How To Achieve Cure in HBV: A Virological Perspective

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Outline of Presentation

1. Can Viral Eradication Be Achieved?
2. Key Aspects of Viral Lifecycle
3. What Models are Available
4. Current Trials
5. Future Opportunities and Challenges
1. Can Viral Eradication Be Achieved?
Eradication

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

VERSUS

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
Why Do We Need New Therapies?

Ultimate goals of HBV treatment

- Eradicate HBV
- Reverse liver damage
- Prevent cirrhosis and HCC

Efficacy of existing treatment

- Suppress but not eradicate HBV
- Little effect on HBsAg levels
- Low rate of HBsAg loss
- Partial reversal of inflammation and fibrosis
- Decrease but not eliminate risk of HCC
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
- **Two therapeutic approaches:**
  (i) direct antiviral agents: lamivudine, adefovir, entecavir, telbivudine and tenofovir
  (ii) immune modulation: interferon alpha and thymosin-alpha
What Would HBV Elimination Look Like?

In the blood: HBV DNA/HBsAg negative
anti-HBs positive

In the liver: no HBV cccDNA
no HBV RC/DSL DNA
HBcAg staining negative
± HBsAg (occasional)

[reflecting integrated HBV DNA]

**Functional Cure:** HBsAg loss/Seroconversion

**Absolute or Complete Cure:** Maintenance of undetectable serum HBV DNA off-treatment
No cccDNA anywhere
Treatment Challenges: Barriers to Curing Chronic Hepatitis B

1. Reservoir of cccDNA
2. Dysfunctional T-cell Response
3. Insufficient or inadequate B-cell Response
4. HBV is NOT a Stealth Virus

Strategies to overcome these barriers

1. Deplete or Silence cccDNA
2. Improve potency of Pol Inhibitors
3. Broaden Viral Targets: combination DAA Therapy
4. Activate Antiviral Immunity
2. Key Aspects of Viral Lifecycle that are Susceptible to Intervention

- Overall lifecycle
- cccDNA
- Reverse transcription
- Entry
- Assembly Inhibitors
  - HBV nucleocapsid assembly (HBcAg)
  - HBV minichromosome
  - Virion Assembly
- HBeAg
HBV Lifecycle Showing Novel Approaches for Viral Targets

Target entry: NTCP blocker (Mycludex B?)
Target cccDNA: ZFNs? DSS? IFN-α? Lymphotoxin-β receptor?
Target HBsAg secretion: Nitazoxanide; tizoxanide? Triazolo-pyrimidine? NAP (Rep9AC“)?
Target envelope: Glucosidase inhibitors?
Target assembly/encapsulation: HAPs? Phenypropenamides?
HBV Replication: cccDNA Pathway

Uncoating
ER
Mature Nucleocapsid
Immature Nucleocapsid
Nuclear Transport
RC-DNA
Transcription
viralRNA
Core Polymerase
Intracellular Conversion Pathway
GOLGI
Translation
Uncoating
ER
Mature HBV virion
HBeAg
Spherical & Filamentous HBsAg
Precore
Surface
Translation
Reverse Transcription
Immature Nucleocapsid
Mature Nucleocapsid
RC-DNA
RC-DNA
The cccDNA is a Minichromosome


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*
Brief Reports

Reactivation of DNA viruses in association with histone deacetylase inhibitor (HDI) therapy: a case series report

David Ritchie, Richard L. Piekarz, Piers Blombery, Laszlo J. Karai, Stefania Pittaluga, Elaine S. Jaffe, Mark Raffeld, John E. Janik, H Miles Prince, and Susan E. Bates

*Department of Haematology and Medical Oncology, Peter MacCallum Cancer Centre and University of Melbourne, Melbourne, Victoria, AUSTRALIA; Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA; Laboratory of Pathology, Hematopathology Section, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, and Metabolism Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
in the presence of HBcAg peak number of nucleosomes increased from 15 to 16, resulting in a 20bp decrease in nucleosomal spacing

Interaction of APOBEC 3A/3B, HBV Core Protein (HBc) and cccDNA

Modified from Lucifora, J et al 2014. Science;343(6176):1221-8

Reverse Transcription: Improved Potency of NA 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.
Inhibitors of HBV Attachment and Entry

Sodium taurocholate cotransporting polypeptide (NTCP) identified as HBV and HDV receptor in 2012

Myrcludex in phase 2 trials in chronic HBV and chronic HDV decrease in HBV DNA and HDV RNA

Yan H, Elife 2012; 1:e00049
Lempp RA, Urban S. Interviro 2014; 57: 151
A Synthetic Peptide Derived from the Large Envelope Protein of HBV Blocks HBV Infection in Susceptible Cells….

Collection of supernatant of days 8-12p.i.

Measurement of secreted HBsAg/HBeAg etc.

Infection of HepaRG cells or PHH

Incubation o/n at 37° C

Collection of supernatant of days 8-12p.i.

Measurement of secreted HBsAg/HBeAg etc.

