HBV Treatment
Endpoints and Research Directions for (Functional) Cure

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Outline of Presentation

1. Current HBV Treatment Guidelines/Endpoints
2. Current Treatment Challenges
3. Useful Tools to Predict Treatment Outcome
4. Can We Cure HBV?
5. Next Steps: Future Directions and new endpoints
More Than 2 Billion People Show Evidence of Hepatitis B (HBV) Infection\(^1\) anti-HBc(+)

240-300 million people are chronically infected with HBV worldwide\(^{1,3}\)

High Replicative, Low Inflammatory
- High HBV DNA
- Trained immunity*
- Normal or low ALT
- HBeAg(+)
- High serum levels of HBeAg & HBsAg
- Mild or no necroinflammation
- No or slow fibrosis progression
- Decreased IL-10, IL-6, IL-8 & TNF-α
- No HBV DNA mutations

Immune Clearance
- High changing to low or undetectable HBV DNA
- High decreasing to normal ALT
- Acute or intermittent hepatitis
- Declining HBeAg & HBsAg
- Eventual loss of HBeAg
- High changing to minimal necroinflammation
- Emergence of core and precore mutations

HBeAg(-) Chronic
- Moderate to high HBV DNA
- High but fluctuating ALT
- Low HBsAg levels
- Persistent hepatitis
- Necroinflammation
- Progressive liver disease
- Immune clearance attempts ineffective

Non-Replicative
- Low or undetectable HBV DNA
- HBeAg(-)
- Very low HBsAg levels
- Normal ALT
- May be seen after immune clearance
- T-cell deletion

HBsAg Loss/Occult Hepatitis B
- Serum HBV DNA phases, alternating undetectable and very low but detectable
- Detectable HBV DNA in the liver
- Intrahepatic replication-competent HBV genomes such as HBV cccDNA
- Integrated HBV DNA

*The immune system is not exhausted or tolerant
Nests of infected cells (cccDNA containing) remain; Constant HBsAg and virus in the blood
Phases of infection with hepatitis B

- Immune Tolerant
  - Trained
  - Exhaustion
  - Clonal deletions

- Immune Active

- Immune Inactive
  - HBsAg
  - HBV DNA
  - ALT
  - T cell dysfunction
  - Deletion

Establishment

Clearance

Time
6 Endpoints in HBV Treatment

Milestone 1: Start of decline of HBV DNA
- HBeAg(+) or anti-HBe(-) status
- HBV DNA level >10⁹ copies/mL
- Milestone 2: HBeAg/anti-HBe seroconversion if there is wild type HBV infection
  - HBV DNA decreased to undetectable
  - HBV Clearance
  - HBV RNA clearance
  - qHBsAg decline
  - Empty particle qHBcrAg

Milestone 3: HBV DNA decreased to undetectable
- Low HBV DNA (<2000 IU/mL) for reduced progression risk
- This is where we would like our patients to be

Milestone 4: Clearance of HBsAg
- Sensitivity test
- Antibody ?

Milestone 5: Clearance of cccDNA

Milestone 6: Clearance of cells with integrated HBV DNA sequences

HBsAg status
- HBsAg+
- HBsAg-

ALT level
- Normal

HBV DNA status
- Undetectable level of HBV DNA
- HBeAg/anti-HBe status
- HBV DNA level >10⁹ copies/mL

Milestone
- Milestone 1: Start of decline of HBV DNA
- Milestone 2: HBeAg/anti-HBe seroconversion if there is wild type HBV infection
- Milestone 3: HBV DNA decreased to undetectable
- Milestone 4: Clearance of HBsAg
- Milestone 5: Clearance of cccDNA
- Milestone 6: Clearance of cells with integrated HBV DNA sequences

Immune status
- Immune low active
- Immune high active/clearance
- Inactive carrier state
- Functional cure
- Absolute cure

Immune control
Eradication

• equates to driving the virus to extinction from the earth. eg: small pox (vaccination)

VERSUS

Functional Cure

• equates to eliminating the virus from the infected host. eg: hepatitis C (treatment)

FOR HEPATITIS B: Yes, it can be eradicated AND maybe cured
To define Endpoints:
we need to know the “Terms” in play:

