **No toxicity reached**
  - *No treatment-related changes in clin chem markers*
  - *No changes in hematology*
  - *No changes in cytokine levels (panel of 23)*

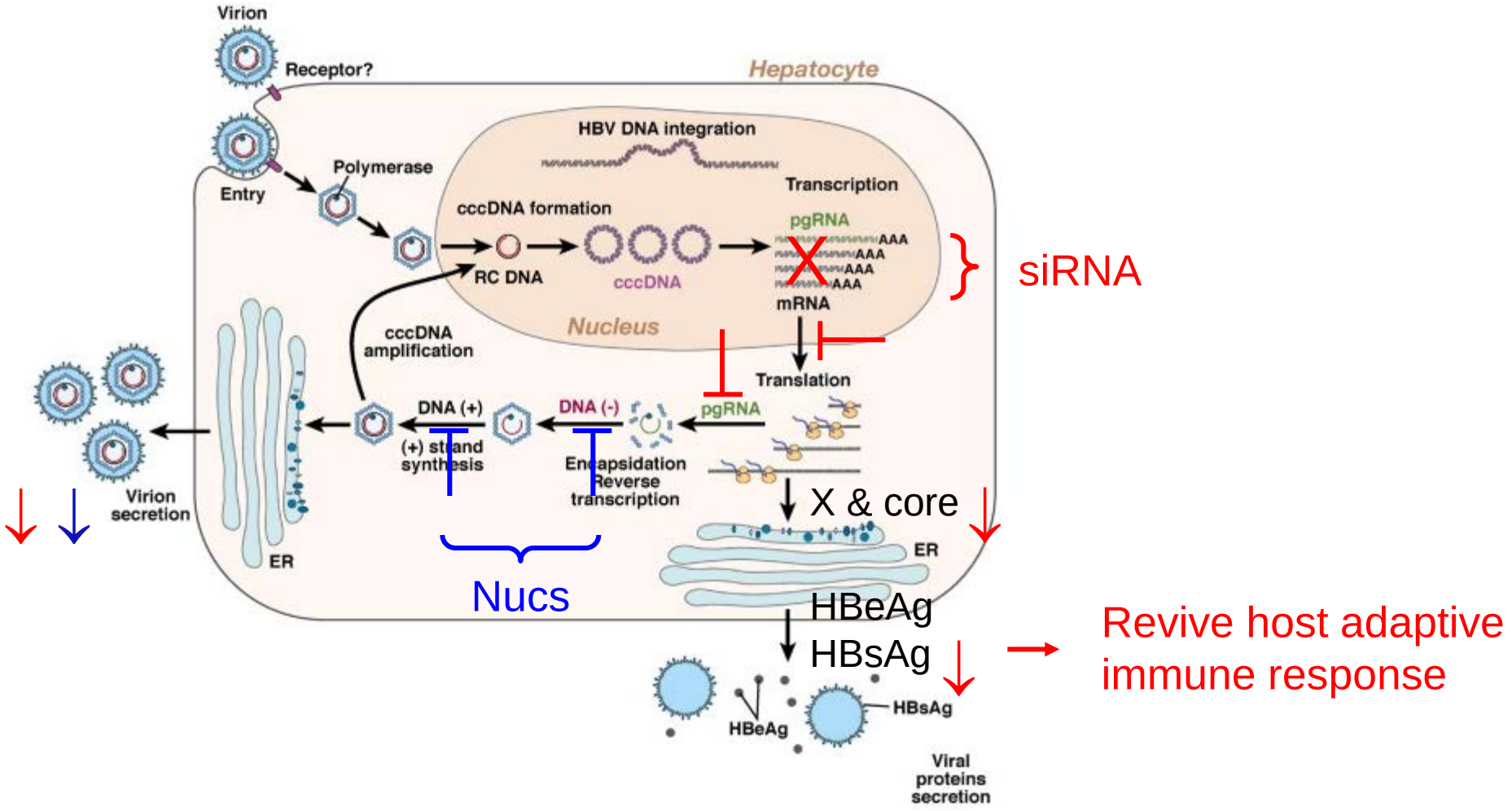



*2 mg/kg chol-siRNA,* Wooddell, et al *Mol Ther (Feb 26, 2013 Epub ahead of print)*

