Progress towards a hepatitis B cure

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Disclosures

• Advisory Board: Allovir
Objectives

- Understand HBV life cycle
- Define HBV cure
- Recognize relevant treatment endpoints in assessing novel therapeutics
- Recognize novel therapeutics in development

HBV, hepatitis B virus
HBV LIFE CYCLE AND HBV CURE DEFINITIONS
HBV Life Cycle: Entry

- Attachment
- NTCP
- Endocytosis
- Uncoating
- Nuclear import
- HBV virion (with dsDNA)
- HBV virion (with rcDNA)
- Recycling
- HBx
HBV Life cycle: Intranuclear

- Endocytosis
- Uncoating
- Nuclear import
- DNA repair
- Pre-core/Core mRNA
- Pregenomic RNA (pgRNA)
- 3.5 kb RNA
- 2.4 kb RNA
- 2.1 kb RNA
- 0.7 kb RNA
- Transcription
- HBx
- Recycling
- Secretion
- HBV virion (with dslDNA)
- DNA integration
- DNA repair
- Minichromosome (cccDNA)
- Integrated HBV DNA (iDNA)
- Relaxed circular DNA (rcDNA)
- Double stranded linear DNA (dslDNA)
HBV Life Cycle: Intranuclear

- HBV virion (with dslDNA)
- Endocytosis
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- Nuclear import
- Double stranded linear DNA (dslDNA)
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- Integrated HBV DNA (iDNA)
- DNA repair
- Relaxed circular DNA (rcDNA)
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- HBx
- 0.7 kb RNA
- 3.5 kb RNA
- Pre-core/Core mRNA
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- 2.4 kb RNA
- 2.1 kb RNA
HBV Life Cycle: Intranuclear
HBV Life Cycle: replication and secretion

- **HBV virion** (with dsDNA)
  - Endocytosis
  - Uncoating
  - Nuclear import
  - DNA repair
  - Integrated HBV DNA (iDNA)
- **Minichromosome** (cccDNA)
- **Integrated HBV DNA (iDNA)**
- **Double stranded linear DNA (dsDNA)**
- **DNA integration**
- **Relaxed circular DNA (rcDNA)**
- **Minichromosome (cccDNA)**
  - Transcription
  - 3.5 kb RNA
  - Pre-core/Core mRNA
  - Pregenomic RNA (pgRNA)
  - 2.4 kb RNA
  - 2.1 kb RNA
  - 0.7 kb RNA
- **HBsAg**
- **Polymerase**
- **Capssid Formation**
- **Reverse Transcription**
- **Capsid**
- **pgRNA**
- **HBx**
- **Attachment**
- **Nuclear Transport Carrier Protein (NTCP)**
- **Secretion**
- **Assembly**
- **Subviral particles (SVP)**
- **Recycling**
- **~10% ~90% DNA integration**
- **Secretion**
- **~90%**
- **~10%**
- **NUC**
- **LHBs**
- **SHBs**
Definitions of HBV cure

No cure (current treatment)
Lifelong Rx in most
Definitions of HBV cure

Partial cure
sAg+, HBV DNA- off Rx
cccDNA/iDNA+

No cure (current treatment)
Lifelong Rx in most

cccDNA, covalently closed circular DNA; iDNA, integrated DNA; sAg+, surface antigen positive
Definitions of HBV cure

**Sterilizing cure**
cccDNA/iDNA-

**Partial cure**
sAg+, HBV DNA- off Rx
cccDNA/iDNA+

**No cure (current treatment)**
Lifelong Rx in most
Definitions of HBV cure

Sterilizing cure
cccDNA/iDNA-

Functional cure
sAg-, HBV DNA- off Rx
cccDNA/iDNA+

Partial cure
sAg+, HBV DNA- off Rx
cccDNA/iDNA+

No cure (current treatment)
Lifelong Rx in most
HBsAg loss is infrequent but possible with current therapy

Not head-to-head trials; different patient populations and trial designs

Extended Treatment With Nucleos(t)ide Analogues* vs 1 Yr Peginterferon Treatment HBeAg positive

*With sustained undetectable HBV DNA.

