Hepatitis B: New Treatment Targets and Possible Cure

Fabien Zoulim
Hepatology Department, Hospices Civils de Lyon
INSERM U1052, Cancer Research Center of Lyon
Lyon University, France
Why a need for new antiviral targets for hepatitis B?

Current antivirals achieve viral suppression in the majority of patients (in western countries)

Issues with antiviral drug resistance in developing countries (use of low barrier to resistance antivirals)

The cure rate (cccDNA / HBsAg loss) remains very low

Life-long therapy is needed in the majority of cases

Treatment with finite duration if:
- cccDNA control or loss
- HBsAg loss

HBsAg clearance is associated with a lower risk of HCC development

Zoulim, Antiviral Research 2012
Definition of HBV cure

Virologic definition

- **Functional cure**
  - Situation where antiviral therapy could be stopped with a minimal risk of viral reactivation
  - HBsAg loss with anti-HBsAb seroconversion
  - cccDNA inactivation and/or control by host mechanisms

- **Complete cure**
  - HBsAg clearance and cccDNA eradication

Clinical definition

- Functional cure associated with a regression in the risk of progression of fibrosis and HCC

Zeisel, Lucifora et al, Gut 2015
Results of current antivirals
Mode of action of antivirals for CHB

Adaptive immune responses
- CD8+ cells
- CD4+ cells
- B cells

Innate responses
- NK cells
- Interferon alpha

Nucleos(t)ide analogues

## Current treatments: virus suppression and sustained disease control

<table>
<thead>
<tr>
<th></th>
<th>Entecavir&lt;sup&gt;1,2&lt;/sup&gt;</th>
<th>Tenofovir&lt;sup&gt;3&lt;/sup&gt;</th>
<th>PEG-IFN α-2a&lt;sup&gt;4,5&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HBeAg positive</strong></td>
<td>n = 354</td>
<td>n = 176</td>
<td>n = 271</td>
</tr>
<tr>
<td>HBV DNA undetectable</td>
<td>67%</td>
<td>76%</td>
<td>25%&lt;sup&gt;a&lt;/sup&gt;</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>
</tr>
<tr>
<td>HBsAg loss</td>
<td>2%</td>
<td>3.2%</td>
<td>2.9%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

| **HBeAg negative**   | n = 325                  | n = 250               | n = 177                    |
| HBV DNA undetectable| 90%                      | 93%                   | 63%<sup>a</sup>            |
| ALT normalisation    | 78%                      | 76%                   | 38%                        |
| HBsAg loss           | 0.3%                     | 0%                    | 0.6%<sup>b</sup>           |

Results at 48 weeks

<sup>a</sup> HBV DNA < 400 copies/mL; <sup>b</sup> At 72 weeks

Persistence of intrahepatic viral DNA synthesis during Tenofovir therapy (HIV-HBV cohort)

New round of infection and/or replenishment of the cccDNA pool occur despite « viral suppression »

Boyd et al, in revision
Long-term therapy is required to maintain viral suppression

- HBV DNA change from baseline (log_{10} c/mL)
  - 0.0
  - -1.0
  - -2.0
  - -3.0
  - -4.0
  - +1.0

- Time

- SERUM
- HBsAg
- HBVDNA

- LIVER
- cccDNA

NUC Therapy
New treatment concepts for a functional cure of HBV infection

- **Antivirals**
- **Immune restoration**
- **Decay or epigenetic control**

**Therapy**

- **HBsAg**
- **HBVDNA**
- **cccDNA**

- **SERUM**
- **LIVER**

- **Time**
Stopping TDF therapy after long-term viral suppression

High rates of viral replapse & ALT elevations

3 patients with HBsAg loss out of 41

HBeAg positive (n=4)

HBeAg negative (n=37)

24-week TFFU Completers (n=41)

Buti et al AASLD 2015
Characterisation of the immune profile in Chronic Hepatitis B, with CyTOF, to identify biomarkers of immune control following NUC therapy discontinuation