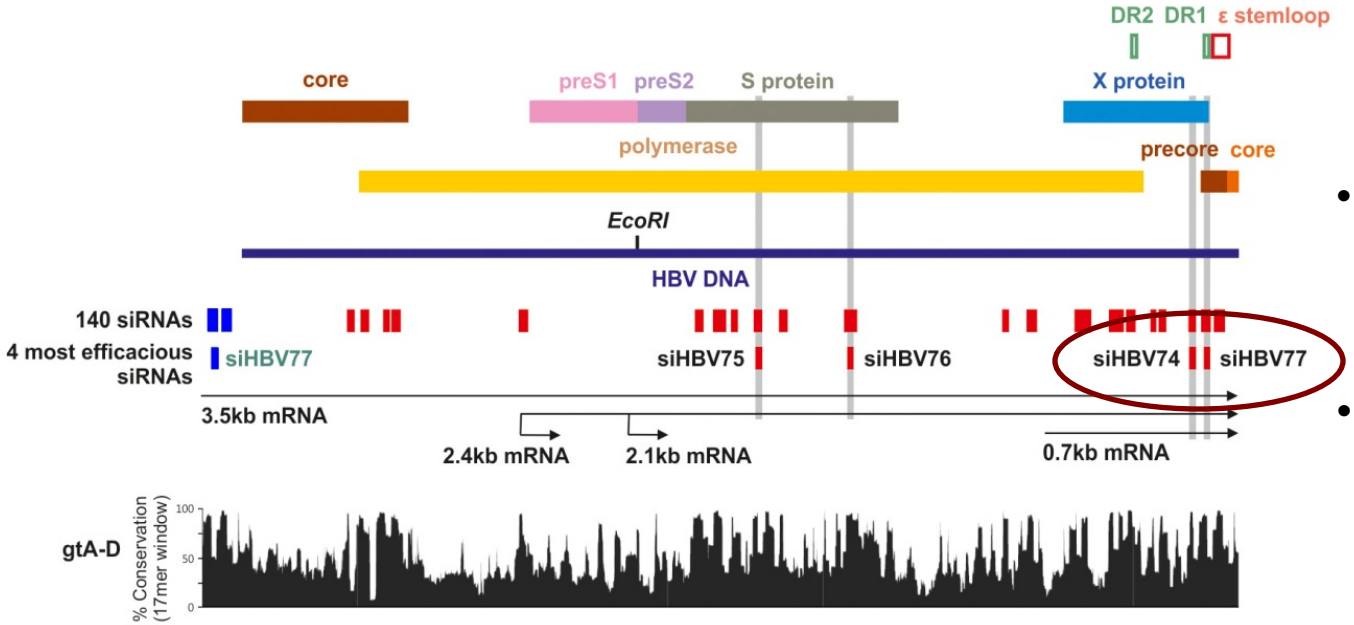
# RNA therapeutics are expected to cause reduction of HBV viral RNAs

