Novel approaches towards HBV cure

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The size of the problem: 292 million people worldwide

The Polaris Observatory, *Lancet Gastroenterol Hepatol*. 2018
HBV lifecycle
**Cure: what dose it mean?**

<table>
<thead>
<tr>
<th>CURE</th>
<th>HBsAg</th>
<th>HBV-DNA</th>
<th>HBsAb</th>
<th>cccDNA</th>
<th>Integrated HBV-DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMPLETE CURE</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FUNCTIONAL CURE</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PARTIAL CURE</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**CLINICAL GOAL**

- Disappearance of inflammation
- Prevention of fibrosis progression
- Reversal of fibrosis and cirrhosis
- Prevention of liver failure
- Reduction of the risk of developing hepatocellular carcinoma

- Impossible to achieve, yet
- IFN therapy
- Spontaneous recovery
- NUCs

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Current treatment limitations

Prophylactic vaccine
- No effect on established infection

NUCs
- No effect on cccDNA
- No effect on integrated DNA
- Long term treatment

peg-IFN
- Low efficacy
- Unsuitable in decompensated cirrhosis
- Side effects
The ideal drug

- Able to eradicate ccc-DNA
- No side effects
- Cheap and easy to administer
- Finite treatment
What Are the Barriers to HBV Cure?

- High viral burden
- Persistence of cccDNA
- Weak immune response
- B-cells
- CD8+ T-cells
- PD-1
- Integrated viral genome
HBV lifecycle and possible targets
Preventing HBV entry: Myrcludex B

**Entry inhibitors**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Manufacturer</th>
<th>Action</th>
<th>Peptide</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myrcludex B (bulevirtide)</td>
<td>MYR Pharmaceuticals</td>
<td>Blocks NTCP</td>
<td>Peptide II</td>
<td>2 mg Myrcludex B + IFNα treatment resulted in 40% responders with HBsAg loss observed in 26.7% of the cohort</td>
</tr>
</tbody>
</table>

![Graph showing undetectable HDV RNA, ALT normalization, and combined response over time.](Image)
Preventing HBV entry: Myrcludex B
Preventing HBV entry: CRV431

- A non-immunosuppressive cyclosporine A analogue with potent inhibition of cyclophilin isomerases
- In cellular and in vivo models of HBV infection CRV431 reduced HBV DNA, HBsAg, HBeAg, pregenomic RNA and cccDNA
- CRV431 appeared to be safe and well tolerated in humans

Trepanier et al, *Pharmaceutics*. 2019
Armas et al, *J Hepatol*. 2019
## Targeting cccDNA

### A. cccDNA Minichromosome

- **Designer Nucleases (Indels)**
  - ZFN, TALEN & CRISPR/Cas9
  - **Targeted Mutagenesis**
  - Translation of non-functional protein, inhibition of viral replication
- **Active**
- **Inactive**

### B. Epigenetic Modification

- **ZF-DNMT3A, HDAC inhibitors & methyltransferases**
- **Targeted Epigenetic modification**
- Inhibition of viral gene transcription and replication

## Comparison of ZFN, TALEN, and CRISPR/Cas9

<table>
<thead>
<tr>
<th>Feature</th>
<th>ZFN</th>
<th>TALEN</th>
<th>CRISPR/Cas9</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DNA binding domain</strong></td>
<td>- Individual ZF proteins recognise nucleotide triplets&lt;br&gt;- Typically arranged in arrays of three to four ZFs&lt;br&gt;- Heterodimers&lt;br&gt;- Targets 18–24 bp</td>
<td>- Individual TALE monomer RVDs recognise a single nucleotide&lt;br&gt;- Modular assembly of TALE repeats&lt;br&gt;- Heterodimers&lt;br&gt;- Targets ~40 bp</td>
<td>- Single complementary guide RNA&lt;br&gt;- Requires PAM and tracrRNA&lt;br&gt;- Targets ~20 bp</td>
</tr>
<tr>
<td><strong>Nuclease domain</strong></td>
<td>- FokI endonuclease fusion protein</td>
<td>- FokI endonuclease fusion protein</td>
<td>- PAM-dependent Cas protein</td>
</tr>
<tr>
<td><strong>Advantages</strong></td>
<td>- Naturally occurring mammalian proteins&lt;br&gt;- Easily assembled, highly specific</td>
<td>- Naturally occurring mammalian proteins&lt;br&gt;- Easily assembled, highly specific</td>
<td>- Very easily synthesized and assembled</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>- Require arduous context-dependent assembly</td>
<td>- Large size limits packaging of both heterodimers into a single delivery vector</td>
<td>- Higher potential for off-target cleavage, large Cas proteins limit packaging into delivery vectors</td>
</tr>
<tr>
<td><strong>Cleavage (%)</strong></td>
<td>- No</td>
<td>- Yes (12–35%) [45,46]</td>
<td>- Yes (10–91%) ** [49,51,54,58,63]</td>
</tr>
<tr>
<td><strong>Reduction (%)</strong></td>
<td>- No</td>
<td>- Yes (60%) [46] ***&lt;br&gt;- No</td>
<td>- Yes (35–80%) ** [50,54–56,59,63] [52,64] ***</td>
</tr>
<tr>
<td><strong>Alternative effector domain</strong></td>
<td>- DNMT3a–catalytic methylation [79]&lt;br&gt;- KRAB-transcriptional repressor [80]</td>
<td>- KRAB-transcriptional repressor [81]</td>
<td>- Cas9 nickase-RGN heterodimer (targets ~40 bp) [51,57]</td>
</tr>
</tbody>
</table>

* Varying methods of introducing the NTCP receptor into HepG2 cells. ** Results from single and/or multiple gRNAs. *** Incorporates co-administration of NAs.