Gripon et al., PNAS, 99 (24) 2002
Urban et al., J. Virol, 79 (3), 2005
Glebe et al., Gastroenterology, 129, 2005
Engelke et al., Hepatology, 43, 2006
Schulze et al., Hepatology, 46, 2007
NTCP Binding Domains to Pre-S1

- NTCP is a 349 amino acid protein

- Pre-S1 binds at 2 regions: codons 84-87 and codons 157-165

- In Southern China the variant S267F is associated with protection against HBV infection

Peng, Zhao et al 2014
(i) HBV Nucleocapsid Assembly

- packaging

- reverse transcription

- Precore/HBeAg effect
Core/-Precore Structures

Role of cysteine 61 in HBc
- Core dimer interface
- For HBeAg dimers
  - 7 cys in precore and 61 cys in core
HBV Nucleocapsid Assembly Inhibitors

- Phenylpropenamide Derivatives (AT-61, AT-130) [Gilead]
- Heteroaryldihydropyrimidines (HAP-1, BAY 41-4109 series) [Bayer]
- Sulfamoylbenzamide Derivatives (DVR-23, DVR-56 and Novira Therapeutics NVR-1221)
- BCM-599 [2-amino-N-(2, 6-dichloropyridin-3-yl) acetamide family]
- Isothiafludine (pgRNA packaging)
Packaging Inhibitors

(A) 

AT-61
C_{21}H_{21}ClN_{2}O_{2}
Mol.Wt.: 368.86

AT-130
C_{22}H_{22}BrN_{2}O_{5}
Mol.Wt.: 488.33

(B) Recombinant HBV Baculovirus: IC Core HBV DNA

Wild-type HBV  AT-130  AT-61  LMV

μM 0 10 100 0 10 50 250 0 0.001 0.001 0 0

SS

Figure: RNA Production in Presence of AT-130 and LMV

Figure: HBV DNA and RNA Production of pTRE-Cell lines in the presence of AT-130 and LMV

Feld, JJ et al 2007. AVR;76:168
Targeting HBV Nucleocapsids

Heteroaryldihydropyrimidines

Destabilization of nucleocapsids

Deres et al, Science 2003
(iii) Virion Assembly

a. HBsAg Production
b. HBV Maturation

**Molecular-Based Therapies (Viral mRNA)**

- ESC-GalNAc-siRNA [Alnylam]
- DPC-NAG targeting ligand for ARC-520 siRNA [Arrowhead]
- Rep 9AC (ampipathic oligonucleotide) [Replicor]
- Anti-Sense Oligonucleotides
- Micro-RNAs
- Ribozymes
Role of Cellular Targets

• Glucosidase Inhibitors (toxicity significant)
• Alisporivir (Novartis)
  – (cyclophylin inhibitors)
• Birinipant (TetraLogic)
  – (apoptosis-promoting)
  – toxicity issues (?)
  – can it be reversed?
• Nitazoxanide (?)
• Triazolo-pyrimidine Derivatives (?)
Role of Cellular Targets

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4. Current Trials
Status of 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.
- Vials for clinical studies have been filled.
- Myrcludex B has been characterized for purity, stability etc.
# Nucleic Acid-Based Approaches

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<thead>
<tr>
<th>Name</th>
<th>Approach</th>
<th>Phase</th>
<th>Company</th>
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<tbody>
<tr>
<td>ARC-520</td>
<td>RNAi</td>
<td>IIA</td>
<td>Arrowhead</td>
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<tr>
<td>ALN-HBV</td>
<td>RNAi</td>
<td>Preclinical</td>
<td>Alnylam</td>
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<tr>
<td>ddRNAi</td>
<td>DNA-directed RNA</td>
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<td>Benitec/Biomics</td>
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<td>BSBI-25</td>
<td>cccDNA inhibitor</td>
<td>Preclinical</td>
<td>Blumberg Institute</td>
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<tr>
<td>TKM-HBV</td>
<td>RNAi</td>
<td>Preclinical</td>
<td>Tekmira</td>
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<tr>
<td>Isis HBV</td>
<td>anti-sense</td>
<td></td>
<td>Isis/GSK</td>
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1. Short interfering ds RNA can lead to transcriptional silencing (if homologous) and translational repression (if mismatched).

2. Involves Drosha and Dicer enzymes, the RNA-induced silencing complex (RISC) and the nuclease Ago.

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**
  - 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

- **DPC polymer complex is enclosed in an endosome. Low pH results in polymer unmasking.**
- **DPC/siRNA complex is taken up into the cell cytoplasm.**
- **siRNA engages the RNA interference machinery, resulting in knockdown of target gene expression.**
- **Polymer induces endosome lysis and release of siRNA payload into the cell cytoplasm.**
RNA therapeutics can reduce HBV RNAs and protein production

Differentiation from nucleos(t)ide reverse transcriptase inhibitors

Revival of adaptive immune response and functional cure
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**

Dr. Robert Lanford, Texas Biomedical Research Institute
**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
6. Future Opportunities and Challenges
Future Directions: New Targets/New Drugs Towards a Cure of HBV Infection

Immune modulation
- Toll-like receptors agonists, e.g. GS-9620
- Anti-PD-1 mAb, e.g. BMS-936559
- CYT107
- GI13000
- Vaccine therapy

Entry inhibitors
- Lipopeptides, e.g. Myrcludex-B

RNA interference, e.g. ARC-520

Inhibitors of HBsAg release, e.g. REP 9AC

Polymerase inhibitors
- Nucleoside analogues, e.g. emtricitabine, amdoxovir, MIV-210
- Non-nucleoside, e.g. LB80380

Inhibition of nucleocapsid assembly, e.g. Bay 41-4109, NVR1221

Development stage: preclinical, clinical

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]
Future Perspectives and Developments

• The goalposts are shifting
• The medium-term aim for the field is to achieve “cure”
  – HBsAg seroconversion
  – An immunomodulator is likely to be required
• New agents for CHB are starting to emerge
  – Identification of the HBV-R (NTCP) may be paradigm shifting
  – Improved delivery to the liver for molecular therapeutics

PALPABLE OPTIMISM