*Differentiation from nucleos(t)ide reverse transcriptase inhibitors*

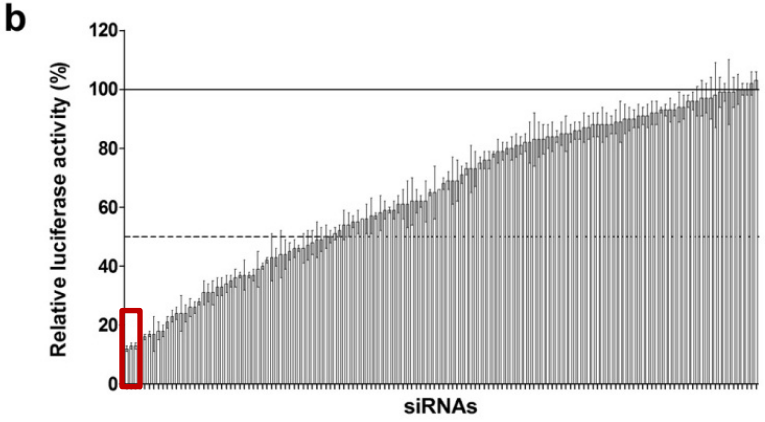


# RNAi treatment for chronic Hepatitis B

*siRNA design and in vitro screening*



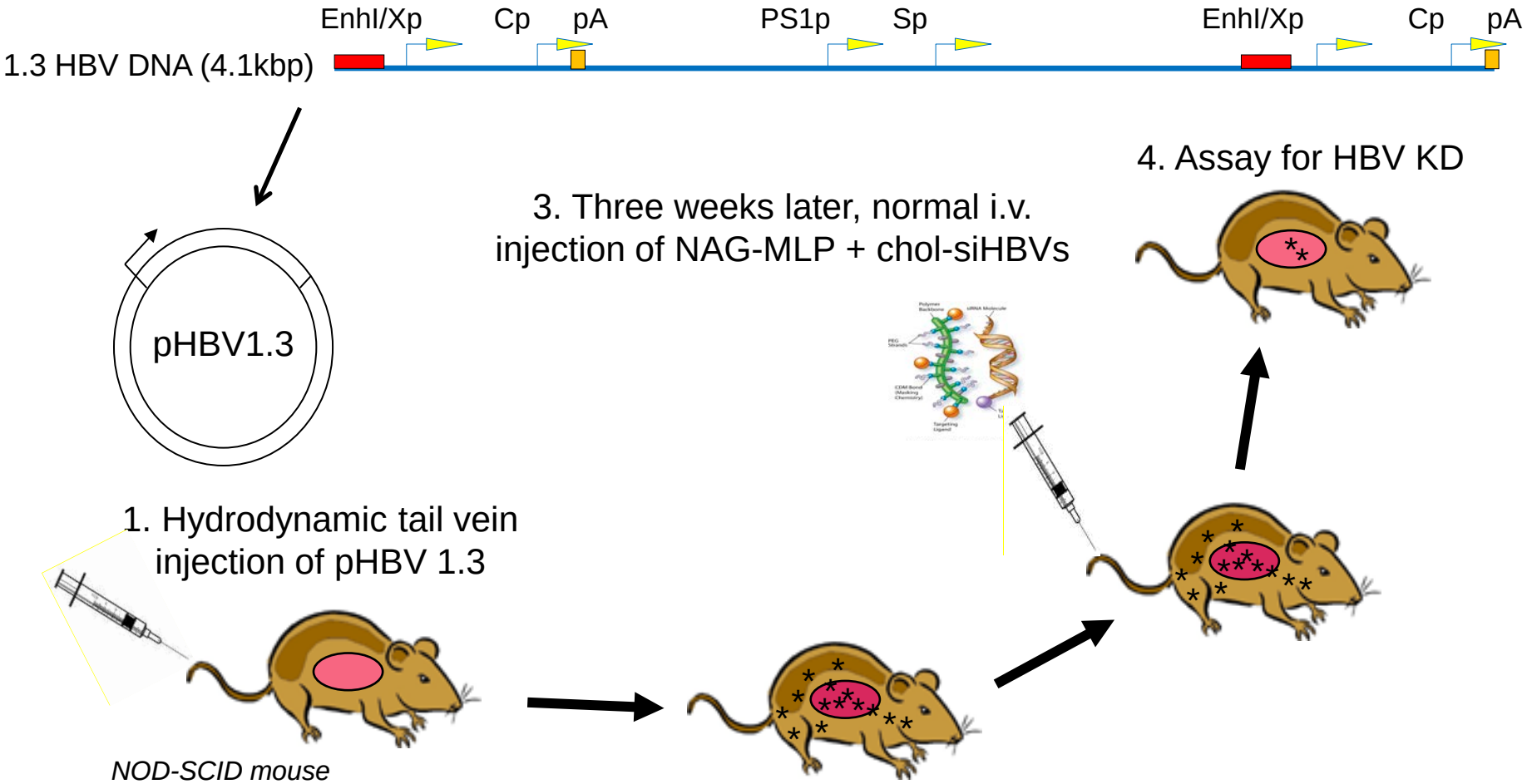

- Designed 140 siRNAs targeting conserved regions of HBV genotypes A-D
- Confirmed conservation in genotypes E-H as well.



