Progress in Hepatitis B Cure Research

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Disclosures

• Research funding from Gilead Sciences
Structure of Presentation

• Global burden of chronic HBV
• CHB natural history and current treatments
• Definitions of cure and (some) new therapeutic targets
• ICE-HBV Global Scientific Strategy
More Than 2 Billion People Show Evidence of Hepatitis B (HBV) Infection\(^1\)

- 257 million people live with chronic HBV infection worldwide\(^1,3\)
- Many reside in Asia/Pacific, Africa and The Americas

Hepatitis B: Global Burden

- 880,000 attributable deaths annually (GBD Stanaway The Lancet 2016)
- 120 million people with CHB in the Asia/Pacific Region
- Viral hepatitis is now the 7th ranked cause of human mortality (GBD Stanaway The Lancet 2016)
- Without appropriate management, 15-25% of people with CHB will develop advanced liver disease &/or HCC (Lavanchy 2004)
- Liver cancer is the 3rd most common cause of cancer mortality globally (GBD report 2016)
  - HBV causes 40% of liver cancers

• Current vaccine is prophylactic and has no effect on existing infections.
• Current treatments reduce but do not eliminate the risk of liver cancer
Current Treatment Paradigm

- **Goal** = sustained suppression of HBV DNA

- **First line drugs:**
  - **NA** = entecavir / tenofovir (**Direct acting antivirals**).
    - Potent viral suppression
    - Well-tolerated, very low rates of resistance
    - HBsAg loss is very rare
    - **Long-term (indefinite?)** treatment
  - **PEG-IFN Immunotherapy**
    - Response rate ~ 25% in HBeAg-positive patients- varies by HBV genotype (A>B>C>D)
    - **Finite therapy**
    - HBsAg loss occurs but uncommon
    - Most suitable for young, HBeAg-positive patients
Current antiviral treatments, while very effective, are not cures!

- Direct acting antiviral therapy reduces HBV replication to below LLOQ but does not target the viral nuclear reservoir of episomal covalently closed circular DNA.
- Replicating virus is still infectious
- Nor does it impact integrated HBV DNA
- Therapy is usually life-long
- Does not eliminate risk of liver cancer
Current Treatments Do Not Completely Suppress HBV DNA

- In the majority of patients, treatment with the most effective nucleos(t)ides results in
  - HBV DNA suppression to <LLOQ (29 IU/mL or 165 copies/mL)
  - ALT normalization; fibrosis improvement
- Despite this, HBV DNA remains detectable in the majority of treated patients with viral load <LLOQ
- Ongoing replication despite nucleos(t)ide therapy may provide a mechanism for long-term viral persistence

Marcellin et al. Hepatology 2014;60:1093A.

Slide courtesy of Fabien Zoulim
Patient Sera with HBV DNA Below LLOQ Under TDF Is Infectious

Patient Sera
HBV DNA Suppressed on NUC

N = 12

All below LLOQ DNA Detected

Burdette et al, EASL ILC 2019
Slide courtesy of Fabien Zoulim
Can we cure chronic hepatitis B?

- The recent development of cures for hepatitis C virus following as little as 4-8 weeks of treatment has raised expectations for HBV cure.
- HCV replication takes place solely in the cytoplasm and there is no viral reservoir.
- HBV has both nuclear (cccDNA) and cytoplasmic phases to its life-cycle.
- It is the nuclear (cccDNA/minichromosome) phase that makes HBV elimination so difficult.
- Produces a continual source of viral mRNAs and proteins which are associated with disease progression.
Definitions of HBV cure

- **Functional cure** (equivalent to resolved acute infection): Sustained/durable HBsAg loss *with or without anti-HBs seroconversion*, with undetectable serum DNA, but persistence of cccDNA which is not transcriptionally active, allowing treatment cessation.