• Natural Cure
  – Clearance of HBsAg without therapy and serum HBV DNA is undetectable

• Functional Cure
  – Based on the clinical outcome, in which the patient’s life expectancy becomes the same as that of an individual who has resolved his HBV infection without therapy

• Apparent Virologic Cure
  – Based on the stable off-drug suppression of HBV viremia and antigenemia and the normalization of ALTs and other laboratory tests

• Absolute Cure/Sterlizing Cure
  – In which an individual with chronic hepatitis B completely resolves the infection, and is then at the same risk of death from liver disease as someone the same age who has never been infected

Block and Gish, et al, AVR 2013
Absolute or Ultimate: Cure will include

- Clearance of all cells with cccDNA
- Elimination of cells with integrated HBV DNA
  - Remove integrated HBV DNA to completely stop risk of HCC
- Prevent risk of HBV reactivation in anti-HBc(+) patients
What Would a True Cure = HBV Elimination Look Like?

In the blood:  
- HBV DNA/HBsAg negative
- anti-HBs positive
- anti-HBc positive
- No HBV RNA
- HBcrAg negative
- HBeAg negative

In the liver:  
- no HBV cccDNA
- no HBV RC/DSL DNA
- HBcAg staining negative
- No HBsAg
- No integrated HBV DNA]
Hepatitis B: Molecular Pathogenesis

- HBV replicates its DNA genome via reverse transcription of pregenomic RNA
- HBV is not generally cytopathic to hepatocytes
- Precore protein / HBeAg essential for establishing persistent infection
- Host immune responses (generally inadequate and/or in appropriate) are responsible for the liver disease of chronic hepatitis B
- **2016: Two therapeutical approaches:**
  - (i) direct antiviral agents: lamivudine, adefovir, entecavir, telbivudine and Tenofovir/TAF
  - (ii) immune modulation: interferon alpha
Integrated DNA is a carcinogen and may produce viral particles

Original Articles

Identification of Integrated Hepatitis B Virus DNA Sequences in Human Hepatocellular Carcinomas

DAVID A. SHAFRITZ AND MICHAEL C. KEW

Departments of Medicine and Cell Biology and The Liver Research Center, Albert Einstein College of Medicine, Bronx, New York 10461 and Department of Medicine and The South African Primary Liver Cancer Research Unit, University of Witwatersrand Medical School, Johannesburg 2001, South Africa

DNA extracts from hepatocellular carcinomas of 13 patients from South Africa were examined for hepatitis B virus (HBV) DNA sequences by molecular hybridization using $[^{32}P]$-labeled recombinant, cloned, and purified HBV-DNA. Eight patients were HBV carriers as demonstrated by the presence of hepatitis B surface antigen (HB,Ag) in their serum, and each of these patients had HBV-DNA sequences in hepatocellular carcinoma tissue. Five patients who were not HB,Ag carriers, did not have HBV-DNA in their tumors. In DNA extracts from all tumors of patients who were HB,Ag-positive, the HBV-DNA was integrated into the host genome. The integration pattern was unique for each tumor, but HBV-DNA bands of a given length were present in more than one specimen and in a human hepatocellular carcinoma cell line (PLC/PRF/5). These results suggest that integration of HBV-DNA into the human genome occurs in conjunction with malignant transformation.
Do we need new Nucs?

- Is Safety an important endpoint?
- TAF, (25mg) tenofovir analogue will be a safety endpoint with little or no renal toxicity
  - Gets across the gut wall easily
  - Stable in Plasma
  - Concentrates in liver cells and mononuclear cells
  - Single digit conc in bone and renal
  - Much less renal tox using biomarkers

- CMX-157 same concept as TAF (from Chimerix) (now at Contravir), may target low doses as with TAF
  - Liver targeting
  - Similar to TAF
  - Probably very low tissue levels, like TAF, of off target drug concentrations
Tenofovir Alafenamide (TAF)

- TAF = orally bioavailable phosphonoamidate prodrug of tenofovir (TDF)
- In comparison with tenofovir, TAF enables enhanced delivery of the parent nucleotide and its active diphosphate metabolite into lymphoid cells and hepatocytes.
- This is attributed to an improved plasma stability and differential intracellular activation mechanism for TAF relative to TDF