Expanded global CD4+ T cell memory populations in ‘non-flare’ vs flare patients

**CD4+ T cells**

- **CD4+ T cells (%)**
  - Naive
  - T
  - CM
  - T
  - EM
  - T
  - EMRA

**CD127+ (IL7R) CD4+ T cells**

- **CD127+ CD4+ T cells (%)**
  - Naive
  - T
  - CM
  - T
  - EM
  - T
  - EMRA

HBV polymerase specific T cell responses on NUC therapy are predictive of hepatic flares with NUC discontinuation

- **On NUC therapy**
  - SFC/10⁵ cells
    - X
    - Core
    - Env
    - Pol

- **NUC discontinuation**
  - SFC/10⁵ cells
    - X
    - Core
    - Env
    - Pol

- Patients with detectable frequencies of circulating HBV-specific T cells controlled HBV replication / no hepatic flare following NUC discontinuation

Gill US, et al. AASLD 2015, San Francisco. #167
Improvement of existing drugs
Example of TAF for tenofovir

- Improved stability in plasma:
  - Enhanced delivery of active form (TFV-DP) to hepatocytes
  - Lower doses are used; systemic exposures of TFV reduced

**TFV**
Tenofovir

**TDF**
Tenofovir Disoproxil Fumarate

**TAF**
Tenofovir Alafenamide

CES1 = carboxylesterase 1; DP= di-phosphate; MP= mono-phosphate.

Agarwal K et al. AASLD 2013, Poster # 973
Murakami E et al. HepDART 2013, Abstract 104
Phase 1B results: HBV DNA kinetics on 28 days

- No differences in viral declines over range of TAF 8 mg to 120 mg

- Viral suppression over 4 weeks with TAF was similar to TDF

Agarwal K et al. AASLD 2013, Poster # 973
GS-US-320-0101 - Clinicaltrials.gov NCT01671787
New targets for HBV therapy
**Vaccine therapy**

- Chimeric antigen Receptors (CAR)
- TLR agonists
- Check-point inhibitors
- Blockade of immune-suppressive cytokines

**Antiviral cytokines**

- Entry inhibitors
- Egress Inhibitors
- Targeting HBx
- RNA interference
- Targeting cccDNA
- Core modulators
- Polymerase inhibitors
- Core modulators

*Testoni and Zoulim, Hepatology 2015*
Model for HBV entry in hepatocytes and development of entry inhibitors

Li et al, elife 2012
Urban et al, Gastroenterology 2014
Myrcludex B, a peptidic inhibitor of NTCP-mediated entry of HBV and HDV

- **Myrcludex B** is an HBV preS-derived lipopetide binding sodium-taurocholate co-transporting polypeptide (NTCP).
- **Myrcludex B inhibits** HBV and HDV receptor function of NTCP in vitro and in animal models ($IC_{50} \sim 80 \text{ pM}$).
- **Myrcludex B inhibits** NTCP-mediated bile salt uptake into hepatocytes ($IC_{50} \sim 100 \text{ nM}$).
- **Myrcludex B specifically targets** liver hepatocytes after subcutaneous administration.
- **Myrcludex B showed safety in** Phase I clinical trials.

⇒ (A) Proof of safety and efficacy in chronically HBV infected individuals.
⇒ (B) Proof of safety and efficacy in chronically HBV/HDV co-infected individuals.

*S Urban Heidelberg U & MyrGmbH*
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
Targeting cccDNA

Lucifora et al, *Science* 2014
Belloni et al, *JCI* 2012
Koeniger et al, *PNAS* 2014
Tropberger et al, *PNAS* 2015

cccDNA formation

cccDNA degradation

cccDNA silencing

Hepatocyte turn-over
Model for cccDNA degradation

IFNalpha /Lymphotoxin beta can induce APOBEC3A/B dependent degradation of HBV cccDNA

Lucifora et al, Science 2014; Shlomai & Rice, Science 2014

Similar observation with IFNγ and TNFα – Xia et al, Gastroenterology 2015
Epigenetics of covalently closed circular (ccc)DNA
Regulation by viral proteins (HBc and HBx)