- **Complete cure**: as for functional cure, but with cccDNA clearance.
Barriers to HBV elimination

Defective CD8+ responses (exhaustion)
Defective B cell responses
Inefficient innate response

Persistence of cccDNA and integrated HBV DNA
Long t1/2
Continuous replenishment
Not affected by NAs and IFN
Generation of the cccDNA nuclear reservoir

HBsAg Pre-S truncation

NTCP

HBx truncation

Nuclear Transport

De-envelopment

GOLGI

ER

Precore

Surface

Core Polymerase

HBx

Transcription

Viral RNA

pc pg preS1 preS2/S HBx

Intracellular cccDNA amplification pathway

Main integration pathway

Mature HBV virion

Spherical & Filamentous HBsAg

RNA-containing particle

Empty virion

Mature Nucleocapsid

Immature Nucleocapsid

Reverse Transcription

HBSP

HBV cccDNA minichromosome

Splicing of pgRNA

Viral Integration

RC-DNA

DSL-DNA

Main integration pathway

Intracellular cccDNA amplification pathway

Mature Nucleocapsid

Immature Nucleocapsid

Reverse Transcription
Although HBV is currently incurable...

- Over a billion people have resolved an acute infection.
- Functional HBV cure of CHB (HBsAg loss/seroclearance) is observed in a small number of persons (1-2% each year) either spontaneously or in the setting of antiviral therapy.
- A therapeutic functional cure (at the very least) is a realistic goal.
The discovery of the sodium taurocholate co-transporting polypeptide (NTCP) HBV entry receptor means..

• It is now possible to interrogate the complete HBV life-cycle, including HBV cccDNA and the minichromosome.
• Again this has raised expectations of cure…
• Numerous approaches for HBV treatment and cure are in “the pipeline” targeting different stages of the HBV replication cycle – direct acting antivirals and immune-mediated therapies.

Yan et. al., Elife 2012
Viral targets & drug discovery to cure HBV infection

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

**RNA interference**
- Arrowhead, Arbutus, Alnylam, Janssen, GSK

**Targeting HBsAg**
- Mab: Gilead
- Release: Replicor

**Polymerase inhibitors**
- Nucleoside analogues, e.g. TAF
  - Gilead, BMS
- Non-nucleoside, e.g. LB80380
- RNAseH inhibitors

**Inhibition of nucleocapsid assembly**
- Novira, AssemblyBiosc, Gilead, Janssen, Roche

The Cure Pipeline

- Direct Acting Antivirals
- Immune therapies
The Cure Pipeline

• Direct Acting Antivirals
  • Viral entry inhibitors
  • cccDNA targeting
  • Capsid modulators
  • siRNAs
  • NAPS
Model for HBV entry in hepatocytes and development of entry inhibitors

Entry inhibitors

Myrcludex (pre-S1 peptide)
Blank et al, J Hepatol 2016
Bogomolov et al, J Hepatol 2016

Ezetimibe
Lucifora, Antiviral Res 2013

Proanthocyanidin
Tsukuda, Hepatology 2017

Cyclosporin analogues
Shimura, J Hepatol 2017

Li et al, elife 2012; Urban et al, Gastroenterology 2014
Myrcludex B with PEG-interferon α 2a: Safety and efficacy in patients with chronic HBV/HDV co-infection in a phase 2 trial (MYR203)

**RESULTS**

- **Safety:** MyrB was well tolerated, with 155 drug-related AEs up to w72 (mild n=122, moderate n=28, serious n=5), primarily increased total bile salts
  - Most AEs (n=524) related to PegIFNα2a
  - All cases resolved; bile salts returned to baseline by follow-up Week 50
  - Two SAEs (anal fistula and proctitis) not-related to MyrB occurred in 1 patient of Arm B in follow-up
- **Efficacy:** MyrB + PegIFNα2a induced a significant enhancement of HDV RNA response
  - 40% (12/30) patients had undetectable HDV RNA at Week 72
  - 2 mg MyrB + PegIFNα2a induces HBsAg response in HBeAg negative patients at Week 72
    - 40% of patients experienced HBsAg response
    - In this group 27% lost HBsAg and 20% seroconverted

**CONCLUSIONS** In contrast to PegIFNα2a monotherapy, MyrB + PegIFNα2a demonstrated high rates of HDV RNA suppression. **HBsAg loss was achieved in 27% of patients**, indicating a potential role for MyrB in future HBV cure regimens.

