Current and future approaches for the treatment of HBV

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Natural history of HBV infection

WONG and LOK, Arch Intern Med 2006;166:9-12
What are the endpoints of therapy in chronic hepatitis B?

• To decrease the viral load, the number of infected cells and the associated inflammation, in order to prevent liver disease progression to cirrhosis and HCC, and death

• Ideally, to eradicate HBsAg, i.e. to achieve a sterilizing cure (infrequent)

• More realistically, to reach a serological profile comparable to that of an inactive carrier:
  – In HBeAg+: seroconversion to anti-HBe
  – In anti-HBe+: persistent abatement of viral load

EASL Clinical Practice Guidelines, *J Hepatol* 2012;57:167-185
Cumulative incidence of HCC according to HBV DNA
A prospective study (REVEAL-HBV Study)
(n=3653; mean FU 11.4 years; 41,779 person-years)

*Subgroup of patients with HBeAg-, normal ALT, no cirrhosis at enrolment (n=2925)
Whom to treat, in 2016

Patients in the immunoactive phase
- HBeAg+, VL > 2,000 UI/mL, elevated ALT
- HBeAg-, VL > 2,000 UI/mL, elevated ALT (but fluctuating!)

Inactive carriers (HBeAg-, VL < 2,000 UI/mL, persistently normal ALT)
- In case of immune-suppressive therapy, to prevent viral reactivation

Immune tolerant patients (HBeAg+, VL > 6 log UI/mL, normal ALT)
- If family history of cirrhosis or HCC (Other criteria? In case of sudden VL reduction? If ALT levels in the upper range of normality? All of them?)

Pregnant women
- If VL > 6 log UI/mL, during the last trimester of pregnancy, to prevent transmission to the newborn (together with HBIG and vaccine)

EASL Clinical Practice Guidelines 2012; AASLD guidelines 2015
HUANG et al, JAMA 2014; PERRILLO et al, JAMA 2015; CHAN et al, Gastroenterology 2014
Two classes of drugs

**INTERFERON**
- Immunomodulating and antiviral effect (rarely leads to HBsAg loss)
- Treatment duration is finite
- Safety issues
- Many contraindications

**ANALOGUES**
- Only antiviral effect
- Lead to suppression of HBV, very rarely to eradication
- Require administration for life (risk of relapse at treatment cessation)
Virological and biochemical response rates to current HBV treatments

<table>
<thead>
<tr>
<th></th>
<th>Entecavir¹,²</th>
<th>Tenofovir³</th>
<th>PEG-IFN α-2a⁴,⁵</th>
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</thead>
<tbody>
<tr>
<td><strong>HBeAg positive</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV DNA undetectable</td>
<td>67%</td>
<td>76%</td>
<td>25%⁺</td>
</tr>
<tr>
<td>HBeAg seroconversion</td>
<td>21%</td>
<td>21%</td>
<td>27%</td>
</tr>
<tr>
<td>ALT normalisation</td>
<td>68%</td>
<td>68%</td>
<td>39%</td>
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<tr>
<td>HBsAg loss</td>
<td>2%</td>
<td>3.2%</td>
<td>2.9%⁺</td>
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<tr>
<td><strong>HBeAg negative</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HBV DNA undetectable</td>
<td>90%</td>
<td>93%</td>
<td>63%⁺</td>
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<tr>
<td>ALT normalisation</td>
<td>78%</td>
<td>76%</td>
<td>38%</td>
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<tr>
<td>HBsAg loss</td>
<td>0.3%</td>
<td>0%</td>
<td>0.6%⁺</td>
</tr>
</tbody>
</table>

Results at 48 weeks; ⁺HBV DNA < 400 copies/mL; ⁺⁺At 72 weeks

A 1-year treatment with NUCs leads to a modest decrease of intrahepatic cccDNA (only 5/117 or 4% had undetectable cccDNA)

WONG et al, Clin Gastroenterol Hepatol 2013;11:1004-10
Long-term NUC therapy and fibrosis improvement

**ETV, n=57, median FU 280 weeks**

- Cirrhosis regression: 71/96 (74%)
- Progression to cirrhosis of non-cirrhotics: 3/252 (1.2%)

**TDF, n=348**

- Cirrhosis regression: 71/96 (74%)
- Progression to cirrhosis of non-cirrhotics: 3/252 (1.2%)

CHANG et al, Hepatology 2010;52:886–93

MARCELLIN et al, Lancet 2013;381:468-475
Propensity-score study of HCC prevention by NUC treatment of chronic hepatitis B: a stratified sub-analysis