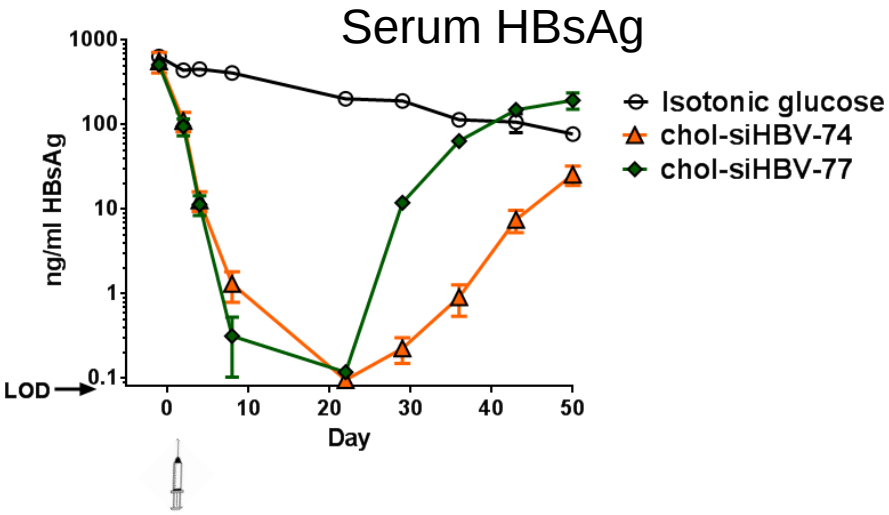

- Screened candidate siRNAs in a cell culture system
- 4 highly potent siRNAs chosen for further testing in animal models
- siHBV-74 and siHBV-77 chosen as leads

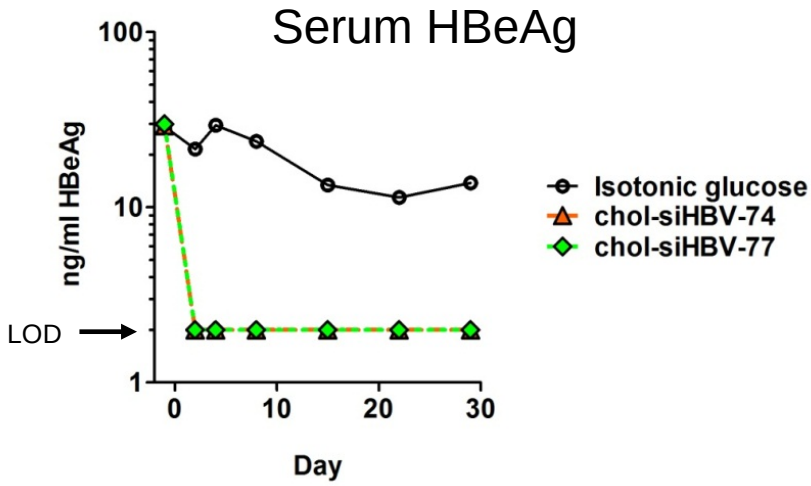
Wooddell, et al *Mol Ther* (Feb 26, 2013 Epub ahead of print)

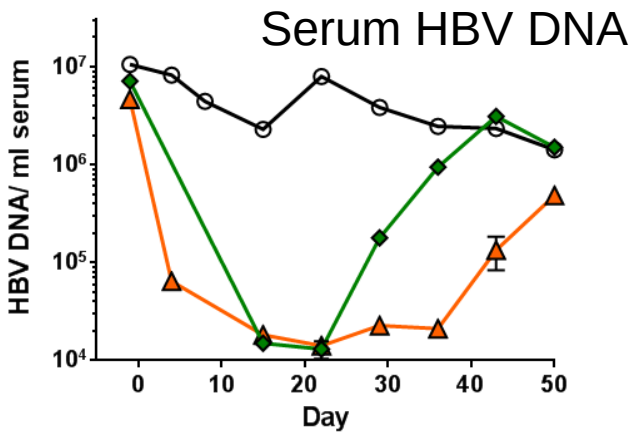
# Non-transgenic mouse model for HBV infection



# 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

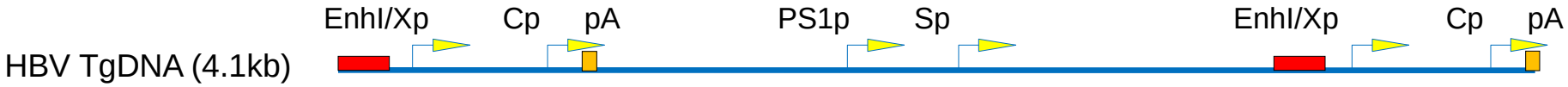
**Decreased HBeAg to LOD**

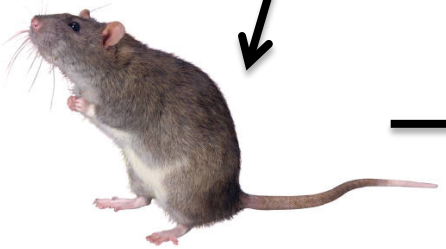
**Decreased HBV DNA**

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

*6 mg/kg NAG-MLP + 6 mg/kg chol-siRNA,* Wooddell, et al *Mol Ther* (Feb 26, 2013 Epub ahead of print)