<table>
<thead>
<tr>
<th>EC_{50} HIV-1 (PBMCs)</th>
<th>Tenofovir</th>
<th>Tenofovir Disoproxil</th>
<th>TAF</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1.2 µM</td>
<td>0.015 µM</td>
<td>0.003 µM</td>
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</table>
**TAF – A Novel Prodrug of Tenofovir**

**Mechanism of Action**

- **GI TRACT**
  - DIANION
  - ESTER
  - AMIDATE

- **RENAL TUBULAR CELL**
  - TFV
  - TDF (tenofovir disoproxil fumarate) 300 mg
  - TAF (tenofovir alafenamide) 25 mg

- **PLASMA**
  - TFV
  - short plasma half-life
  - ~90% LOWER PLASMA TFV

- **HEPATOCELL**
  - TFV
  - TFV-DF

- **RENAL TUBULAR CELL**
  - TFV

† T1/2 based on in vitro plasma data - TDF = 0.4 minutes, TAF = 90 minutes.


Agarwal K et al. J Hepatology 2015; 62: 533-540; Buti EASL 2016, Oral GS06; Chan, EASL 2016, Oral GS12
Study 108 and 110: Phase 3 CHB Studies: TAF vs TDF

Results: Renal Safety

Subjects receiving TAF experienced significantly less change in eGFR\textsubscript{CG} and sCr at Week 48 compared to TDF.

Continuous data are expressed as mean (SD)
sCr, serum creatinine; eGFR\textsubscript{CG}, creatinine clearance by Cockcroft-Gault
Buti EASL 2016, Oral GS06
Chan, EASL 2016, Oral GS12
Gilead Sciences, Data on File
Tenofovir Alafenamide (TAF)

– Improved safety profile vs TDF in HIV patients:

## New Polymerase inhibitors

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Target</th>
<th>Compound</th>
<th>State of Development</th>
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<tbody>
<tr>
<td>Tenofovir alafenamide (TAF)</td>
<td>HBV polymerase</td>
<td>Prodrug of Tenofovir</td>
<td>Phase III</td>
</tr>
<tr>
<td>CMX157</td>
<td>HBV polymerase</td>
<td>Prodrug of Tenofovir</td>
<td>Phase II</td>
</tr>
<tr>
<td>AGX-1009</td>
<td>HBV polymerase</td>
<td>Prodrug of Tenofovir</td>
<td>Phase I, China</td>
</tr>
<tr>
<td>Besifovir</td>
<td>HBV polymerase</td>
<td>Acyclic nucleotide phosphonate</td>
<td>Phase III, Korea</td>
</tr>
</tbody>
</table>
Orally Bioavailable Anti-HBV Dinucleotide Acyloxyalkyl Prodrugs

John E. Coughlin, Seetharamaiyer Padmanabhan, Guangrong Zhang, Cassandra J. Kirk, Chandrika P. Govardhan, Brent E. Korba, Kathleen O'Loughlin, Carol E. Green, Jon Mirsalis, John D. Morrey, and Radhakrishnan P. Iyer

a Spring Bank Pharmaceuticals Inc., Milford, MA
b Division of Virology and Molecular Biology, Georgetown University, Rockville, MD
c Biosciences Division, SRI International, Menlo Park, CA
d Institute for Antiviral Research, Utah State University, Logan, UT

New Targets for HBV “Cure”

Entry Inhibitors
- Myrcludex
- Cyclosporine
- Ezetimibe

Immunodulators
- TLR 7 and 9 agonists
- T-cell vaccines
- PD-1/PD-L1 blockade

cccDNA silencing

Inhibit protein translation by siRNA
- Arrowhead
- Tekmira
- Alnylam
- GSK

RT Pol Inhibitors
- Nucleotide analogues
- Non-Nuc analogues
- RNAseH inhibitors

HBsAg release Inhibitor
- NAP

Core inhibitors
- Novira
- Bayer (Aicuris)
- Assembly
- Gilead
- Janssen
- Roche
# Inhibitors of HBsAg release