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Untreated</th>
<th>Treated</th>
<th>HR (95% CI)</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>HCC</td>
<td>N</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
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<tr>
<td>(0, 40]</td>
<td>8945</td>
<td>713</td>
<td>9390</td>
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<tr>
<td>(40,50]</td>
<td>6039</td>
<td>1410</td>
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<tr>
<td>(50,100]</td>
<td>6611</td>
<td>2331</td>
<td>6884</td>
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<td>Gender</td>
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<tr>
<td>Female</td>
<td>4982</td>
<td>976</td>
<td>5281</td>
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<tr>
<td>Male</td>
<td>16613</td>
<td>3478</td>
<td>16314</td>
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<td>Cirrhosis</td>
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<td>Diabetes</td>
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<td>4081</td>
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<td>Yes</td>
<td>1574</td>
<td>373</td>
<td>1590</td>
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<tr>
<td>Total</td>
<td>21595</td>
<td>4454</td>
<td>21595</td>
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</tbody>
</table>

WU et al, Gastroenterology 2014;147:143-51
Cumulative incidence of HCC in 1666 chronic hepatitis B patients treated with entecavir and/or tenofovir, by liver disease severity

Cumulative probability of HCC

Years since ETV/TDF initiation

Number at risk

CHB 1085
Comp. cirrhosis 476
Decomp. cirrhosis 55

0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00

p < 0.001
Prevention of HCC by NUC treatment in advanced HBV-related liver disease is suboptimal

- Reversal of cirrhosis is not universal
- Persistence of additional causes of liver disease (metabolic syndrome, surreptitious alcohol drinking)
- Pre-existing somatic mutations due to long-standing exposure to carcinogens (aflatoxin)
- Persistence of integrated HBV DNA at sensitive sites
- Epigenetic changes (miR602, TFIIH, miR148a...)

(adapted from FARAZI & DEPINHO, Nat Rev Cancer 2006)
Main limitations of current treatments

- **Limited access to care**
- **Not all patients are treated**, especially during the immuno-active phase (minimal hepatitis or non-inflammatory phase are beyond current guidelines)
- **IFN-α is poorly tolerated and has a low response rate**
- **Nucleos(t)ide analogues must be given for life**, potentially leading to the emergence of RAS (especially in case of use of low barrier to resistance drugs in resource poor countries) and safety issues
- **The cccDNA decline rate is very slow** (partly due to continuous replenishment of the pool due to incomplete viral suppression)
- **HBsAg clearance is rare** (although the most desirable endpoint) with potential, continuing effects on adaptive immune response
- **The risk of HCC is not eliminated**, despite viral «response»

MAISON and ZOULIM, Gut 2012; GISH et al, Lancet Infect Dis 2014; AASLD/APASL/EASL guidelines
With current technologies, a sterilizing HBV cure is unlikely.

Antiviral therapy can be stopped
Low risk of spontaneous reactivation

cccDNA is inactivated (but HBV DNA persists integrated)

DURANTEL and ZOULIM, J Hepatol 2016
What about the future?
Target #1: viral entry


Entry inhibitors
Myrcludex (pre-S1 peptide)
NTCP expression supports WMHBV infection of HepG2 cells

ZHONG et al, J Virol 2013;87:7176-7184

NTCP (un)conjugated Na+ taurocholate T3- and T4-sulfate rosvastatin

L form of HBsAg
Target #2: the viral minichromosome (cccDNA)

**cccDNA degradation**
- IFNα or Lymphotixin-β-induced, core-mediated activation of cytidine deaminase APOBEC3A/B
  
  **LUCIFORA et al, Science 2014**

**cccDNA deletions**
- CRISPR/Cas9
  
  **SEEGER et al, Mol Ther Nucleic Acids 2014**

**cccDNA transcription suppression**
- Via inhibition of p300/CBP histone acetyltransferase
  
  **BELLONI et al, Proc Natl Acad Sci USA 2009**
  **TROPBERGER et al, Proc Natl Acad Sci USA 2015**
- Via increased binding of transcriptional repressors to IFN-stimulated RE
  
  **BELLONI et al, J Clin Invest 2012**
- Blocking Smc5/6 degradation
  
  **DECORSIERE et al, Nature 2016**
Specific and Nonhepatotoxic Degradation of Nuclear Hepatitis B Virus cccDNA

Julie Lucifora,¹ ² ² Yuchen Xia,¹ ² Florian Reisinger,¹ Ke Zhang,¹ Daniela Stadler,¹ Xiaoming Cheng,¹ Martin F. Sprinzl,¹,³ Herwig Koppensteiner,¹ Zuzanna Makowska,⁴ Tassilo Volz,⁵ Caroline Remouchamps,⁶ Wen-Min Chou,¹ Wolfgang E. Thasler,⁷ Norbert Hüser,⁸ David Duranet,⁹ T. Jake Liang,¹⁰ Carsten Münk,¹¹ Markus H. Heim,⁴ Jeffrey L. Browning,¹² Emmanuel Dejardin,⁶ Maura Dandri,²,⁵ Michael Schindler,¹ Mathias Heikenwalder,¹†† Ulrike Protzer¹,²††