Silencing
Interferon alpha,
Capsid inhibitors,
Epigenome modifiers

Pollicino et al. Gastroenterology 2006
Levrero et al. J Hepatol, 2009
Lucifora et al, J Hepatol 2012
Belloni et al, PNAS 2009
Belloni et al, J Clin Invest 2012
Strubin et al, HBV conference 2015
Challenges in targeting cccDNA

- cccDNA formation: involves nuclear enzyme / DNA repair machinery
- cccDNA degradation: is the whole pool of cccDNA susceptible to degradation? will all infected cells be susceptible?
- cccDNA damage: CRISPR/cas9 technologies and others. Issues with delivery?
- cccDNA silencing: targeting virus-specific mechanisms to avoid safety issues
- Hepatocyte turn-over: may trigger the clonal selection of hepatocytes in the context of an oncogenic virus
- Small molecules needed!
Targeting the HBV capsid

1) Capsid Assembly
- Inhibition of Viral replication

2) cccDNA Amplification
- Inhibition of Viral replication

3) ISG Inhibition
- Restoration of host innate immune response
- cccDNA silencing inhibits viral replication & restores host immune response

4) Maintenance of cccDNA in Active State
- Core dimer bound to virus mini-chromosome (cccDNA)
- Core dimer bound to host chromosome

NVR 3-778
Targeting HBV nucleocapsids

Heteroaryldihydropyrimidines
Destabilization of nucleocapsids
Deres et al, Science 2003
Klumpp et al, PNAS 2015

Phenylpropenamide derivatives
Prevent pgRNA encapsidation

Novel classes of capsid inhibitors based on the 3D structure of HBc
Novira, Assembly Biosciences, Janssen, Roche, and others
Phase 1 studies with Novira completed
Effective reduction of serum HBV DNA by NVR 3-778 (Novira)

All treatments reduced HBV DNA

ETV and NVR 3-778 monotherapies show similar antiviral activity (p>0.05)

PEG-IFN + NVR 3-778 combination provides highest antiviral efficacy

5/5 mice achieve serum HBV DNA BLQ

Lam A, et al. AASLD 2015, San Francisco. #33
Phase 1b clinical trial: NVR 3-778 reduces serum HBV DNA and RNA (Novira)

Serum HBV DNA: mean 1.7 log reduction (600 mg BID)

Serum HBV RNA: mean 0.86 log reduction (600 mg BID)

Yuen M-F, et al. AASLD 2015, San Francisco. #LB-10
RNA Interference

Targeted Gene Silencing

mRNA degradation

Natural Process of RNAi

RISC

Complementary pairing

Strand separation

Cleavage

dicer

dsRNA

Cleavage Strand separation Complementary pairing Cleavage

Synthetic siRNA

mRNA

(A)ₙ

mRNA (A)ₙ

(A)ₙ
Reductions in cccDNA under NUC and ARC-520 therapy in chimpanzees with chronic hepatitis B virus infection implicate integrated DNA in maintaining circulating HBsAg

ARC-520 reduced total liver DNA and cccDNA beyond levels achieved in HBeAg-pos NUC treatment during lead-in

ARC-520 and NOT NUC reduced intrahepatic HBV RNA and antigens


#32
ARC-520 produces deep and durable knockdown of viral antigens and DNA in a phase II study in patients with chronic hepatitis B

**HBV antigen reduction in ETV experienced HBeAg-positive patients with a single 4 mg dose (cohort 5)**

**HBsAg reduction in ETV naïve patients with a single 4 mg dose (cohort 7)**

Direct antiviral effect lasted up to 57 days after a single dose of ARC-520, delayed response duration >85 days

Small dose-related reduction in HBsAg

Maximum effective dose not reached

Yuen M-F, et al. AASLD 2015, San Francisco. #LB-9
Restoration of antiviral immunity

http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1003784
Antiviral activity of a TLR7 agonist in HBV infected chimpanzees

Lanford et al, Gastroenterology 2013
The Oral Toll-Like Receptor-7 Agonist GS-9620 in Patients with Chronic Hepatitis B Virus Infection

Gane et al, Journal of Hepatology, 2015
Restoration of defective T-cell immune control

Patients who have resolved HBV

CD8 T cells

Infected hepatocytes

Effective T-cells control virus

Patients with chronic HBV

Infected hepatocytes

Exhausted T-cells lose control of virus

Can an effective antiviral T-cell response be recovered?

