Novel targets for HBV therapy

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Chronic Hepatitis B (CHB) - a global health problem
from viral suppression to cure

- 240 million CHB worldwide
- 1.7 million CHB treated worldwide
- Hepatocellular Carcinoma (HCC) : 2\textsuperscript{nd} cause of cancer death worldwide

![Liver and Blood Diagram]

UNTREATED

![Normal, Cirrhosis, Hepatocellular carcinoma Diagram]
Chronic Hepatitis B (CHB) - a global health problem
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**Untreated**

Liver

Blood

HBV-DNA

Rc-DNA

ccc-DNA

HBsAg

Risk of HCC reduced (after 5 yrs) but not eliminated

**NUCs**

Liver

Blood

Normal

Cirrhosis

Hepatocellular carcinoma

Normal

Cirrhosis

Hepatocellular carcinoma
Chronic Hepatitis B (CHB) - a global health problem
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**UNTREATED**

**NUCs**

**NMEs**

"Cure"

Direct Antiviral Agents (DAAs)

Immunomodulatory strategies
Definition of HBV cure: what do we want to achieve?

Lok et al, Hepatitis B Cure: From Discovery to Regulatory Approval; Hepatology / J Hepatol joint publication; 2017
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

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Current available therapies inhibit complete virion formation and release, but are not able to eliminate cccDNA → no real « cure » of the infection

A few copies of cccDNA per liver can (re)initiate a full-blown infection
Viral Targets under investigation

- Entry inhibitors
- Egress Inhibitors
- Targeting cccDNA
- Core inhibitors
- RNA interference
- Polymerase/RNAsH inhibitors
Entry/egress 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*

**Nucleic Acid Polymers (NAP)**
*Noordeen, F et al. AAC. 2013*

Effect on HBV/HDV co-infection
Opportunity to combine
Long term effect on cccDNA pool? HBsAg?
HBV Serum DNA-levels decline during Myrcludex B treatment

⇒ HBV DNA levels decline significantly during Myrcludex B treatment in all groups.
⇒ Pronounced effects by > 1log in 6/8 patients were observed in the 10 mg dosing group.
⇒ 7/40 showed > 1log HBV reduction in lower dosing groups.

S Urban Heidelberg U & MyrGmbH, AASLD 2014
NAPs ± TDF and pegIFNa2a in treatment naïve HBeAg(-) CHB patients

REP 2139-Mg or REP 2165-Mg used in combination with TDF and peg-IFN alpha-2a in treatment-naive Caucasian patients with chronic HBeAg-negative HBV

Serum HBsAg (A), anti-HBs (B), and HBV DNA (C) dynamics in the REP 401 protocol

Bazin et al. EASL 2017, Amsterdam. #THU-154
## Challenges of entry/egress inhibitors

<table>
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<tr>
<th>Myrcludex</th>
<th>NAPs</th>
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<td>SC administration</td>
<td>Mode of action still under investigation</td>
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<td>IV infusion</td>
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Myrcludex:
- SC administration
- Inhibition of NTCP and increase of bile salts
- Slow kinetics of cccDNA decay and slow hepatocyte turn-over; which combination with other DAAs?

NAPs:
- Mode of action still under investigation
- IV infusion
- ALT exacerbation
- Long-term safety profile
Capsid assembly modulators (CAMs)

Inhibition of nucleocapsid entry into the nucleus
Inhibition of encapsidation
Inhibition of HBeAg secretion?
Different classes of CAMs

Heteroaryldipyrimidine derivatives (HAP)

Phenylpropenamide derivatives (AT series)

Compounds in evaluation
BAY41-4109
HAP-12
AT-130
NVR3-778
JNJ-379
ABI-H0731
ABI-H0808
GLS4JHS
HAP_R01
SBA_R01
AB-423

Phase 1b clinical trial of JNJ-379

Field:

- Pooled placebo (n=8)
- JNJ-379 25 mg QD (n=8)
- JNJ-379 75 mg QD (n=8)

HBV DNA Change from Baseline

Time (weeks)

0 1 2 3 4

HBV DNA change from baseline (log₁₀ IU/mL)

0 -1 -2 -3

Shown are mean values ± SD

* and *** refer respectively to 1 and 3 patients with HBV DNA <LLOQ of the HBV DNA assay

