HBV Cure: Possible Lessons for HIV

Professor Stephen Locarnini
WHO Regional Reference Laboratory for Hepatitis B, Victorian Infectious Diseases Reference Laboratory, Doherty Institute
Melbourne, Victoria 3000, AUSTRALIA
## Disclosure

<table>
<thead>
<tr>
<th></th>
<th>Gilead Sciences</th>
<th>Arrowhead Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consulting Fees</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Fees for Contract Research and/or Clinical Trials</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>
Outline of Presentation

1. Introduction
2. Barriers to Curing Chronic Hepatitis B
3. Overcoming the Barriers
   a) Virological
   b) Immunological
4. Challenges and Opportunities Ahead
Hepatitis B: Global Burden

- 240 - 350 million people living with CHB globally

- 786,000 attributable deaths from hepatitis B annually in 2010;
  1.3 million from viral hepatitis B & C collectively (GBD 2010)

- Viral hepatitis was the 9th ranked cause of human death;
  similar numbers of deaths to HIV, malaria and TB (GBD 2010)

- Without appropriate management, 15-25% of people with CHB will develop advanced liver disease &/or HCC

- Liver cancer is the 2nd most common cause of cancer death globally
  - GBD report 2013
Natural History

<table>
<thead>
<tr>
<th>Phase</th>
<th>Immunotolerance</th>
<th>Immunoelimination</th>
<th>Inactive</th>
<th>HBeAg-Negative CH-B</th>
<th>Occult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral Load (IU/ml)</td>
<td>&gt;20,000</td>
<td>&gt;20,000</td>
<td>&lt;2,000</td>
<td>&gt;2,000</td>
<td></td>
</tr>
<tr>
<td>Years</td>
<td>0 10 20 30 40 50 60 70</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Disease
- Minimal necroinflammatory activity
- Chronic hepatitis
- Inactive
- Cirrhosis/HCC
- Occult

Serology
- Anti-HBe
- HBeAg
- HBsAg

ALT level

HBV DNA level

IFN-α, LMV/LdT, ADV/TVF, ETV

THERAPY
Current Treatment Challenges and Why we Need a Cure

• if use low genetic barrier NUCs, drug resistance a serious problem
• in China, > 3 x 10^6 LAM-resistant cases
• long term therapy with NUCs (> 3 years) affects patient compliance and typically has little effect on HBsAg levels
• Peg-IFN has substantial toxicity, low (<20%) efficacy
• Cost: very few countries in high prevalence regions have reimbursement policies
• HBsAg-positivity poor prognosis
Importance of HBsAg Clearance/Seroconversion

- ↓ Hepatic decompensation
- ↓ HCC
- ↑ Survival
- ↓ Levels of cccDNA

- As close to cure as we can expect to achieve in chronic hepatitis B

Major Barriers to Curing CHB

- **Viral**
  - cccDNA
  - HBsAg
  
    Both unaffected by NUC therapy

- **Immunological**
  - T-cell exhaustion
  - emerging role of (inadequate) B-cell responses
Productive HBV Replication: cccDNA Pathway

1. RC DNA $\rightarrow$ cccDNA
   - DNA repair
   - TDP-2

2. HBeAg (early protein)
   - synthesised from precore mRNA

Non-Productive HBV Replication: HBsAg (from Integrated DNA) Pathway
The cccDNA is a Minichromosome


Low Replication Phenotype
Quiescent or active
Medium to Low Viraemia

High Replication Phenotype
Transcriptionally Active
High Viraemia

HBV Minichromosomes and Chromatin Modelling

- **Relaxed Chromatin**
  - **Activation of Gene Expression**: Histone Acetylase (HAT)
    - transcription activation complex containing HATs
    - HATs acetylate lysine residues of the histone tails

- **Compacted Chromatin**
  - **Repression of Gene Expression**: Histone Deacetylases (HDAT)
    - transcription repression complex containing HDAC
    - HDACs deacetylate histone lysine tails

- **Conclusion**
  - acetylation status of HBV minichromosome (cccDNA-bound H3 & H4 histones) regulates HBV transcription/replication and is reflected in viral load

*Pollicino, T. et al 2006. Gastroenterology;130:823*

*Haematologica. 2009;94(11):1618-22*
HBV and Subviral (HBsAg) Particles

- HBsAg secreted in vast excess over virions (3-4 orders of magnitude)
- circulate in blood 100-400 μg/ml
- half-life is ?
- NUC therapy has minimal effect on HBsAg levels or its clearance
HBsAg as An Immune Regulator

• mounting evidence for HBsAg proteins playing a key role in HBV persistence
• can suppress both innate (TLR-2, TLR-9 and IFN-α) as well as adaptive (mDC) responses to infection
• “immuno competence” of host can affect HBsAg “set-points”
• co-existence of HBsAg and anti-HBs (include heterologous sub-type specificity)

HBsAg Major Neutralisation Domain

The major anti-HBs neutralisation domain contains major immunogenic epitopes located within **Loop1** (aa107-138) and **Loop2** (aa139-147).