**Restoration of defective T-cell immune control**

**Patients who have resolved HBV**

- CD8 T cells
- Granzyme
- Perforin
- INF-γ
- TNF-α
- IL-2

**Effective T-cells control virus**

**Patients with chronic HBV**

- Infected hepatocytes
- INF-γ
- TNF-α
- IL-2

**Exhausted T-cells lose control of virus**

**Prolonged nucleotide therapy in a subset of patients**¹

Restoration of defective T-cell immune control

Patients who have resolved HBV

CD8 T cells

Infected hepatocytes

Effective T-cells control virus

Patients with chronic HBV

Infected hepatocytes

Exhausted T-cells lose control of virus

Specific immunomodulation of existing T-cells e.g. PD-1 blockade

Triple combination therapy of ETV treatment, DNA vaccination and in vivo PD-L1 blockade in WHV infected woodchucks

In vivo PD-L1 blockade synergizes with therapeutic vaccination to control WHV replication

New treatment concepts for a functional cure of HBV infection

- Antivirals
- Immune restoration
- HBsAg
- HBV DNA
- cccDNA
Target & drug discovery to cure HBV infection

Immune modulation
- Toll-like receptors agonists, Gilead, Roche
- PD1 blockade, BMS, Merck etc.
- Vaccine therapy: Transgene, Gilead, Roche Innovo, Medimmune, ITS

Cyclophilin inhibitors
- Arbutus

Entry inhibitors
- Lipopeptides, e.g. Myrcludex-B

Targeting cccDNA
- Polymerase inhibitors
  - Nucleoside analogues, e.g. Gilead, BMS
  - Non-nucleoside, e.g. LB80380
  - RNAseH inhibitors

RNA interference
- Arrowhead, Arbutus, Alnylam, GSK

Targeting HBsAg
- Mab, Gilead
- Release, Replicor

Polymerase inhibitors
- Nucleoside analogues, e.g. Gilead, BMS
- Non-nucleoside, e.g. LB80380
- RNAseH inhibitors

Can we cure the liver disease?

- Mechanisms of HBV-induced HCC involve several factors: inflammation, fibrosis, chromosomal integration, HBx etc.
- Improvement of liver inflammation & fibrosis with long-term NUC
- HCC not always seen on a background of cirrhosis
- Clonal expansion of hepatocytes not supporting HBV replication occurs even before cirrhosis
- NUCs decrease, but don’t eliminate, the risk of HCC
- Early treatment intervention & better antivirals needed

Zoulim & Mason, Gut 2012; 61 : 333-336
Acknowledgements

Hepatology Unit
David Durantel
Barbara Testoni
Julie Lucifora
Malika Ait-Goughoulte
Souphalone Luangsay
Marion Gruffaz
Nathalie Isorce
Fanny Lebossé
Maelenn Fournier
Maud Michelet
Judith Fresquet

INSERM U1052
Maelle Locatelli
Valentina d'Arienza
Pascal Jalaguier
Thomas Lahlali
Dulce Alafaiate
Lucyna Cova
Romain Parent
Anna Salvetti
Birke Bartosch
Eve Pecheur
Boyan Grigorov
Christophe Combet

Collaborations
C. Caux, Lyon CRCL
FL. Cosset, Lyon CIRI
K. Lacombe, Paris
M. Levrero, Rome/Lyon
JP Quivy, Institut Curie
“Save the date”

Third ANRS “HBV cure” Workshop
HBV pathobiology and target discovery

Scientific coordination: Fabien Zoulim

Tuesday, May 31st, 2016
Union internationale des chemins de fer (UIC)
16, rue Jean Rey - 75015 PARIS