Virology
Getting Rid of a Persistent Troublemaker to Cure Hepatitis
Amir Shlomai and Charles M. Rice
The HBV X protein promotes the Smc5/6 degradation in PHH by hijacking the cellular DDB1-containing E3 ubiquitin ligase ➔ release of the Smc5/6 transcription inhibition of episomal DNA templates

Target #3: the viral envelope

Strategies under study:
- RNA interference (siRNA)
- Nucleic acid polymers (NAPs)

Goals:
- To inhibit virion production
- To restore the antiviral activity of exhausted T cells
ARC-520 (anti-HBs siRNA) induces a profound and durable knockdown of viral antigens and DNA in a phase II study

**Impact of integrated sequences on siRNA efficacy**

- cccDNA
- S promoter
- X promoter
- Precore, core promoters
- Host DNA
- Integrated HBV DNA
- S mRNA
- S protein
- ARC-520 siRNAs

**HBsAg reduction in therapy naive patients with a single 4 mg/Kg dose**

**Challenges:**
- HBV sequence diversity (10 genotypes, 7.5% heterogeneity)
- Uptake by appropriate cell type (liposomes, cholesterol conjugation)
- Endosomal escape (cationic lipids, melitin-like peptide)
- Avoid innate immune response (complex lipids, chemical modifications)
- IV administration!

*YUEN et al, AASLD 2015, abstract #LB-9*
REP-2139 (Nucleic Acid Polymer) 500 mg IV qW monotherapy
(REP 101 study, n=7)

Anti-HBs seroconversion observed in patients treated with REP-2139 + Zadaxin
Phase II study in hepatitis B started in October 2015 in Moldova
Target #4: the viral capsid

1) Capsid Assembly
   - Inhibition of Viral replication

2) cccDNA Amplification
   - Inhibition of Viral replication

3) ISG Inhibition
   - Restoration of host innate immune response

4) Maintenance of cccDNA in Active State
   - cccDNA silencing inhibits viral replication & restores host immune response

(from FLORES O, Novira Therapeutics, presented at HepDart 2015)
NVR 3-778 targets the N-terminal assembly domain of HBV core, leads to defective core particles and reduces serum HBV DNA and RNA.

Cohort I: 600 mg BID

serum HBV RNA change from baseline

YUEN et al, AASLD 2015, abstract #LB-10
KLUMPP K et al, Proc Natl Acad Sci USA 2015;112:15196-201
Target #5: the host immune system

• HBV persists with **virus-specific and global T-cell dysfunction** mediated by multiple regulatory mechanisms, but without distinct T-cell–based immune signatures for clinical phenotypes
  
  **PARK et al, Gastroenterology 2015**

• Children and young adults with chronic hepatitis B have a Th1-cell cytokine profile and a **partial profile of T-cell exhaustion**

• Young patients with CHB have more HBV-specific T cells with the ability to proliferate and produce cytokines than adult patients with CHB

• HBV infection in younger patients is **not associated** with an immune profile of T-cell tolerance
  
  **KENNEDY et al, Gastroenterology 2012**

• HBV exposure **in utero** induces a robust Th1-polarized response in the newborn (« trained immunity »)

  **HONG et al, Nature Comm 2015**
Immune checkpoint inhibitors may restore pre-existing immune responses: the example of anti-PD-1

• Blockade of PD-1 increases responses of liver HBV-specific T cells
  
  FISICARO et al, Gastroenterology 2012

• Blockade of PD-1 with BMS-936558 (Medarex-1106, nivolumab), a fully human anti-PD-1 monoclonal IgG4 in a single, ascending dose, phase I trial in hepatitis C led to significant HCV RNA reductions (one patient aviremic one year after dosing)

  GARDINER et al, PLoS One 2013
Conclusions

Current treatments for HBV:
- Allow suppression of viral replication in most patients who have access to potent NUCs
- Must be prolonged for life, with limited effects on HBsAg and cccDNA
- Do not eliminate completely the risk of complications

Future treatments for HBV:
- Will allow reaching a functional / complete cure (a sterilizing cure is unrealistic with current technologies)
- Ideally, should be short-term
- May require a paradigm shift, e.g. targeting host gatekeepers (innate and adaptive responses)