The ‘a’ determinant is highly conformational, with a raft of **cysteine & proline** residues


HBsAg ‘a’ determinant topology and/or epitope availability influence the HBV **neutralisation phenotype**

Selective immune (anti-HBs) pressures can influence epitope availability and HBsAg profile (loss or gain of binding)

- **Potentially a predictive biomarker for HBsAg response on-treatment**
- developed a 19plex panel of anti-HBs mAbs covering HBsAg ‘a’ determinant and C-terminal domain (residues 99-226)
In a treatment naïve cohort of genotype A chronic hepatitis B (CHB) patients receiving tenofovir disoproxil fumarate (TDF) therapy (TF103 trial):

**HBsAg clearance profile (CP)**

HBsAg epitope pressure (reduced recognition) at *both* loop 1 **AND** loop 2 epitopes
- associated with HBsAg response/decline (>1log) and potentially HBsAg loss/seroconversion

**HBsAg non-clearance (or escape) profile (NCP)**

No change in HBsAg epitope profile, OR reduced epitope binding at *only* one loop
- associated with no HBsAg response/decline (<1log)

**Conclusion/Findings**

Significant association (p <0.02) between the development of a HBsAg CP and HBsAg Loss/Seroconversion [PPV 83%] by 48 weeks of treatment

*Walsh, R et al (2015), submitted*
## Nucleic Acid-Based Approaches

<table>
<thead>
<tr>
<th>Name</th>
<th>Approach</th>
<th>Phase</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARC-520</td>
<td>RNAi</td>
<td>Ila</td>
<td>Arrowhead</td>
</tr>
<tr>
<td>TKM-HBV</td>
<td>RNAi</td>
<td>Preclinical</td>
<td>Tekmira/OnCore</td>
</tr>
<tr>
<td>ALN-HBV</td>
<td>RNAi</td>
<td>Preclinical</td>
<td>Alnylam</td>
</tr>
<tr>
<td>ddRNAi</td>
<td>DNA-directed RNA</td>
<td>Preclinical</td>
<td>Benitec/Biomics</td>
</tr>
<tr>
<td>Isis HBV</td>
<td>anti-sense</td>
<td></td>
<td>Isis/GSK</td>
</tr>
</tbody>
</table>
RNA Therapeutics can Reduce HBV RNAs and Protein Production

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

Wooddell, CI et al 2013. *Molecular Therapy;*21(5):976-985
TKM-HBV: Targeting Multiple HBV Transcripts

- Design an anti-HBV RNAi Trigger ‘payload’ that is:
  - **Potent** - reduces viral protein production, especially HBsAg
  - **Universal** - effective against all genotypes

- All three triggers target the 2.1/2.4 kb sAg encoding mRNAs and also cleave 3.5 kb and 0.7 kb mRNA and pgRNA with potential for additional therapeutic benefit by reducing eAg, HBx, and core Ag

*Kindly provided by Dr Mike Sofia & Dr Tom Frohlich*
TKM-HBV: Reduction in Multiple HBV Markers

- deep reduction in HBsAg
- strong inhibition of HBeAg
- viral DNA and cccDNA are reduced by TKM-HBV

![Graph showing reduction in HBsAg, HBeAg, cccDNA, and HBV DNA over time.](image)

**Kindly provided by Dr Mike Sofia & Dr Tom Frohlich (Tekmira/OnCore)**

**Arrowhead Research**

- Achieved similar effects in HBV-infected chimpanzees

*Lanford, RE 2013. Hepatology;58(S1):705A-730A*
ARC-520 in CHB Patients

- Phase 2 multicenter, randomized, double-blind, placebo-controlled, dose-escalation study in HBsAg+ (>1000 IU/ml), HBeAg-neg CHB patients with viremia controlled on ETV
  - randomized 1:3 (placebo or ARC520) for up to 24 patients
- Single IV dose at 1, 2 and 3 mg/kg
- Safe, well-tolerated, no SAE’s or dose-limiting toxicities
- 1mg/kg group:
  - mean HBsAg nadir: -39% (-22 to -57)
  - mean HBsAg change on day 85: -31% (-14 to -39)
- 2mg/kg group:
  - mean HBsAg nadir: -51% (-46 to -59)
  - mean HBsAg change on day 85: -22% (range -7 to -40)
  - **Statistically significant difference vs placebo from days 3 to 43 post-dose**
- 3mg/kg group: Results not yet available