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Target</th>
<th>Compound</th>
<th>Stage of Development</th>
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<tbody>
<tr>
<td>REP-2139 (REP 9AC)</td>
<td>Subviral particle formation</td>
<td>Phosphorothioated oligonucleotides</td>
<td>Phase II</td>
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<tr>
<td>BM601</td>
<td>Inhibits HBsAg secretion</td>
<td>Benzimidazole derivative</td>
<td>Preclinical</td>
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<tr>
<td>NVPO18</td>
<td>Inhibits HBsAg secretion</td>
<td>Cyclophilin inhibitor</td>
<td>Preclinical</td>
</tr>
<tr>
<td>CPI-431-32</td>
<td>Inhibits HBsAg secretion</td>
<td>Cyclophilin inhibitor</td>
<td>Preclinical</td>
</tr>
<tr>
<td>PBHBV-001</td>
<td>Inhibits HBsAg secretion</td>
<td>Triazolo-pyrimidine derivatives</td>
<td>Preclinical</td>
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<tr>
<td>PBHBV-2-15</td>
<td>Inhibits HBsAg secretion</td>
<td>α-glucosidase inhibitors / Iminosugar derivatives of butyldeoxynojirimycin</td>
<td>Preclinical</td>
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<tr>
<td>DNJ</td>
<td>Inhibits HBsAg secretion</td>
<td>Recombinant Hepatitis B Human Immunoglobulin</td>
<td>Phase I</td>
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<tr>
<td>GC1102</td>
<td>Neutralizing HBsAg</td>
<td></td>
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<tr>
<td>Humabs</td>
<td>Inhibits HBsAg secretion</td>
<td>High affinity oligoclonal aby prep</td>
<td>PreClinical</td>
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## Inhibitors of nucleocapsid assembly

<table>
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<th>Target</th>
<th>Compound</th>
<th>Stage of Development</th>
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<tbody>
<tr>
<td>GLS4</td>
<td>Interfere with capsid formation/stability</td>
<td>Heteroaryldihydropyrimidines (HAPs)</td>
<td>Phase II</td>
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<tr>
<td>Bay 41-4109</td>
<td>Viral nucleocapsid inhibitor</td>
<td>HAPs</td>
<td>Phase I</td>
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<tr>
<td>AT-130</td>
<td>Inhibition of HBV capsid assembly</td>
<td>Phenylpropenamide derivatives</td>
<td>Preclinical and early clinical phase</td>
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<tr>
<td>NVR-3-778 (NVR1221)</td>
<td>Inhibition of HBV capsid assembly</td>
<td>Small molecule</td>
<td>Phase Ib</td>
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</tbody>
</table>
REP-2139 (REP 9AC, Replicor), i.v qw

REP-2139 prevents subviral particle (SVP) formation in HBV-infected hepatocytes and inhibits HBsAg release

* NAPs: nucleic acid polymers
New Targets for HBV “Cure”