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Wedemeyer H, et al. ILC 2019; GS-13
Directly targeting the cccDNA reservoir

- cleave cccDNA molecules via cccDNA sequence-specific endonuclease using
  - zinc-finger nucleases (Hoeksema, KA & Tyrell, DL. 2010. Meth Mol Biol;649:97-116)
  - CRISPR/Cas9 technology (Seeger, C & Sohn, JA. 2014. Mol Ther Nucleic Acids;3:e216)

- Inhibited HBV replication up to eight fold
- Over 90% of HBV DNA cleaved by Cas9
- Off target effects?
A first-in-class orally available HBV cccDNA destabilizer ccc_R08 achieved sustainable HBsAg and cccDNA reduction in the HBV circle mouse model

**BACKGROUND & AIMS**

- Persistence of cccDNA is a major barrier to cure in CHB patients with existing therapies
- **Aim:** To evaluate the effect of a novel small molecule ccc_R08 on the level of pre-existing cccDNA both *in vitro* and *in vivo*

**METHODS**

- HBV-infected primary human hepatocytes (PHH) were used for evaluating antiviral activities *in vitro*
- ccc_R08 was orally administered in HBV circle mouse model* to study its in vivo efficacy
  - Levels of HBV DNA, HBsAg, HBeAg, pgRNA, and cccDNA were measured

**RESULTS**

**HBV-infected PHH**

- Potent inhibition of HBV DNA, HBsAg, HBeAg, and pre-existing cccDNA
- No effect on mitochondrial DNA level and cytotoxicity

**HBV circle mice**

- Levels of serum HBV DNA, pgRNA, HBsAg, HBeAg reduced significantly
  - Sustained during the off-treatment period
- Levels of cccDNA in the liver of ccc_R08 treated mice were < LLOQ
  - ETV had no such effect on cccDNA level in this model

Wang L, et al. ILC 2019; PS-074

Gao et al ILC EASL 2019
Small molecules targeting the cccDNA pool

Gao et al, EASL ILC 2019

Slide courtesy of Fabien Zoulim
HBx, DDB1 & SMC5/6 - CRITICAL FOR CCCDNA TRANSCRIPTION

- SMC5/6 associates with cccDNA to repress transcription
- HBx interacts with DDB1 to hijack cellular mechanisms, targeting SMC5/6 for degradation via proteasomal pathways
  - Relieves repression of cccDNA transcription

Damaged DNA-Binding Protein 1 (DDB1)

- 127kDa subunit of UV-damaged DNA binding complex (UV-DDB) implicated in DNA repair
- High evolutionary conservation
- Functions as adaptor protein for CRL4 complex

IS THE HBX / DDB1 INTERACTION A POTENTIAL DRUG TARGET?

AASLD, 2018.
Sekiba et al. Discovery of a novel therapeutic agent targeting HBx-DDB1 interaction for HBV cure.
Nitazoxanide disrupted HBx-DDB1 interaction and impacted HBV RNAs.

ILC 2019.
Binqian et al. Targeting the HBx-DDB1 Cullin complex inhibits transcription from HBV covalently closed circular DNA in susceptible hepatoma cells.

International HBV Meeting, Melbourne, 2019.
Plank et al. HBx SUMOylation promotes DDB1 binding and efficient host substrate degradation.

