Viral and Immune Targets to cure HBV infection

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The global burden of chronic HBV infections

HBsAg prevalence
- High ≥ 8%
- Intermediate 2% to 7%
- Low < 2%

HCC prevalence

Flowchart:
1. HBV susceptible
2. Acute HBV
3. Chronic HBV
4. Cirrhosis / HCC

Treatment options:
- Vaccine
- Universal precautions
- Antiviral treatment

From A Lok, AASLD 2016
Current treatments: virus suppression and sustained disease control
(Why not treating more patients?)

- Decreased inflammation/fibrosis
- Decreased progression
- Reversal of fibrosis
- Decreased progression
- Decreased incidence but not eliminated
- HBsAg loss rate Max 10% after 5 years
- Life-long therapy

Barriers to eradicating HBV

- **cccDNA reservoir**
  - Long t1/2
  - Continuous replenishment
  - Not affected by NAs and IFN

- **Integrated forms**
  - HBV persistence

- **Defective CD8+ responses**
- **Defective B cell responses**
- **Inefficient innate response**
- **Defective immune responses**

Goals of future therapies to cure HBV infections

**Therapy**
- HBV DNA change from baseline (log $10$ c/mL)
  - $0.0 - 1.0$
  - $2.0 - 3.0$
  - $4.0$

**Time**
- HBsAg
- Partial Cure
- Functional Cure
- Virus suppression
- +/- Anti-HBsAb
- Sterilizing Cure

**SERUM**
- Anti-HBsAb

**LIVER**
- cccDNA

Cure

Access to care

Clinical development

Biomarkers

Antivirals

Immunotherapy

Drug Discovery
Curative approaches: Targeting the pool of cccDNA

Entry inhibitors
- Enveloped RC-DNA virion
- Inhibitors of HBsAg release

Controlling viral replication: Pré- and Post-cccDNA targets

Antiviral approaches

Immunomodulatory approaches

Targeting cccDNA

Targeting HBx

RNA interference

CpAMs “Capsid inhibitors”

NAs “Polymerase inhibitors”

Specific hepatocyte killing

Innate immunity modulation
- Toll-like receptor agonist
- RIG-1
- STINGs

Virus neutralization

Adaptive immunity modulation
- Anti-PD-1 mAb
- TCR engineering
- Vaccine therapy

Specific hepatocyte killing

Dysfunctional T-cell response

Insufficient B-cell response

Curing hepatocytes

IFNs and other antiviral cytokines

IL-12

pDC

NK

NKT

IL-1β

KC

IL-6

CpAM: core protein allosteric modulators; HBx: hepatitis B X protein; IFN: interferon; IL: interleukin; KC: Kupffer cells; mAb: monoclonal antibody; NA: nucleos(t)ide analogue; NK: natural killer; NKT: natural killer T cell; pDC: plasmacytoid dendritic cell; PD-1: programmed cell death-1; TCR: T cell receptor

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
HBV conference, Taormina 2018

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)

**BACKGROUND & AIMS**
- Myrcludex B (MyrB, Bulevirtide) is a first-in-class entry inhibitor for HBV/HDV infection
- In a phase 2 study MYR202, MyrB monotherapy led to HDV RNA decline and improvement of ALT levels
- End-of-treatment data from a MyrB ± PegIFNα2a 48 weeks combination study (MYR203) have been reported
- Here, the 24-week treatment-free follow-up data are presented

**METHODS**
- Primary endpoint: undetectable serum HDV RNA at Week 72 (w72)
- Secondary endpoints: ALT normalization, combined treatment response*, and HBsAg reduction >1 log₁₀

*≥2 log serum HDV RNA decline + normal ALT levels.
**Median HDV RNA levels**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median log reduction</th>
<th>Week 48</th>
<th>Week 72</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-IFNα</td>
<td>-1.30</td>
<td></td>
<td>-0.26</td>
</tr>
<tr>
<td>2mg MyrB + PEG-IFNα</td>
<td>-4.81</td>
<td></td>
<td>-4.04</td>
</tr>
<tr>
<td>5mg MyrB + PEG-IFNα</td>
<td>-5.59</td>
<td></td>
<td>-1.48</td>
</tr>
<tr>
<td>2mg MyrB</td>
<td>-2.84</td>
<td></td>
<td>-1.08</td>
</tr>
</tbody>
</table>

**Primary endpoint:**
undetectable HDV RNA at week 72

**Two-tailed Fisher’s Test**
- * p = 0.0209
- ** p = 0.0022
Pros & Challenges for entry inhibitors

Inhibition of new rounds of infection

Decrease the pool of cccDNA on the long term

Opportunity to treat HBV/HDV co-infections

Effect on NTCP and elevation of bile salts

Slow kinetics of cccDNA decay and slow hepatocyte turn-over; which combination with other DAAs?
Different classes of capsid assembly modulators

Heteroaryldipyrimidine derivatives (HAP)

Phenylpropenamide derivatives (AT series)

Compounds in evaluation

BAY41-4109
HAP-12
AT-130
NVR3-778
JNJ-6379
RO7049389
ABI-H0731
ABI-H0808
GLS4
GLP26
HAP_R01
SBA_R01
AB-423
AB-506
EP-027367

CpAMs inhibit viral genome replication and prevent cccDNA formation when administered prior to HBV inoculation.