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Bloom, Genes. 2018
Targeting cccDNA

- Mouse model
- Small molecule ccc_R08
- Orally administered

Wang et al, ILC 2019
Targeting viral gene expression: transcriptional control

• Reducing expression of viral antigens and regulatory proteins by silencing cccDNA transcription

  • Modification of epigenetic control of cccDNA using general epigenetic modifiers → significant adverse events

• Specifically targeting viral factors involved in the regulation of cccDNA transcription

  • Pevonedistat, an NEDD8-activating enzyme inhibitor
  • Nitazoxanide, a thiazolide anti-infective agent
  • GS-5801
Targeting viral gene expression: siRNA e ASO

- Reducing expression of viral antigens and regulatory proteins by degrading viral RNA

<table>
<thead>
<tr>
<th>Translation inhibitors</th>
<th>mRNA degradation</th>
<th>siRNA</th>
<th>ASO</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>JNJ3989 Janssen</td>
<td>mRNA degradation</td>
<td>siRNA</td>
<td>II</td>
<td>Most patients had HBsAg levels &lt;100 IU mL⁻¹ after 3 doses. Range of 1.3–3.8 (at nadir) log decrease in HBsAg levels 55</td>
<td></td>
</tr>
<tr>
<td>ARB-1467 Arbutus</td>
<td>mRNA degradation</td>
<td>siRNA</td>
<td>II</td>
<td>7 of 11 patients had &gt;1 log decrease in HBsAg levels after 10 weeks of dosing (responders). Biweekly dosing better than monthly dosing 173</td>
<td></td>
</tr>
<tr>
<td>GSK3389404 GlaxoSmithKline</td>
<td>mRNA degradation</td>
<td>ASO</td>
<td>II</td>
<td>Safe and well tolerated in healthy volunteers 174</td>
<td></td>
</tr>
</tbody>
</table>

Fanning et al, Nat Rev Drug Discov. 2019
RNA interference

1. dsRNA is processed by DICER
2. RISC is assembled
3. RISC processes dsRNA
4. RISC, ready to target mRNA
5. Cutting of target sequences
6. Target mRNA is silenced

Infected hepatocyte

Target gene mRNA / viral mRNA
Target gene DNA

A. Lombardi

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## Targeting capsid assembly

### Virus particles level (in association with NUCs) → HBV-specific immune response

<table>
<thead>
<tr>
<th>Capsid assembly inhibitors</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABI-H0731</strong></td>
<td>Assembly</td>
<td>Core binding</td>
<td>Small molecule</td>
<td>II</td>
</tr>
<tr>
<td><strong>JNJ6379</strong></td>
<td>Janssen</td>
<td>Core binding</td>
<td>Small molecule</td>
<td>II</td>
</tr>
<tr>
<td><strong>JNJ0440</strong></td>
<td>Janssen</td>
<td>Core binding</td>
<td>Small molecule</td>
<td>I</td>
</tr>
<tr>
<td><strong>GLS4</strong></td>
<td>HEC Pharma</td>
<td>Core binding</td>
<td>Small molecule</td>
<td>II</td>
</tr>
<tr>
<td><strong>RO7049389</strong></td>
<td>Roche</td>
<td>Core binding</td>
<td>Small molecule</td>
<td>II</td>
</tr>
<tr>
<td><strong>AB-506</strong></td>
<td>Arbutus</td>
<td>Core binding</td>
<td>Small molecule</td>
<td>I</td>
</tr>
</tbody>
</table>
Preventing HBsAg release

- HBsAg is the most abundant circulating viral antigen
  - Inhibits innate and adaptive immunity
  - Exhausts the HBsAg specific B- and T-cell responses

- Nucleic acid polymers (NAPs)
  - Broad spectrum viral attachment/entry inhibitors whose activity increases with polymer length and with increased amphiphatic (hydrophobic) character
  - Naturally accumulate in the liver and enter into hepatocytes

SVP: sub-viral particles

Vaillant, Antiviral Research. 2016
Preventing HBsAg release

**HBsAg secretion inhibitors**

<table>
<thead>
<tr>
<th>REP 2139 and Replicor</th>
<th>At the end of the trial, 60% of patients had HBsAg loss (53% had HBsAg loss at 24 weeks and 50% had HBsAg loss at 48 weeks). Anti-HBs antibodies were detectable in 56% of patients at 48 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>REP 2165</td>
<td>Nucleic acid-based polymer</td>
</tr>
<tr>
<td>HBsAg binding</td>
<td></td>
</tr>
</tbody>
</table>

**Outcome after removal of all therapy**

- **Viral rebound**
  - HBV DNA + 2000IU normal ALT
- **Inactive HBV**
  - HBV DNA, HBsAg TTN
- **Functional cure**
  - HBV DNA, normal ALT

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Bazinet et al, ILC 2019.
Fanning et al, Nat Rev Drug Discov. 2019

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What Are the Barriers to HBV Cure?