Zoulim et al, AASLD 2017
Pros & challenges for CAMs

**Decrease the pool of cccDNA on the long term**

**Opportunity to combine with NUCs, pegIFN and other DAAs**

**Oral administration**

**Long-term safety profile**

**Mainly suppressive**

**How to combine with other DAAs to be curative?**
cccDNA targeting or functional silencing
siRNA Candidate Development

- Contains a hepatocyte targeted, reversibly masked membrane active peptide (NAG-MLP)
- Endosomal release of two synthetic siRNAs
- PEG modification to inhibit membranolytic activity

ESC-GalNAc-Conjugate for subcutaneous administration

Journal of Controlled Release, Volume 209, 2015, 57–66
Decreased serum HBsAg levels in patients receiving ARC-520 every 4 weeks with daily entecavir

Impact of integrated sequences on siRNA efficacy
Will the decrease of viral antigen load result in restoration of immune responses?

Yuen MF et al, EASL ILC 2017; Wooddell, Science Trans Med 2017
Pros & challenges for siRNAs

Decrease of HBsAg
Possibility of immune restoration?
Opportunity to combine with NUCs, pegIFN and other DAAs
Combination with immunotherapeutic approaches?

IV infusion
Long-term safety profile
Mainly suppressive
Impact of integrated sequences
How to combine with other DAAs to be curative?

As of November 2016, the NAG-MLP containing drug platform was discontinued due to animal toxicology findings, not due to safety signals in humans. New formulations are being evaluated.
Direct cccDNA targeting

IFNalpha /Lymphotoxin beta induced APOBEC3A/B dependent degradation ; other cytokines
Lucifora et al, Science 2014; Xia et al, Gastroenterology 2015

CRISPR/cas9 cleavage

cccDNA silencing through virus specific mechanisms
Belloni et al, JCI 2012; Liu et al, Plos Path 2013; Tropberger et al, PNAS 2015
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Immune Targets under investigation

1. Therapeutic vaccines
2. Blockade of immunosuppressive pathways
3. Stimulation of innate immunity
4. Engineering of T cells

Check-point inhibitors

TLR agonists
The Oral TLR-7 Agonist GS-9620 in CHB patients

Gane et al, Journal of Hepatology, 2015
PD-1 blockade enhances HBV-specific Tcell response

In liver and blood

- LIVER
  - $\Delta%\text{IFN-}\gamma+/\text{CD8+CD3+}$
  - $P = 0.035$
  - $P = 0.02$

- BLOOD
  - $\Delta%\text{IFN-}\gamma+/\text{CD4+CD3+}$
  - $P = 0.028$
  - $P = 0.01$

With differential impact based on HBeAg status

<table>
<thead>
<tr>
<th>Antigen</th>
<th>S</th>
<th>Core</th>
<th>RT2</th>
<th>Flu</th>
</tr>
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<tbody>
<tr>
<td>$\alpha\text{PDL1}$</td>
<td>0.048</td>
<td>0.01</td>
<td>0.008</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Stimulation Index

HBeAg status

- Park J, Gastroenterol 2016; 150: 684

Fisicaro P, Gastroenterol 2010; 138: 682
Phase 1 anti-PD-1 with or without GS-4774 in CHB patients

Gane et al, EASL ILC 2017 PS-044
Pros & challenges for immunemodulators

- Induction of ISG
- Restoration of adaptive immunity
- Possibility of combination with NUCs, pegIFN
- Combination with other DAAs or other immunotherapeutics

Not effective in humans (vs animal models)

Side effects: potential for cytokine storm/autoimmunity
Conclusions

Combination therapies required
Direct cccDNA targeting remains a priority
Biomarkers in evaluation to assist drug development

Dane particles

cir HBV-RNAs

HBsAg

HBcrAg

NUCs

exclusive

CAMs

subgenomic RNAs

cccDNA drugs

integrated sequences

Testoni et al, Sem Liver Dis, 2017
Open questions

HBsAg clearance is an **endpoint of therapy**

Decline in HBsAg levels may **restore the antiviral activity of exhausted T cells? Other factors?** *(Fisicaro et al, Nat Med 2017; Schurich, Cell Rep 2016)*
Open questions

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**HBV integration?**

In HBeAg(-) patients, HBsAg mainly comes from integrated sequences

Lebossé, Testoni et al J Hep 2017
Wooddell et al., Sci Transl Med 2017

HBV integration and clonal expansion of hepatocytes found in all CHB phases (major risk factor for HCC)

Mason et al, Gastroenterology 2016
Open questions

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**Early treatment intervention?**
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