PEG, pegylated interferon; TDF, tenofovir disoproxil fumarate

Marcellin et al, Lancet 2013 381:468
HBV Life Cycle: replication and secretion

- Endocytosis
- NTCP
- Nuclear import
- Minichromosome (cccDNA)
- Integrated HBV DNA (iDNA)
- DNA repair
- Relaxed circular DNA (rcDNA)
- Double stranded linear DNA (dsDNA)
- Attachment
- Secretion
- Assembly
- Subviral particles (SVP)
- Polymerase
- pgRNA
- Capsid

- Pre-core/Core mRNA
- 3.5 kb RNA
- 2.4 kb RNA
- 2.1 kb RNA
- 0.7 kb RNA

- Reverse Transcription
- Capsid Formation
- Polyadenylation

- Core
- HBx

- Secretion

- ~10% DNA integration
- ~90% Secretion

- HBV virion (with dslDNA)
- HBV virion (with rcDNA)
- pgRNA
- Capsid
- Formation
- Subviral particles

- SHBs
- MHBs
- LHBs

- HBsAg

- NUC
HBsAg loss with nucleos(t)ide analogues decreases HCC risk

HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma

Yip et al J Hepatol 2019 70:361
HBsAg clearance with treatment is durable

Yip et al, J Hepatol 2018 68(1):63
TREATMENT ENDPOINTS
Is HBsAg decline a useful intermediate endpoint in evaluating treatments?
HBsAg <100 IU/ml predicts sustained response off of treatment

Chang ML et al Aliment Pharmacol Ther 2015;42:243
**qHBsAg <100 IU/ml at EOT in HBeAg negative increases likelihood of cure**

**DARING-B:**
- 57 HBeAg neg stopped NAs after ≥ 4 yrs and HBV DNA undetect ≥ 3 yrs
- HBCrAg not assoc
- qHBsAg not predictive of relapse

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Papatheodiordi et al, JVH 2020; 27:118
Virological relapse still occurs with HBsAg <100 IU/ml

135 individuals HBsAg <100 IU/ml
- stopped entecavir or TDF
- Relapse >2000 IU/ml

IU, international units; TDF, tenofovir disoproxil fumarate

Tseng et al, Clin Gastro and Hepatol April 2020 epub
NOVEL MARKERS
Novel markers for cccDNA transcription

• HBV RNA
• Hepatitis B core-related antigen (HBCrAg)
HBV Life Cycle: replication and secretion

- HCV virion (with dsDNA)
- Attachment
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- 0.7 kb RNA
HBV RNA

• Primarily pgRNA
HBV RNA declines with nucleos(t)ide analogues

HBV RNA greater declines in responders vs. non-responders

- HBeAg neg who received PEG-IFN x 48 wks
- Responders: HBV DNA <2000 IU/ml & nI ALT 6 mos after treatment
- 3.2 log IU/ml cutoff at wks 12 and 24: NPV 91% and 93% but PPV 30%

NPV, negative predictive value; PPV, positive predictive value

Farag et al 2021 72(2): 202
HBV RNA limitations

• Needs standardization
  – Difficult to compare between studies
• Applicability to all genotypes
• Sensitivity low
HBCrAg

- Measures products of precore/core gene:
  - Core antigen
  - Denatured HBeAg
  - 22kDa precore protein
- Theoretically only from cccDNA
Lower HBCrAg at end of NA treatment decreases virological relapse

HBCrAg levels at EOT

Virological relapse HBsAg loss

Percent with outcome

<table>
<thead>
<tr>
<th>&lt;3</th>
<th>≥ 3</th>
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<tbody>
<tr>
<td>40</td>
<td>60</td>
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</tbody>
</table>

AUROC to predict virological relapse

HBCrAg + HBV RNA

0.742

AUROC, area under receiver operator curve; EOT, end of treatment; NA, nucleos(t)ide analog

It looks like there's something partly covered over here

Steven McGuire, 4/11/2021
iTACT-HBcrAg has improved sensitivity

161 HBeAg-negative NA-treated patients with HBCrAg tested on two platforms. Sensitivity of iTACT-HBcrAg 2.1 log IU/ml


NA, nucleos(t)ide analog
APPROACHES TO HBV CURE
Three strategies

Inhibit HBV replication
Three strategies

- Inhibit HBV replication
- Decrease HBsAg production
Three strategies

- Inhibit HBV replication
- Decrease HBsAg production
- Enhance anti-HBV immune response
Targets to inhibit HBV replication