Marchetti et al. Host DNA Damage Binding Protein 2 is involved in HBV cccDNA formation.
The Cure Pipeline

- Immunotherapeutic approaches
HBV persistence and immune control

**Acute resolving infections**

Robust, coordinated adaptive immunity

1. 2 – 3 months to clear acute HBV infection
2. CD8 T cells mediate clearance of infected cells
3. B cells - anti-HBs marker of resolution
4. CD4 T helper cells support CD8 & B cells
5. Supported by innate response?
6. Persistence of cccDNA

**Chronic infections**

Weak Adaptive Immune response

1. Low T cell frequency
2. Inhibitory receptors – PD-1, CTLA-4, Tim-3
3. HBV-specific T cells are prone to apoptosis
4. Metabolic regulation

Pipeline to restore antiviral immunity

Innovations in immunotherapy

T cell engineering and immunotherapy

Non-lytic Lymphocytes Engineered to Express Virus-specific T-cell Receptors Limit HBV Infection by Activating APOBEC3

Koh et al, Gastroenterology 2018
Antiviral approaches  Immunomodulatory approaches

Direct antivirals

Curative approaches  cccDNA targets

Control approaches  Post-cccDNA targets

Specific hepatocyte killing

Immunotherapy

Curing hepatocytes

Virus neutralisation

Adaptive Immunity Modulation
- Checkpoint Inhibitors
- TCR engineering, CAR
- Vaccine therapy

Immune response
- PD-1
- CD8+ T-cell
- B cell
- Insufficient B-cell response
- Dysfunctional T-cell response

Antiviral cytokines
- pDC
- IL12
- IFN-α
- IL-1β
- IL-6
- NK
- NKT
- KC

Innate Immunity Modulation
- Toll-like receptors agonists
- RIG-I agonists
- STING agonists

adapted from Levrero et al. Curr Opin Virol 2016
A number of new approaches are in Phase 1 or Phase 2 clinical trials

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<th>Approach</th>
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<td>HBV Locked Nucleic Acid (LNA) RO7062931</td>
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<td>Liquid nano-particle (LNP) RNAi (ARB-1462)</td>
<td>Arbutus Biopharma</td>
<td>Phase 2 (IMPACT study)</td>
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<td>NCT02981602 (R), NCT03020745 (R)</td>
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<td>Myr-pharma</td>
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<td>Roche</td>
<td>NCT03762681 Not yet recruiting</td>
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HBV cure – Towards Combination Therapy

**SERUM**

- HBV DNA change from baseline (log_{10} c/mL)

**LIVER**

- cccDNA

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Slide courtesy of Fabien Zoulim

Testoni et al, Liver International 2017
This is an urgent need for a coordinated approach for HBV cure

• Prior to 2016, global HBV cure efforts were not coordinated.

• Given that chronic HBV infection is a global problem, we believed there was an urgent need for global collaboration for HBV cure – similar to the HIV field.
ICE-HBV was formed in 2016 and aims to fast-track the discovery of a safe, effective, affordable and scalable cure to benefit all people living with CHB, including children and people living with HCV, HDV and HIV co-infection. ICE-HBV intends to contribute to the elimination of CHB as a global public health challenge.
Key Stakeholders Group advises our researchers

- **Stakeholders**
  ANRS; ASHM; Asian Liver Centre; Bill and Melinda Gates Foundation (Beijing); Biomed Central/WHO CC; CEVHAP; NCHHSTP, CDC; DZIF; Hepatitis Australia; Hepatitis B Foundation; Hepatitis Education Project; Inno Community Development; MRC Unit – The Gambia; NIAID; Pasteur Institute/International Network of Pasteur Institutes; The Forum for Collaborative Research; The World Hepatitis Alliance; World Indigenous Peoples' Conference on Viral Hepatitis; WHO; WHO Collaborating Centre for Viral Hepatitis; Yellow Warriors.
ICE-HBV Position Paper

A Global Scientific Strategy towards Cure of Chronic Hepatitis B Virus Infection.

ICE HBV working groups have identified two key strategies for cure of chronic HBV infection

1. Cure HBV infection without killing HBV infected cells

2. Induce Immune Control to safely eliminate HBV infected cells.
Immediate and Future Actions Required to Achieve HBV Cure – the ICE-HBV Strategy

**INCREASE** funding for individual and collaborative cure-related research projects by governmental and private funding agencies and philanthropic benefactors.