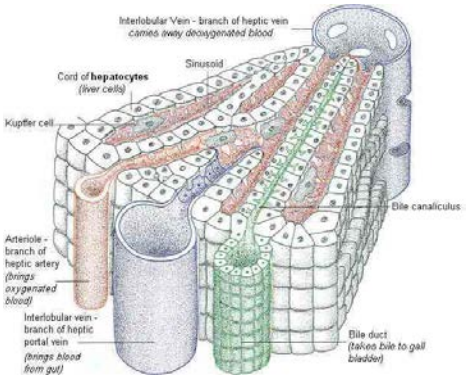
# Transgenic mouse model of chronic HBV infection



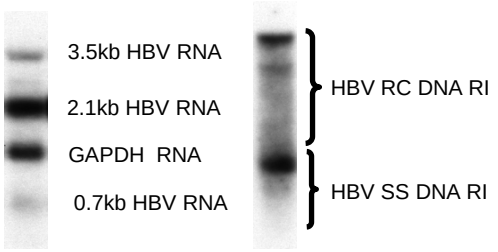



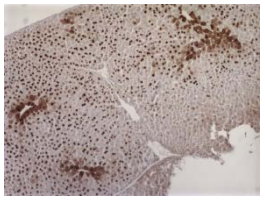
Transgenic HBV mouse

HBV expression in all hepatocytes in liver




Multiple efficacy readouts in liver



HBV core antigen

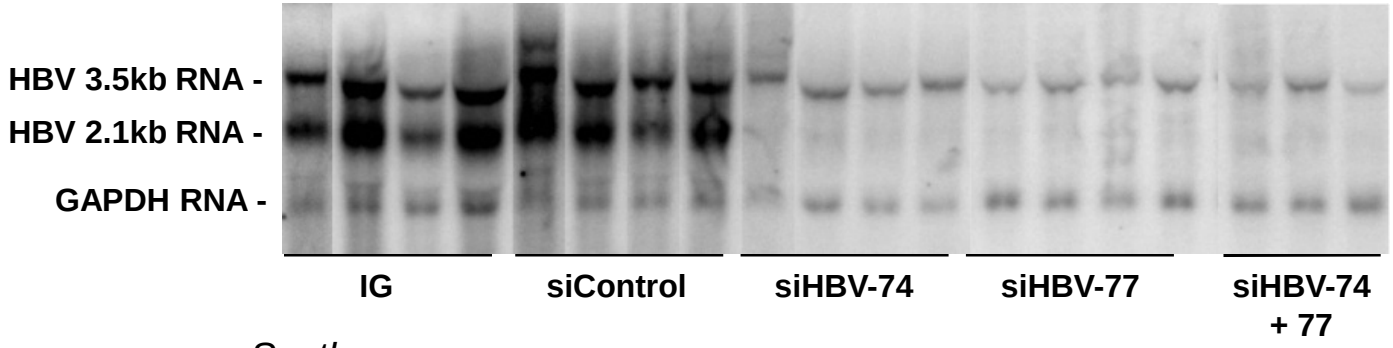


*Dr. Alan McLachlan, University of Illinois-Chicago Medical Center*