Regression of fibrosis and cirrhosis during long-term Tenofovir therapy

348 HBeAg(+) and HBeAg(-) CHB patients from phase 3 studies who enrolled in a long-term rollover study were evaluated for long-term liver histology outcomes. **51% of patients had regression of fibrosis, including 71/96 patients with cirrhosis (Ishak score ≥ 5) at phase 3 study baseline.**

Japanese cohorts: Entecavir reduced HCC incidence, compared with controls

PS-matched cohort multivariate cox regression analysis:

HR 0.37 (95% CI 0.15–0.91) p = 0.030

*Adjusted for age, sex, alcohol, smoking, cirrhosis, HBV genotype, HBeAg status, HBV-DNA, ALT, albumin, γGTP, total bilirubin and platelet count.

Log-rank test: p<0.001

Cumulative HCC rates (%)

<table>
<thead>
<tr>
<th>Treatment duration (years)</th>
<th>No. at risk ETV</th>
<th>No. at risk Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>316</td>
<td>316</td>
</tr>
<tr>
<td>1</td>
<td>316</td>
<td>316</td>
</tr>
<tr>
<td>2</td>
<td>264</td>
<td>277</td>
</tr>
<tr>
<td>3</td>
<td>185</td>
<td>246</td>
</tr>
<tr>
<td>4</td>
<td>101</td>
<td>223</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>200</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>187</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>170</td>
</tr>
</tbody>
</table>


HR, hazard ratio; PS, propensity score
The incidence of HCC after clearance of HBsAg was 36.8 per 100,000 person-year (95% CI 13.5-80.0) which was significantly lower than the rate in those who remained HBsAg-positive (195.7 cases per 100,000 person-years of follow-up [95% CI 141.1-264.5; P < 0.001])
Liver Damage and HBV infection

HCC not always seen on a background of cirrhosis

Liver damage results of immune killing of hepatocytes

Clonal expansion of hepatocytes not supporting HBV replication occurs even before cirrhosis

Experimental models show that clonal hepatocyte repopulation is a major risk factor for HCC

Zoulim & Mason, Gut 2012; 61 : 333-336
Mechanisms of HBV-related hepatocarcinogenesis

Chronic HBV Infection
Viral replication

Chronic liver inflammation, hepatocyte injury, proliferating fibroblasts, fibrosis/cirrhosis

Host DNA mutations due to high hepatocyte turnover, cytokine and growth factor release

Chromosomal integration

Chromosomal instability, altered gene expression or function

HBV protein X

Interference with cellular transcription and signaling pathways

Hepatocellular carcinoma

Clonal expansion of hepatocytes not supporting active viral replication

Analysis of liver fragments for clonal expansion of cells by serial dilution - woodchucks

Analysis of liver tissue for variability in levels of HBV infection - Chimpanzee

Similar observations in HBV infected chimpanzees and patients

Reductions in Serum HBV DNA, Total Intrahepatic HBV DNA and cccDNA During Adefovir Therapy

- 48 weeks of ADV resulted in significant reductions in:
  - serum HBV DNA > total intrahepatic HBV DNA > cccDNA
  - > 14 years of therapy to clear completely viral cccDNA

Werle et al, Gastroenterology 2004
0.8 log_{10} (84%) decline in cccDNA, not paralleled by a similar decline in the number of HBcAg+ cells

Suggests cccDNA depleted primarily by non-cytopathic mechanisms or new rounds of hepatocyte infection occurred during therapy

Werle et al, Gastroenterology 2004
cccDNA formation: identification of TDP2

TDP2: Tyrosyl DNA Phosphodiesterase

Cortes Ledesma et al, Nature 2009

Koeniger et al, PNAS 2014
Targeting Hepatitis B Virus With CRISPR/Cas9

Induction of deletions in cccDNA
Decreased number of cells expressing viral antigens

Mol Ther Nucleic Acids. 2014; Seeger et al
Histone modifications, Pol2 binding and transcriptional profile of cccDNA

Tropberger et al, PNAS 2015
In vivo proliferation of hepadnavirus-infected hepatocytes induces loss of cccDNA in mice

Dandri et al, Hepatology 2010