Yuen, MF et al 2014. Hepatology;60:1267A-1290A
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

Rozema, DB et al 2007. Proc Natl Acad Sci(USA);104:12982
Overcoming the Immunological Barriers

i. Role of Immune Regulatory Receptors

• in CHB, immune regulatory receptors (IRR) have been shown to be the key drivers of T-cell dysfunction [eg: PD-1]
  
  (Fisicaro, P et al 2010. Gasto;138:682-693.,

• blocking these inhibitory IRRs has the potential to restore T-cell function [eg: anti-PD-1/PD-L1]
  

ii. Follicular Helper T-Cells (Tfh)

• Tfh (CXCR5⁺ CD4⁺) under influence of IL-21 provide help to B-cells

• elevated serum IL-21 levels associated with HBeAg seroconversion (Ma, S-W et al 2012. J Hepatol;56:775-781)
# Immunotherapy: Results Reported at AASLD 2014

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABX-2013</td>
<td>Therapeutic vaccine: HBsAg, HBcAg</td>
<td>Center for Genetic Engineering and Technology, Cuba AbiVax</td>
</tr>
<tr>
<td>GS-4774</td>
<td>Therapeutic vaccine: yeast based, express HBV S, X and core proteins</td>
<td>Gilead</td>
</tr>
<tr>
<td>GS-9620</td>
<td>Oral agonist of TLR-7</td>
<td>Gilead</td>
</tr>
<tr>
<td>DV-601</td>
<td>Therapeutic vaccine with recombinant HBsAg and HBcAg and adjuvant</td>
<td>Dynavax</td>
</tr>
</tbody>
</table>

**Why have attempts at therapeutic vaccination failed?**

- approaches not HBV-specific or target only 1 HBV epitope
- HBV replication and HBsAg production not shut down
- inappropriate selection of patients
GS-9620: Oral TLR-7 Agonist

- **TLR-7**
  - intracellular pathogen sensor
  - endolysosomal RNA
  - agonism induces anti-viral response via innate immune activation

- **GS-9620**
  - oral
  - nanomolar potency
  - selective (TLR-7 >>> TLR-8)
  - pharmacodynamic effects in mouse, cyno, chimp, human
  - efficacy in woodchuck model

GS-9620: Reduction in HBV DNA, and Serum HBsAg and HBeAg in Chimpanzee

Lanford, RE et al. 2013. Gastroenterol;144(7):1508-1517
Reverse T Cell Exhaustion by PD-1/PD-L1 Pathway Blockade

Exhausted

- Proliferation
- Cytokine secretion
- Cytotoxicity

Functional

- Proliferation
- IFN-γ, TNF-α, IL-2
- Cytotoxicity
*In vivo* PD-L1 Blockade Synergizes with Therapeutic Vaccination to Control WHV Replication.

Companies Developing the Anti-PD-1/Anti-PD-L1 Therapies
BMS, Merck & Co, Novartis, Roche, MedImmune

Stopping Treatment

**APASL Recommendation to Stop Antiviral Treatment**

In HBeAg-positive patients: when HBeAg seroconversion has developed > 6 months

In HBeAg-negative patients: when HBV DNA remaining undetectable for three separate occasions 6 months apart

- **Outcomes**
  - 25-50% develop viral relapse with hepatitis
  - up to 40% remain treatment free (SVR)
  - half of these lose HBsAg

- **Factors**
  - HBV DNA undetectable at stop
  - HBsAg < 100 IU/ml [low]
  - duration of AV therapy (4-5 years)

---

Liang, Y et al 2011. Aliment Pharacol Ther;34:344.
International Efforts to Cure CHB

- Coalition to Eradicate Viral Hepatitis from Asia-Pacific (CEVHAP): policy & advocacy
- ANRS – Collaborate Workshop *(Zeisel, MB et al 2015. GUT;0:1-13)*
- ICE-HBV - International Collaboration to Eradicate HBV (being modelled on IAS approach): viral and immunological targets
- Philanthropy – focused on vaccination
- Pharmaceutical Companies
  - post-HCV era
- Professional Societies (EASL, AASLD, APASL)
- Hepatitis B Foundation (USA): community engagement
Future Perspectives and Developments

• The goalposts are shifting
• The medium-term aim for the field is to achieve “cure”
  – HBsAg seroconversion
• New agents for CHB are starting to emerge
  – identification of a HBV-Receptor (NTCP) is paradigm shifting
  – improved delivery to the liver for molecular therapeutics now a reality

PALPABLE OPTIMISM
What Might a HBV Curative Regimen Look Like?

- **Potent NA**
  - Agent to prevent viral spread and cccDNA re-amplification

- **cccDNA Inhibitor**
  - Safe and selective agent to reduce or silence cccDNA

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

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