- Block entry
- Eliminate cccDNA
- Inhibit encapsidation
- Inhibit RT or RNAse H
Novel drug targets

Eliminate cccDNA

HBV virion (with dsIDNA)

Endocytosis

Uncoating

Nuclear import

DNA repair

Integrated HBV DNA (iDNA)

Double stranded linear DNA (dsIDNA)

DNA integration

Relaxed circular DNA (rcDNA)

Minichromosome (cccDNA)

Transcription

0.7 kb RNA

3.5 kb RNA

Pre-core/Core mRNA

Pregenomic RNA (pgRNA)

2.4 kb RNA

2.1 kb RNA

Transcription

Recycling

HBx

HBV virion (with rcDNA)

Attachment

Secretion

Assembly

~10% ~90%

DNA integration

Secretion

Block entry

HBsAg

Polymerase

Core Formation

Recycling

HBx

pgRNA

Capsid

Subviral particles (SVP)

Assembly

Secretion

Encapsidation inhibitor

0.7 kb RNA

0.7 kb RNA

~10% ~90%

~10% ~90%

NUC

Core Formation

Polymerase

pgRNA

Capsid

Subviral particles (SVP)
CpAM: core protein allosteric modulator
CAM: capsid assembly modulator

Core is essential for
• HBV genome packaging
• Reverse transcription
• Intracellular trafficking
• Maintenance of chronic infection as encapsidated HBV genomes are imported into the nucleus.
JNJ-6379 x 24 w in non-cirrhotics

Minimal effect on HBeAg and HBsAg levels

Janssen H et al. EASL 2020
NAs may reduce integrated DNA

RCT TDF vs placebo x 3 yrs
• Treatment naïve, DNA >2000 IU/ml
• RNA seq: viral-human chimera
• 3.28 vs 1.81 fold reduction in # of distinct viral integrations Y1 to Y3

Hsu et al, Gastro 2022 online and in press
Targets to decrease HBsAg production

- Silence HBV RNA
  - RNAi
  - ASO (anti-sense oligonucleotide)
- NAPs (nucleic acid polymers)
- Eliminate cccDNA
**RNAi – JNJ-3989 (ARO-HBV)**

- Off-treatment persistence of HBsAg decline → translates to lower HBcrAg, HBeAg and HBV RNA as well
- What is the mechanism – immune control?

Gane et al EASL 2020

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3 SC doses at days 0, 28 and 56 – long-term follow-up

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*Graph showing sustained off-treatment response in 39%*
The first combination

Core Assembly Modulators

2 mechanisms:
1. Block encapsidation – decrease HBV DNA & RNA
2. Block cccDNA formation

2 mechanisms
1. Block viral replication – pgRNA
2. Block Ag production – restore immunity
**REEF-1: siRNA + CAM**

siRNA (3989) (40, 100 or 200) vs (6379) vs combination (siRNA + CAM) x 48 wks + 24 wks f/U

Non-cirrhotic, nuc suppressed or naïve, HBsAg>100 IU/mL – stratified by HBeAg & treatment

All patients on NUCs (oral TDF, TAF, or ETV qd) during treatment

- **Surprisingly combo did worse!**
- **siRNA alone at 200 mg most effective…but still not the answer**

Yuen MF et al, AASLD 2021 LB10
HBsAg results

Initial rapid decline...with plateau after about 24 weeks – why?
A high proportion achieved HBsAg<100 but no HBsAg loss!
CAM had no effect on HBsAg alone and inhibitory effect on siRNA – why?