Core inhibitors
- Novira
- Bayer (Aicuris)
- Assembly
- Gilead
- Janssen
- Roche
<table>
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<tr>
<th>Strategy</th>
<th>Target</th>
<th>Agents/Company</th>
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<tr>
<td>HBV life cycle</td>
<td>HBV Pol</td>
<td>TAF</td>
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<tr>
<td>Viral entry</td>
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<tr>
<td></td>
<td>Myrcludex-B,</td>
<td>MYR GmbH</td>
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<tr>
<td></td>
<td>Cyclosporine</td>
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<td></td>
<td>Ezetimibe</td>
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<td></td>
<td>cccDNA</td>
<td>Zinc finger nucleases</td>
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<td></td>
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<td>cccDNA conversion inhibitors</td>
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<tr>
<td></td>
<td>mRNA transcription/ stability</td>
<td>Zinc finger proteins</td>
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<td></td>
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<td>Epigenetic silencers</td>
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<td>Ribozymes</td>
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<tr>
<td>Viral assembly</td>
<td>HAPs</td>
<td>Phenylpropenamides</td>
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<tr>
<td>HBV antigen secretion</td>
<td>REP 9AC’</td>
<td>Small molecule inhibitors of HBsAg secretion e.g. glucovirs</td>
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<td></td>
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<td>e.g. triazolo-pyrimidines</td>
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<tr>
<td>mRNA</td>
<td>Antisense iRNA</td>
<td>Ionis OAS product 3rd Gen DNA</td>
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<td>Arrowhead iRNA (ARC520)</td>
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<td>Tekmira iRNA (TKM HBV)</td>
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<td></td>
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<td>Analyym siRNA (formally Merck now Arrowhead IP sharing as well as separate development)</td>
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<tr>
<td>Capsid</td>
<td>Multiple</td>
<td>Novorio Emery</td>
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<tr>
<td>Immuno-therapeutic</td>
<td>PegIFN-λ1a (IL29)</td>
<td>BMS Nanogen</td>
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<td>Cytokines</td>
<td>rIL-7</td>
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<td>Gilead</td>
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<td>TLR agonists</td>
<td>TLR7 (GS-9620)</td>
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<td></td>
<td>Therapeutic vaccines</td>
<td>Adeno-virus approaches (TG1050)</td>
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<td></td>
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<td>NASVAC</td>
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<td>Tarmogen (GI-13020)</td>
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<td></td>
<td></td>
<td>Gilead</td>
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<tr>
<td></td>
<td></td>
<td>Abivax-failed</td>
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<tr>
<td></td>
<td>Blocking T cell inhibitory receptors</td>
<td>Anti-PD-1 moAB (BMS936558)(MedImmune)</td>
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<td></td>
<td>Anti-PD-L1 moAb (BMS936559)</td>
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<tr>
<td></td>
<td>Intrahepatic blocking of suppressive cytokines / regulatory T cells</td>
<td>TGF-β inhibitors</td>
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<td></td>
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<td>T reg depletion (e.g. α-CD25, daclizumab)</td>
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Inhibition of HBV Replication by Non-Nucleoside Analogues

AT/HAPs

Attachment and Penetration

DNA repair

MINICHROMOSOME

HBV polymerase protein

pregenomic RNA

HBV RNA transcripts

transport to cell nucleus

CCC DNA

Nucleus

Envelope proteins S, M, L

core proteins

uncoating

Release

Golgi complex

AT compounds/B-HAP

HBV Nucleocapsid Inhibitors

Heteroaryldihydropyrimidines (HAPs)
- bind to core particles to reduce both HBV DNA and HBcAg levels, the latter via degradation by the proteasome pathway.
- enhance viral assembly
  - favour assembly of aberrant particles, indicating that HAPs interfere with capsid formation/stability in a complex manner.
- Similar to phenylpropenamide derivatives, HAPs are able to efficiently inhibit NA resistant viral variants
The HBV Core Protein is a Promising Antiviral Target

- HBV Core plays multiple essential roles – High Efficacy Potential
- Core proteins are highly conserved – Broad Spectrum Potential Across Genotypes
- No related proteins in human cells – High selectivity potential

1. Capsid Assembly
2. cccDNA Amplification
3. Nuclear Function

- Viral replication
- Suppression of Host Innate Immune response
- cccDNA Maintenance & Transcription
HBV Core Inhibitors Can Disrupt Multiple Steps Required for HBV Replication and Persistence
HBV Core Inhibitors Can Disrupt Multiple Steps Required for HBV Replication and Persistence

1) Capsid Assembly
   - Inhibition of Viral replication

2) cccDNA Amplification
   - Inhibition of Viral replication

3) ISG Inhibition
   - Restoration of host innate immune response

4) Maintenance of cccDNA in Active State
   - cccDNA silencing inhibits viral replication & restores host immune response
NVR 3-778 induces rapid mis-assembly of HBV core proteins in vitro and inhibits encapsidation of HBV RNA in cells.