# Efficacy in a *transgenic* HBV mouse model

Liver - Day 8 post-treatment
6 mg/kg NAG-MLP + 6 mg/kg chol-siRNA

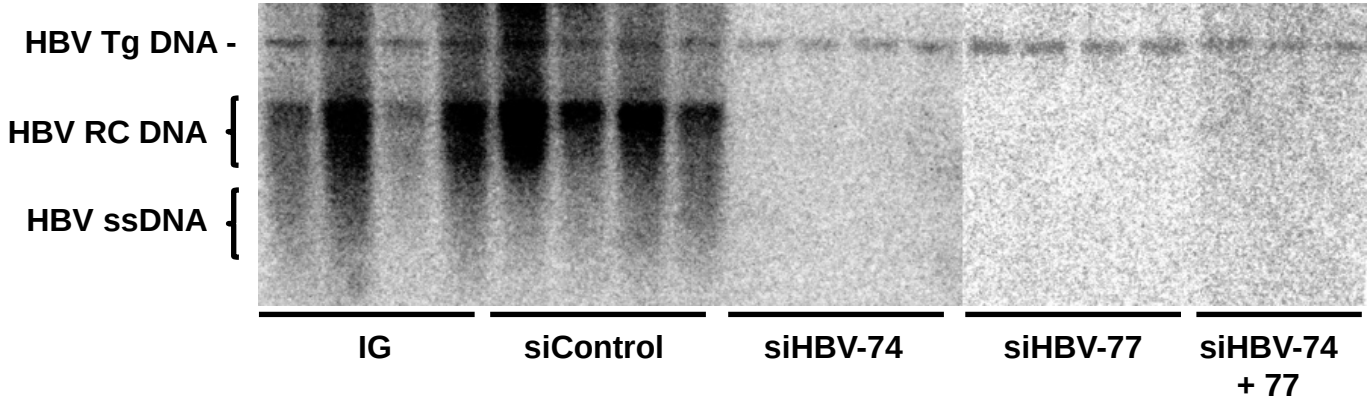
*Northern*




*Substantial reduction in HBV 3.5 kb with all siHBVs*

*Nearly complete elimination of 2.1 kb mRNA*

*Southern*



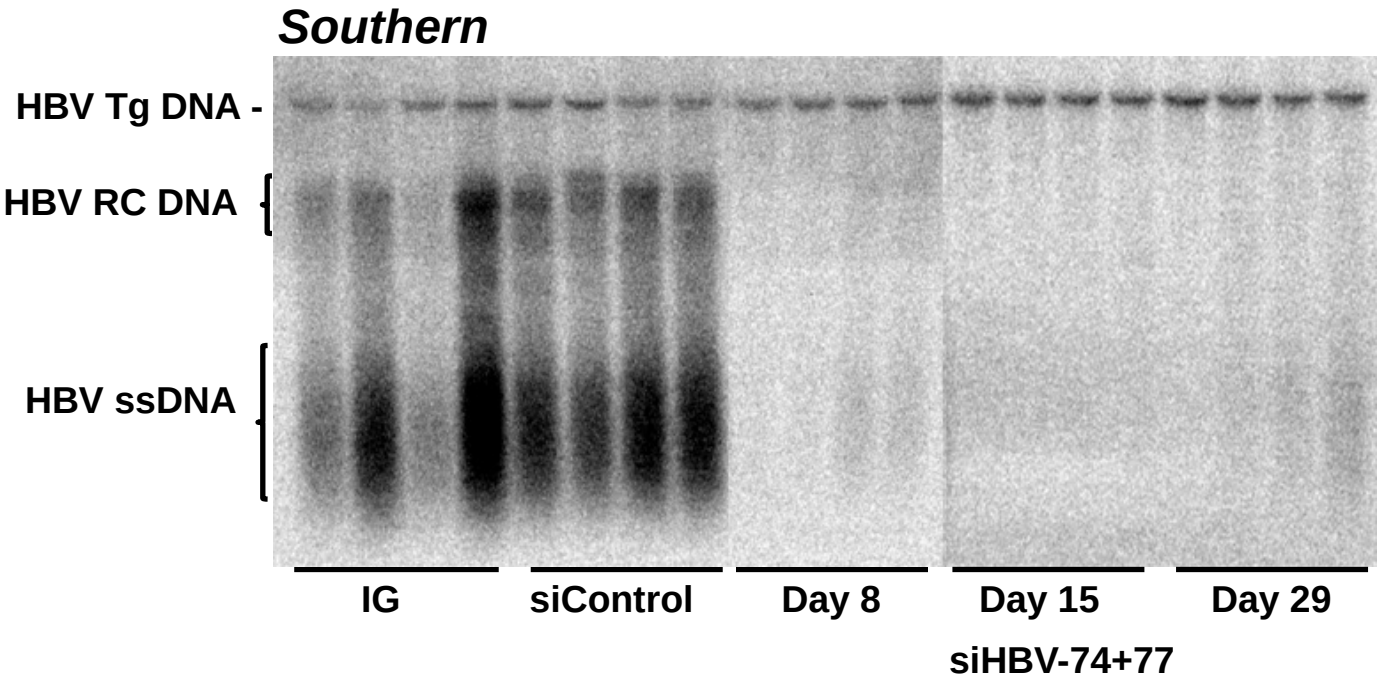

*Nearly complete elimination of HBV intermediates*

NAG-MLP + chol-siHBVs significantly reduces viral mRNA and DNA replicative intermediates in liver.