- Consideration should be given to establishing international research consortia, similar to the Martin Delaney Collaboration for HIV research managed by the NIH in the USA. HBV cure research investment strategies should be prioritised in national HBV plans globally.
- We support recent calls from the Hepatitis B Foundation for increased HBV cure research funding.
- ICE-HBV played a leading role in ensuring hepatitis virus elimination was included in the agenda of the Global Fund replenishment meeting in Lyon, October, 2019.
Immediate and Future Actions Required to Achieve HBV Cure – the ICE-HBV Strategy

**CONCENTRATE** on the discovery of interventional strategies that will permanently reduce the number of productively infected cells and/or permanently silence the cccDNA in those cells AND that will stimulate HBV-specific T cells and the production of antibodies that will prevent viral spread to uninfected cells, mimicking spontaneous resolution of HBV infection
Immediate and Future Actions Required to Achieve HBV Cure – the ICE-HBV Strategy

**ESTABLISH** repositories of standardised HBV reagents and protocols and facilitate access to all researchers across the world and support the development of a new animal model.
Repository for Advancing HBV Cure
Research is underway (NIAID, NIH, USA)

- Peptide libraries for clinical immunology studies
- Monoclonal antibodies against HBV proteins
- Viral DNA, RNA and protein standards
- Replication competent HBV clones of various genotypes
- Compound libraries for studies in experimental models
- Cell lines susceptible to HBV replication and cell to cell spread
- Mouse and primate models susceptible to HBV infection
- Collection of serum and liver biopsy samples from cohort study for research purposes

In parallel, ICE HBV is establishing an on line Open Access Protocols Database

ICE-HBV
International Coalition to Eliminate HBV
PROMOTING GLOBAL COLLABORATION IN HBV CURE RESEARCH
HBV Research Protocols

• Complement the upcoming NIAID reagents repository by making corresponding quality-controlled research protocols available freely for all researchers around the world.
• Led by Haitao Guo and the ICE-HBV working group members including Lena Allweiss, Maura Dandri, Jianming Hu, Jake Liang, Margaret Littlejohn, Fabien Zoulim, Peter Revill, and Barbara Testoni. Coordinated by Marley EasterbrooK.
• Current Categories:
  – In vivo Models
  – Cell Cultures
  – HBV Antigen Analyses
  – HBV Biochemical Assays
  – HBV Nucleic Acid Analyses
  – Immunology Assays
  – In Vitro HBV Infection Systems

Submit protocol ideas to info@ice-hbv.org
How far are we from HBV Cure?

- Considerable advances in the past 5 years
- Huge interest from Pharmaceutical Industry
- Global coordinated approach
- Research Funding is increasing

Together these factors will contribute to a marked increase in HBV Functional cure rates in the next ten years.
The Melbourne Declaration

- We support the recent strategic Roadmaps for HBV Cure from the Hepatitis B Foundation, International Collaboration to Eliminate HBV (ICE-HBV), and the World Hepatitis Alliance’s “Find the Missing Millions” campaign. We endorse the World Health Organization’s Global Health Sector Strategy on Viral Hepatitis 2016-2021, including the specific 2030 elimination goals that were agreed upon by all members of the World Health Assembly. To achieve these goals, we call for a substantial increase in government and industry funding dedicated for increased testing and molecular diagnostics, treatment and curative HBV research, and to facilitate equitable, affordable and universal access to an HBV cure within the next ten years.
2015 was the 50th anniversary of the discovery of the Australia Antigen

- A "NEW" ANTIGEN IN LEUKEMIA SERA.

- ICE-HBV and our partners are determined it will not be another 50 years before we have a cure!
Acknowledgements

- ICE-HBV Governing Board
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ICE-HBV
International Coalition to Eliminate HBV
PROMOTING GLOBAL COLLABORATION IN HBV CURE RESEARCH

Doherty Institute

Australian Academy of Science

DZIF

Australian Centre for Hepatitis Virology

International Meeting