Yuen MF et al, AASLD 2021 LB10
siRNA + PegIFN

siRNA (2218) alone or + PegIFN 180 mcg SC started together or after siRNA lead-in
Non-cirrhotic, nuc suppressed, HBsAg>50 IU/mL

Cohort 1
VIR-2218 200 mg sc q4w (n=15)

Cohort 2
VIR-2218 200 mg sc q4w (n=15)
pegIFNα 180 µg sc qw

Cohort 3
VIR-2218 200 mg sc q4w (n=18)
pegIFNα 180 µg sc qw

Cohort 4
VIR-2218 200 mg sc q4w (n=16)
pegIFNα 180 µg sc qw

pegIFNα D/C if HBsAg <LLOQ is reached at 2 consecutive visits

Yuen MF AASLD 2021
**siRNA + PegIFN**

- **VIR-2218 only**
- **VIR-2218 lead-in + pegIFNα (12 wk)**
- **VIR-2218 + pegIFNα (24 wk)**
- **VIR-2218 + pegIFNα (≤48 wk)**

<table>
<thead>
<tr>
<th>Week 4, n</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
<th>Cohort 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean change in HBsAg (Log_{10} IU/mL)</td>
<td>−0.51</td>
<td>−0.51</td>
<td>−0.92</td>
<td>−1.01</td>
</tr>
<tr>
<td>Week 12, n</td>
<td>14</td>
<td>15</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Mean change in HBsAg (Log_{10} IU/mL)</td>
<td>−1.39</td>
<td>−1.42</td>
<td>−1.98</td>
<td>−2.05</td>
</tr>
<tr>
<td>Week 24, n</td>
<td>15</td>
<td>15</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Mean change in HBsAg (Log_{10} IU/mL)</td>
<td>−1.89</td>
<td>−2.03</td>
<td>−2.55</td>
<td>−2.30</td>
</tr>
</tbody>
</table>

- 3 patients lost HBsAg (started low) and most below 100 IU/mL
- **Combination better but no advantage to ‘lead-in’ with Ag reduction**
- Perhaps not surprising...Ag reduction may be **more important for adaptive immune response**

Yuen MF AASLD 2021
Approaches to enhance anti-HBV immune response

- Innate immune response
- Cell-mediated immune response
- Antibody/B cell immune response
TLR8 agonist: selgantolimod

24 eAg+ and 24 eAg- NA suppressed → SLGN + NA x 24 weeks with 24 wks post-SLGN f/U

- Greater sAg decline (25%>0.1 log)
- 2/39 (5%) with HBsAg loss – 1 1.5 mg and 1 3 mg
- 3/19 (16%) lost HBeAg

- Modest effect
- Fairly well-tolerated
- Some suggestion of stronger immune activation in those with loss

f/U, follow-up; NA, nucleos(t)ide analog; SLGN, selgantolimod

Gane *Hepatol* 2021 74(4):1737
qHBsAg change on anti-PD1 0.3 mg/kg (Nivolumab)

- PD-1 receptor occupancy 69-88% at wk 6 and up to 85 d in some in 0.3 mg/kg
- 3/24 with > 0.5 log reduction in HBsAg. 1/24 lost HBsAg 24 weeks after injection
- Of 24 patients, 2 with gr1 ALT flares and 1 with gr 3 (lost HBsAg)
- Peripheral T cell response did not consistently increase- higher wk 24 in HBsAg loss

Gane et al J Hepatol 2019
B cell and antibody antiviral functions

Maini and Burton, 2019, Nat Rev Gastro Hepatol
Lenvervimab, a mAb against HBsAg, can induce sustained HBsAg loss in a chronic hepatitis B (CHB) mouse model

**CONCLUSIONS**

Removal of HBsAg by lenvervimab resulted in restoration of HBV immune responses. Sustained HBsAg loss was achieved by elimination of HBV+ hepatocytes. This study provides proof of concept for Ab-based therapies for CHB functional cure.
HBV Cure Approach

- Careful patient selection (low HBV DNA)
- Risks of tissue injury
siRNA + vaccination therapy achieves response off therapy in AAV-HBV transduced mouse model

Core expression in liver (Week 22) HBc\(^+\) = brown

AAV, adeno-associated virus; siRNA, small inhibitory RNA

Michler T, et al. ILC 2018, #PS-025
Key Learnings

• Current goal is functional cure (HBsAg loss, cccDNA/iDNA remains)
  – Achievable based on current therapy

• Novel markers being developed

• Cure strategy will likely involve combination of inhibiting replication, decreasing HBsAg production, and increasing the immune response
Thank you for your attention