Wooddell, et al *Mol Ther* (Feb 26, 2013 Epub ahead of print)

# Duration of effect of siHBV DPCs on HBV replication in liver of *transgenic* HBV mice

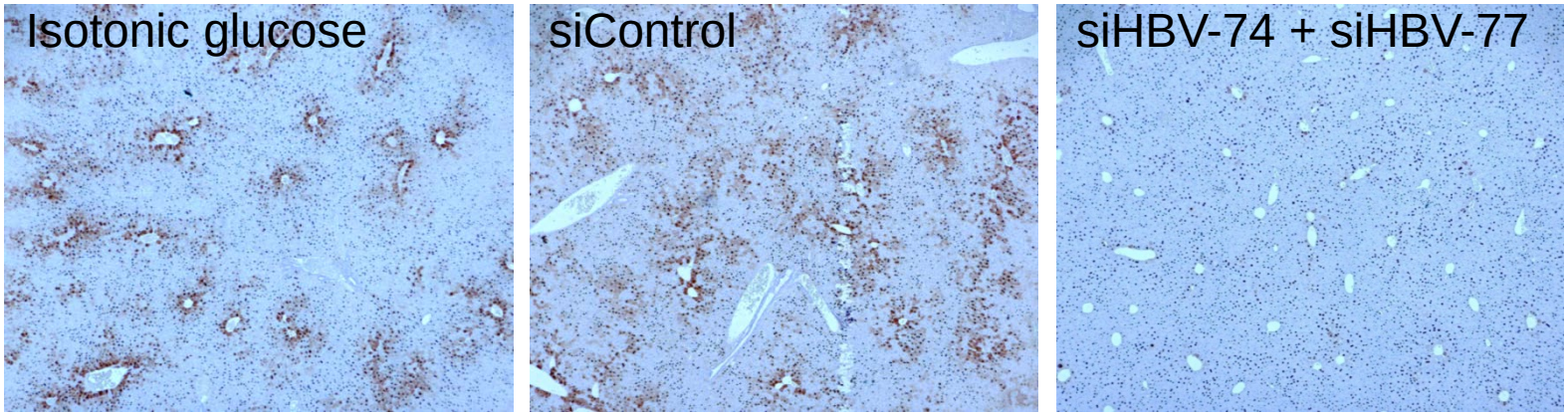
Liver
6 mg/kg NAG-MLP + 6 mg/kg chol-siRNA




Replicative intermediates reduced 1-2 logs for 1 month after single injection.
Duration of effect >1 month.

# Effect of siHBV DPCs on HBV core antigen expression in livers of HBV transgenic mice

Anti-HBcAg immunostain




Strong reduction of core antigen in all liver hepatocytes in animals receiving NAG-MLP + chol-siHBV-74,-77

**ARC-520:**
**NAG-MLP + chol-siHBV-74 + chol-siHBV-77**

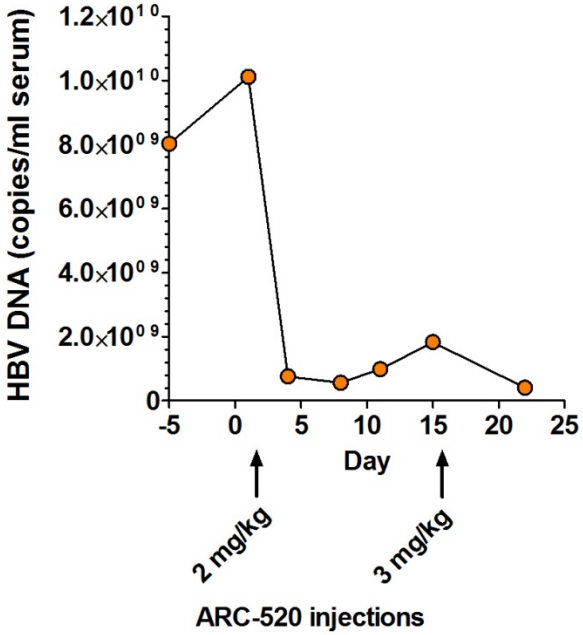
Wooddell, et al *Mol Ther* (Feb 26, 2013 Epub ahead of print)

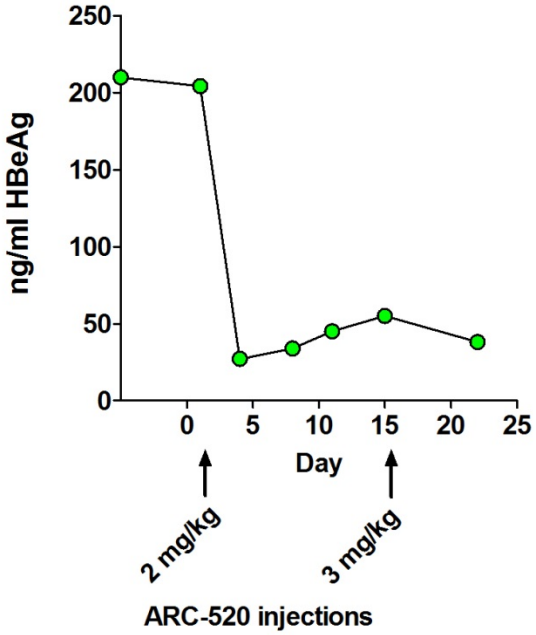
# ARC-520 Produces Knock-down in Chimpanzee Chronically Infected with Human HBV