- High viral burden
- Persistence of cccDNA
- B-cells
- CD8+ T-cells
- Weak immune response
- PD-1
- Integrated viral genome
- Persistence of cccDNA
Immune-based strategies
# Boosting the innate immunity

<table>
<thead>
<tr>
<th><strong>Innate immunity activators</strong></th>
<th><strong>Company</strong></th>
<th><strong>Description</strong></th>
<th><strong>Phase</strong></th>
<th><strong>Results</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Inarigivir</td>
<td>Springbank</td>
<td>RIG-I agonist and polymerase inhibitor</td>
<td>II</td>
<td>Dose-dependent decrease in HBV DNA levels (1.54 log decrease with 200 mg). After switch to TDF, 88% of participants had DNA levels below the LOQ</td>
</tr>
<tr>
<td>RO7020531</td>
<td>Roche</td>
<td>TLR7 agonist</td>
<td>I</td>
<td>Immune activation observed in all patients. No viral data reported</td>
</tr>
<tr>
<td>GS-9620</td>
<td>Gilead</td>
<td>TLR7</td>
<td>II</td>
<td>No change in HBsAg levels. Transient dose-dependent induction of ISG15 and change in NK cell and T cell phenotype observed</td>
</tr>
<tr>
<td>GS-9688</td>
<td>Gilead</td>
<td>TLR8</td>
<td>I</td>
<td>Dose-dependent IL-12 and IL-1β production noted in healthy volunteers</td>
</tr>
</tbody>
</table>

Boosting the innate immunity: GS-9620

Li et al., J. Hepatol. 2018

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Boosting the adaptive immunity

<table>
<thead>
<tr>
<th>Adaptive immunity activators</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TG-1050 (T101)</td>
<td>Transgene/Talsy</td>
<td>Vaccine</td>
<td>Ad5 delivery</td>
</tr>
<tr>
<td>HepTcell</td>
<td>Alimmune</td>
<td>Vaccine</td>
<td>Peptide plus IC31 (adjuvant)</td>
</tr>
</tbody>
</table>

HBV-specific T cells are rare

HBV-specific T cells are exhausted

Low efficacy of attempts to boost HBV-specific response

High levels of HBsAg


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Recovering adaptive immunity: HBsAg blockade

A

Infected hepatocyte

HBV envelope proteins

HB virion release

HBsAg-mediated immunosuppression

Impaired
Ag-specific B-cell responses
Ag-specific T-cell proliferation
Cytokine signaling

B

Infected hepatocyte

HBV envelope proteins

HBV envelope proteins

HBs-humAbs

HBsAg-mediated immunosuppression is removed

Restored
Ag-specific B-cell responses
Ag-specific T-cell proliferation
Cytokine signaling

Long term control of HBV infection

Recovering the adaptive immunity: lenvervimab

BACKGROUND & AIMS

• Sustained loss of HBsAg is regarded as a marker for functional cure in HBV.
• As HBsAg is known to suppress HBV immune responses, this study hypothesized that HBsAg removal could result in immune response restoration.

METHODS

• Therapeutic potential of surrogate lenvervimab (sLenvervimab) was evaluated in an HDI-based 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.
Checkpoint inhibitors for immune-exhaustion

Lombardi & Mondelli, *Aliment Pharmacol Ther*. 2019
Reversing the immune exhaustion: theory

**WOODCHUCK MODEL**

**EX-VIVO**

<table>
<thead>
<tr>
<th>Condition</th>
<th>CD8</th>
<th>CD4</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV-DNA≤1x10^6 copies/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV-DNA&gt;1x10^6 copies/mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fisicaro et al, Gastroenterology. 2010**

**Liu et al, PLoS Pathog. 2014**

- **C** Control
- **E** ETV only
- **ED** ETV in combination with DNA vaccinations
- **EDA** ETV and DNA vaccination in combination with anti-PDL1 antibody
Reversing the immune exhaustion: reality

Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial

PS-044
A phase 1 study evaluating anti-PD-1 treatment with or without GS-4774 in HBeAg negative chronic hepatitis B patients

reductions in HCV RNA. No patients had reactivation of HBV, and no instances of anti-HBs seroconversion were noted among patients infected with HBV.

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El-Khoueiry, Sangro et al, The Lancet. 2017

Gane et al, J Hepatol. 2017
A multi-step strategy?
Conclusions

• With current treatment only a scarce minority reach functional cure
• New treatments should aim to complete cure
• Best approaches should target both viral and host elements
• Results interesting but presently far from applicability
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