- In vitro activity against HBV genotypes A, B, C, D and NUC RAVs
- Additive antiviral activity with nucleoside analogs (Angela Lam Poster P0640)
- Phase 1A completed:
  - Excellent dose-ranging safety and tolerability
  - Excellent, predictable dose-related systemic exposure
- Phase 1b (4-week dosing) clinical study in patients ongoing
Study 1: Serum HBV DNA Reduction

- ETV and NVR 3-778 monotherapies show similar antiviral activity ($p > 0.05$)
- PEG-IFN + NVR 3-778 combination provides highest antiviral efficacy
  - 5/5 mice achieve serum HBV DNA BLQ
HBV Transcription Inhibitor by blocking x Protein
The cccDNA is a Minichromosome

Low Replication Phenotype
Quiescent or active
Medium to Low Viraemia

High Replication Phenotype
Transcriptionally Active
High Viraemia


Current therapy

Chronic HBV infection
Liver

Future therapy?

Viral cccDNA

Injected IFN-α

HBV core protein

Degradation

CURE (<10%)

Viral cccDNA

LTβR agonist

APOBEC3B
HBV core protein

Degradation

CURE (7%)
Gene Editing and cccDNA targeting

cccDNA synthesis and histone stabilization
- measure cccDNA, histone status in cells, nucleus and serum/plasma
- Interferon alpha (Pegasys, approved)
- Lymphotoxin-B receptor agonist (BS1, CBE11, preclinical)
- DNA cleavage enzymes (cccDNA targeted endonucleases) Gene editing, all early pre-clinical
  - Zinc-finger nucleases (ZFNs)
  - Transcription-activator like effector nucleases (TALENs)
  - CRISPR-Cas9 (Intellia Therapeutics and 2 other companies)
- Epigenetic control (histone methylation, acetylation) Arbutus, preclinical
  - Acetyl/methyl transferase inhibitors
  - Deacetylase activators
HBV CURE: A Multi-Step Approach

Deplete or silence cccDNA

Activate antiviral immunity

NUCs Core inhibitors siRNAs

CD8+ T cell

B cell

Courtesy of Ed Gane
## RNA interference

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Target</th>
<th>Compound</th>
<th>Stage of Development</th>
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<tbody>
<tr>
<td>ARC-520</td>
<td>HBV mRNA</td>
<td>siRNA</td>
<td>Phase II / III</td>
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<tr>
<td>TKM-HBV</td>
<td>HBV mRNA</td>
<td>siRNA</td>
<td>Phase I</td>
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<tr>
<td>Ionis-HBVRx</td>
<td>HBV mRNA</td>
<td>Anti-sense RNA</td>
<td>Phase I</td>
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<tr>
<td>dd-RNAi compound</td>
<td>HBV mRNA (Pol)</td>
<td>shRNA</td>
<td>Preclinical</td>
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<tr>
<td>ALN-HBV</td>
<td>HBV mRNA</td>
<td>siRNA - LNP</td>
<td>Preclinical</td>
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</table>
Mechanism of RNA Interference (RNAi)

**Natural Process of RNAi**

- dsRNA
- dicer
- cleavage
- strand separation
- RISC
- complementary pairing
- cleavage
- mRNA degradation
- cleaved mRNA

**Therapeutic Gene Silencing**

- Synthetic siRNAs
- cleavage
- strand separation
- RISC
- complementary pairing
- cleavage
- mRNA degradation
- cleaved mRNA
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
Dynamic Polyconjugate (DPC) technology for siRNA delivery *in vivo*

- DPC polymer composition and physical characteristics
  - Amphipathic peptide
  - peptide amines reversibly “masked” with CDM
  - Slightly negatively charged

- Cellular uptake of peptide is ligand-driven (N-acetyl galactosamine (NAG)) for hepatocytes

- siRNA is made liver tropic by attachment of lipophilic ligand (e.g. cholesterol)

- ↓ pH in endosomes drives peptide unmasking

- Unmasked peptide disrupts endosomal membrane

- siRNA released to cytoplasm
Reduction in HBV after administration of ARC-520 in a chronically infected chimp

- Log_{10} reduction in HBV DNA (95%), HBeAg (90%) and HBsAg (90%)
- First demonstration of RNAi efficacy in the chimp HBV model
- KD comparable to that achieved in mouse HBV models at similar dose level
- Further reduction after a subsequent dose