CpAMs “Capsid inhibitors”

NUCs
“Polymerase inhibitors”

Capsid Assembly Modulators (CAMs)

JNJ-6379 PO OD x 28 d in non-cirrhotic HBeAg+ and HBeAg- CHB

- Well tolerated at increased dose
- Potent HBV DNA suppression with limited dose response at higher dose
- Higher dose may be required to prevent cccDNA replenishment
- Ongoing Phase 2 trial

No effect on HBeAg or HBsAg levels

Zoulim et al AASDL 2018, Abstract 74
Pros & challenges for CpAM

Decrease the pool of cccDNA on the long term

Other MoA?

Opportunity to combine with NUCs, pegIFN, other DAAs and immune interventions

Oral administration

Long-term safety profile

Mainly suppressive

How to combine with other approaches to be curative?
siRNA Candidate Development

- Lipid Nanoparticles for IV infusion
- GalNAc-Conjugate for subcutaneous administration

Wooddell et al, Science Transl Med, 2017
All patients receiving 3 monthly doses have achieved > 1 log reduction in HBsAg

<table>
<thead>
<tr>
<th>Mean HBsAg reductions from baseline</th>
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<tbody>
<tr>
<td>100 mg (C2b)</td>
</tr>
<tr>
<td>200 mg (C3b)</td>
</tr>
<tr>
<td>300 mg (C4b)</td>
</tr>
<tr>
<td>400 mg (C5b)</td>
</tr>
<tr>
<td>300 mg E+</td>
</tr>
<tr>
<td>NUC naive (C9)</td>
</tr>
<tr>
<td>300 mg E+</td>
</tr>
<tr>
<td>NUC exp (C9)</td>
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Shorter dosing intervals do not accelerate HBsAg decline

<table>
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<tr>
<th>Monthly dosing intervals</th>
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<td>Mean HBsAg reductions from baseline</td>
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- JNJ-3989 rapidly reduces HBsAg to thresholds possibly associated with improved chances of HBsAg seroclearance in many patients, even after only 3 doses
  - 88% of patients achieved HBsAg <100 IU/mL
  - 100% of patients achieved ≥ 1.0 Log10 IU/mL HBsAg reduction

MF Yuen, et al, EASL ILC 2019
Pros & challenges for siRNA

Decrease of HBsAg
Potential for immune restoration?
Opportunity to combine with NUCs, pegIFN and other DAAs or immunotherapeutic approaches?

Parenteral administration
Long-term safety profile
Mainly suppressive
Impact of integrated sequences
How to combine with other approaches to be curative?

Other technologies under investigation

**Antisense OGN:** Billioud et al, J Hepatol 2016
**Locked Nucleic Acids:** Javanbakht et al, Mol Ther Nucleic Acids. 2018
**RNA destabilizers:** Mueller er al, J Hepatol 2018, Zhou et al, Antiviral Res 2018
Nucleic acid polymers (NAPs)

- **Nucleic Acid Polymers (NAP)** have entry and post-entry antiviral effects in HBV infection *in vitro.*
  
  *Noordeen, F et al. AAC. 2013*

**Inhibition of HDV entry by preventing attachment of the virus to cell surface glycosaminoglycans**

*Belstein et al, J Virol 2018; Quinet et al, Hepatology 2018*
Nucleic Acid Polymers (NAPs) – Reducing HBsAg

- NAPs block assembly/release of subviral particles
- Aim to restore immune response → viral control

Marked and seemingly durable HBsAg loss & gain of anti-HBs
Interesting…need to confirm ALT flares due to immune activation → plan for Phase 2 ACTG trial to clarify

Bazinet et al, AASLD 2018, Abstract 393
Pros & challenges for NAPs

Decrease of HBsAg

Immune restoration?

Opportunity to combine with NUCs, pegIFN and other DAAs

Mode of action under investigation

IV infusion

ALT exacerbation

Long-term safety profile
Restoration of antiviral immunity

The Oral Toll-Like Receptor-7 Agonist GS-9620 in Virally suppressed Patients with Chronic HBV Infection

Janssen et al, Journal of Hepatology, 2017
Inarigivir (RIG-I agonist) – a novel approach with dual antiviral activity

- Dose-dependent decline in HBV DNA & HBV RNA > in HBeAg-neg patients and those with low qHBsAg levels
- HBV RNA effects persisted after cross-over to tenofovir – ‘new set-point’? **Interesting proof-of-concept**

Yuen et al, AASLD 2018, Abstract 75
Inarigivir Acts Through Modulation of the Innate Immune System Involving RIG-I

**Novel mechanism of action**

- Actively transported into hepatocytes via OATP-1 and OAT-1 with 30:1 liver to plasma ratio
- Binds to RIG-I and causes induction of IFN signaling
- Demonstrated activation of immune system in HCV patients and healthy volunteers at 400mg daily
- DAA effect to prevent interaction of HBV Pol and pgRNA in cell systems
- Active against polymerase and capsid resistant strains
- Activates “host” targets instead of viral targets – potential for higher barrier to viral resistance

ACHIEVE Phase 2 Dose Escalation Study

Inarigivir monotherapy 12 weeks followed by switch to Tenofovir 300 mg for 12 weeks

<table>
<thead>
<tr>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
<th>Cohort 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inarigivir - 25 mg</td>
<td>Inarigivir - 50 mg</td>
<td>Inarigivir - 100 mg</td>
<td>Inarigivir - 200 mg</td>
</tr>
<tr>
<td>Placebo</td>
<td>Tenofovir 300 mg daily</td>
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Up to 80 non-cirrhotic HBV subjects, randomized 4:1 between inarigivir and placebo.