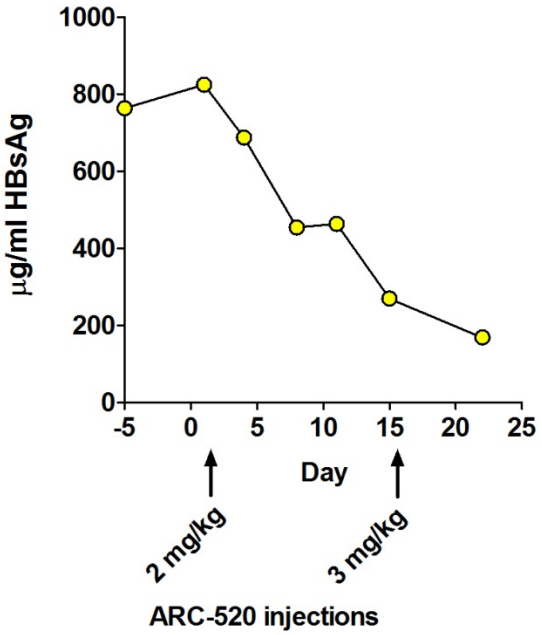
- Key historical attributes
  - 36 year old female, weight 113 pounds
  - Chronic HBV since 1979
  - Multiple prior anti-viral drug and therapeutic vaccine exposures
  - Liver biopsy shows near 100% staining for HBV
  - Exceptionally high titers of circulating DNA (high viral load) and HBsAg ($10^{10}$ vs. $10^{7}$ in average CHB patient)

  - **Genotype B with mismatch for one of the two siHBVs in ARC-520**
    - 8% of genotype B sequences in GenBank are mismatches for this siHBV
    - 5% of genotype B sequences mismatch to the second siHBV (100% match for this chimp)
    - ARC-520 has at least one perfect match siHBV for 99.6% of all GenBank sequences (>2750)

# Reduction in HBV after a single ARC-520 injection




- 80-95% reductions in HBV DNA, HBeAg and HBsAg
- First demonstration of RNAi efficacy in the chimp HBV model
- KD comparable to that achieved in mouse HBV models
- ARC-520 was well tolerated with no changes in LFTs after two doses

# ARC-520 Summary

- RNAi-based therapeutic for chronic hepatitis B
- Targeted, fully biodegradable DPC delivery peptide + 2 chol-siHBVs
- Highly effective in HBV mouse models with multi-log reduction of HBV mRNAs, proteins, DNA and long DoE (~1 month) after single injection
- Early data in a chronically infected chimpanzee shows
  - Significant, rapid reductions in viral load and antigenemia
  - Occurs despite mismatch for one of two siHBVs in ARC-520
  - Well tolerated with respect to clinical chemistries and clinical observation

# Clinical Plan for ARC-520

- Single dose Phase I study in healthy volunteers
  - Standard assessment of safety and tolerability
  - Submissions anticipated Q2 with study start early Q3
- Followed by single dose pilot efficacy study in chronic HBV patients
  - Plan is to apply for ethics and regulatory permission in the fall
  - Chimpanzee study allows prediction of clinical dose
  - Will follow measures of viral load and antigenemia and determine depth / duration of effect
- Multiple dose toxicity studies to begin in second half 2013 to allow expected initiation of multiple dose Phase II studies in second half 2014

Arrowhead Research
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# Dr. Chris Anzalone

# Conclusions

- Large market with unmet need and functional cure opportunity
- De-risked program:
  - Extensive NHP safety data with DPCs
  - Efficacy in multiple rodent models
  - Tolerability, dosing, and efficacy signals in Chimpanzee
    - "Worst case scenario" study animal
      - Extremely high viremia and antigenemia: high bar
      - HBV genotype with uncommon mismatch to one of the ARC-520 sequences, representing potential resistance
    - However, safe and highly effective treatment
      - Rapid and deep reductions of HBV virus and antigens
- Aggressive and achievable timeline

# Q & A