REVIVAL OF IMMUNE RESPONSES AND FUNCTIONAL CURE

Human data? At 1 and 2mg/kg dosing 50% reduction in HBsAg: AASLD 2014
iRNA works in a human SAD dosing study

Figure 1.- Quantitative HBsAg in serum

![Graph showing quantitative HBsAg in serum over time for Placebo, 1 mg/kg, and 2 mg/kg dosages. Error bars indicate SEM, and * indicates P < 0.05.]
Mechanistic comparison of RNAi therapeutics vs. reverse transcriptase inhibitors (NUCs)
Integration of HBV DNA into the host chromosome

- HBV DNA integration occurs throughout infection largely via dsDNA and introduces deletions near the DR sites.
- S protein ORF and trx control elements remain intact and would allow expression of HBsAg.
- A significant proportion of total HBV DNA in HBeAg negative chimps may be integrated DNA.

HepDart 2015
Second Generation of Antisense Oligonucleotides (ASO)

DNA → mRNA → Recruitment → Degradation → Translation

- DNA in gap supports RNase H mediated degradation of target RNA
- Phosphorothioate backbone provides stability and uptake to tissues (including liver)
- MOE in wings provides potency, stability, and tolerability benefit

5’-wing: GCCTC
Gap: AGTCTGCTTC
3’-wing: GCACC

2’-methoxyethyl (MOE) → DNA → 2’-methoxyethyl (MOE)

Very Specific, Clinically Safe and Accumulate in the Liver
ASO and Entecavir act Independently

Serum HBsAg
Chimps and DNA

Baseline Serum HBsAg

Serum HBsAg or DNA (% of pre-treatment)

- saline
- ASO
- ETV
- ETV+ASO
## Immune modulation

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Targets</th>
<th>Compounds</th>
<th>Stage of Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>INO-1800</td>
<td>Therapeutic vaccine</td>
<td>DNA plasmids encoding HBsAg and HBcAg</td>
<td>Phase I</td>
</tr>
<tr>
<td>SB-9200</td>
<td>Induction of host immune responses via activation of RIG-I and NOD2</td>
<td>Small molecule nucleic acid hybrids (SMNH)</td>
<td>Phase II</td>
</tr>
<tr>
<td>Birinapant (TL32711)</td>
<td>Cellular inhibitor of apoptosis proteins (cIAPs)</td>
<td>SMAC inhibitor</td>
<td>Phase I / IIa</td>
</tr>
<tr>
<td>Alinia</td>
<td>Activation of Protein kinase R (PKR)</td>
<td>Nitazoxanide</td>
<td>Preclinical</td>
</tr>
</tbody>
</table>
New Targets for HBV “Cure”

Immunodulators
- TLR agonists
- T-cell vaccines
- PD-1/PD-L1 blockade

Innate responses
- NK cells
- Stimulation
- IFN-α
- Induction of antiviral effectors: e.g., APOBEC3A/B
- Hepatocyte effect through IFN-γ or cytotoxicity

Adaptive immune responses
- CD8+ cells
- CD4+ cells
- B cells

Virion
- hNTCP
- Entry
- Polymerase
- cccDNA formation
- Transcription
- pgRNA
- mRNA
- Translation
- Encapsidation reverse transcription
- Viral proteins secretion
- Nucleos(t)ide analogs
- DNA+ + strand synthesis
- DNA-
Activating the Immune System to Fight HBV
## Immune modulation

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<tr>
<td>ABX-203</td>
<td></td>
<td>Therapeutic vaccine</td>
<td>Recombinant antigen containing HBsAg and HBcAg</td>
<td>Phase IIb / III</td>
</tr>
<tr>
<td>GS-4774</td>
<td></td>
<td>Therapeutic vaccine</td>
<td>Recombinant antigen containing X, Env, Core epitopes</td>
<td>Phase II</td>
</tr>
<tr>
<td>GS-9620</td>
<td></td>
<td>TLR7 agonist</td>
<td>Oral TLR7 agonist</td>
<td>Phase II</td>
</tr>
<tr>
<td>CYT107</td>
<td></td>
<td>Immune-modulator</td>
<td>Recombinant human IL-7</td>
<td>Phase I / IIA</td>
</tr>
<tr>
<td>TG-1050</td>
<td></td>
<td>Immunotherapeutic</td>
<td>Non-replicative adenovirus serotype 5 encoding a large fusion protein (truncated Core, modified Pol and two Env domains)</td>
<td>Phase I</td>
</tr>
<tr>
<td>T cell receptor insertion</td>
<td>Immunotherapeutic</td>
<td></td>
<td>Electroporation of Tcells with mRNA stimulated by HBV sAg</td>
<td>Preclinical Mice Bertoletti</td>
</tr>
</tbody>
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# Host-targeting agents