All patients switch to tenofovir 300 mg monotherapy.
**Primary Endpoint:** Mean Change from Baseline in HBV DNA to Week 12 in Placebo (PL) and IRIG cohorts

**Secondary Endpoint:** Mean Change from Baseline in HBV RNA to Week 12 in Placebo (PL) and IRIG cohorts

**HBeAg negative patients:** Change from Baseline in HBV DNA at Week 12 and Week 24

- **P < 0.01:** IRIG 100mg and 200mg versus PL
- **18 of 22 (82%) patients undetectable at week 24**

**HBeAg negative patients:** Change from Baseline in HBV RNA at Week 12 and Week 24

- **P = 0.05:** All cohorts combined versus PL at week 12
- **3 placebo and 6 IRIG undetectable HBV RNA at baseline. 1 placebo became replicative and detectable at week 12**

**Inarigivir ACHIEVE trial in chronic HBV patients**
PD-1 blockade enhances HBV-specific T cell function

In liver and blood

With differential impact based on HBeAg status

Fisicaro P; Gastroenterol 2010; 138: 682

Park J, Gastroenterol 2016; 150: 684
Randomized phase II study of GS-4774 as a therapeutic vaccine in virally suppressed patients with chronic hepatitis B

GS-4774 is a heat-inactivated, yeast-based, T-cell vaccine designed to elicit HBV-specific T-cell responses

Lok et al, J Hepatol 2016
A Phase 1 Study Evaluating Anti-PD-1 Treatment With or Without GS-4774 in HBeAg Negative Chronic Hepatitis B Patients

![Graph showing treatment outcomes](image)

- Virally-suppressed, HBeAg negative CHB patients (single center New Zealand)

- 2/22 (9%) at Week 12 and 3/22 (14%) at Week 24 with a >0.5 log10 reduction in HBsAg

Gane et al, EASL ILC 2017 PS-044
Curative approaches: Targeting the pool of cccDNA

Entry inhibitors
- Inhibitors of HBsAg release

Controlling viral replication:
- Post-cccDNA targets

Antiviral approaches
- Targeting cccDNA
- Targeting HBx
- RNA interference

Immunomodulatory approaches
- CpAMs “Capsid inhibitors”
- NAs “Polymerase inhibitors”

Curing hepatocytes
- IFNs and other antiviral cytokines
- Innate immunity modulation
  - Toll-like receptor modulation
  - RIG-I
  - STINGs
- Adaptive immunity modulation
  - Anti-PD-1 mAb
  - TCR engineering
  - Vaccine therapy
- Specific hepatocyte killing
- Virus neutralization

CpAM: core protein allosteric modulators; HBx: hepatitis B X protein; IFN: interferon; IL: interleukin; KC: Kupffer cells; mAb: monoclonal antibody; NA: nucleos(t)ide analogue; NK: natural killer; NKT: natural killer T cell; pDC: plasmacytoid dendritic cell; PD-1: programmed cell death-1; TCR: T cell receptor

Towards combined therapies

- **Combination of CpAm + RNA destabilizer + NUC**
  
  HDI mouse model
  
  Gindin Y, et al. ILC 2018, #3503 (PS-027)

- **Combination of CpAM and TLR-7 agonist**
  
  AAV-HBV mouse model
  
  Gao L, et al. ILC 2018, #4008 (PS-028)

- **RIG-I agonist (Inarigivir) and NUCs**
  
  Clinical trial
  
  Walsh R, et al. ILC 2018, #2694 (PS-160)

- **SiRNA followed by therapeutic vaccination**
  
  AAV-HBV mouse model
  
  Michler T, et al. ILC 2018, #1044 (PS-025)
HBV cure - New treatment concepts – Will we need combination of DAA and immune therapy?

- Antivirals
- Therapy
- Immune restoration
- Check point inhibitors
- TLR agonist
- Tx Vaccine
- NUC
- Capsid
- SiRNA Ag load
- HBVDNA
- HBsAg
- Anti-HBsAb
- cccDNA

Testoni et al, Liver International 2017
Need for Novel Biomarkers to Assess Target Engagement and Treatment Endpoints

Innovations and novel perspectives for cure

- cccDNA biology and targeting strategies

  Koh et al, Gastroenterology 2018

  Gao et al, EASL ILC 2019

T cell engineering and immunotherapy

Koh et al, Gastroenterology 2018
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