## Immune modulation

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</tr>
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</table>

Therapeutic vaccine: GS-4774

- Recombinant antigen containing X, Large S (env) and Core epitopes
- GS-4774 activates dendritic cells after phagocytosis
- Recombinant antigen epitopes are displayed via MHC class I and II and stimulate CD4⁺ and CD8⁺ T cells

<table>
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<tr>
<th><strong>Anti-PD-1 / Anti-PD-L1 Immune Therapy</strong></th>
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<tr>
<td><strong>• Bristol-Myers Squibb (BMS)</strong></td>
</tr>
<tr>
<td>– BMS-936558 (anti-PD-1)</td>
</tr>
<tr>
<td>– [Nivolumab]</td>
</tr>
<tr>
<td>– With Gilead</td>
</tr>
</tbody>
</table>
|     [https://www.anzctr.org.au/Trial/Registration/TrialReview.asp
|     x?id=369374&isReview=true](https://www.anzctr.org.au/Trial/Registration/TrialReview.asp?id=369374&isReview=true) with TLR7 |
| **• Merck & Co (Merck Research Laboratories)** |
|   – MK-3475                              |
|   – [Lambrolizumab]                      |
| **• Novartis**                           |
|   – Co Stim Pharmaceuticals               |
| **• Roche**                              |
|   – MPDL 3280A (anti-PD-L1)              |
| **• Other**                              |
|   – Sino Biologicals                    |
What Might a HBV Curative Regimen Look Like?

Potent NA

Agent to prevent viral spread and cccDNA re-amplification

Agent(s) to reduce or silence cccDNA

Safe and selective agent to reduce or silence cccDNA

Agent(s) to activate specific antiviral immune responses or relieve repression/exhaustion of the immune system

Agent(s) to block/inhibit the HBV life-cycle [entry, cell-spread, capsid assembly, HBx, HBeAg, HBsAg]

Modified from S Locarnini
HBV Treatment Strategies

Therapeutic targets

1. Viraemia
   - Viral particles cause (re)infection

2. Antigen load (HBe, HBs)
   - Subviral particles overhaul host immune system

3. cccDNA
   - Master template causes persistence

Block

---

Boost

- T-cell responses
  - Recovery of HBV-specific T-cell responses

- Humoral responses
  - Robust anti-envelope neutralizing responses

- Innate immunity
  - Suppression through cytokine- and APC-mediated mechanisms

---

Courtesy of Ed Gane
The HBV Therapeutic Development Landscape as of Dec, 2016

Pre-clinical

- TTP sAg
- GLS-4 capsid
- Benza capsid
- CpAMS capsid
- DVR capsid
- cccDNA forma
- NV100
- Editope
- HDAC
- Chimigen e HBV
- Altravax HBV
- STING

Human Phase Trials

- AGX1009 prodrug
- CMX157 prodrug
- NVR122 1 capsid
- ?Bay4110 9 capsid
- ALN-HBV
- ddRNAi HBV
- TKM-HBV
- Isis HBV antisense RNAi
- MycB entry*
- Rep2139 sAg
- GS7340 Pro-ten
- Roche 7795
- GS477 4 vac
- GS962 0 Toll
- DV501 Vac
- *HDV active

Indirect Host modifier

- Indirect Immunomodulator

DAA

TAF
Acknowledgements to my HBV Gurus!

- Tim Block
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- Stephan Urban
- Fabien Zoulim
- Harry Janssen
- Henry Chan
- Novira development team
- Tekmira/Oncore/Arbutus leadership
- Marion Peters