Hepatitis B challenges towards a cure – Virological perspectives

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Natural History of hepatitis B

Acute infection

Chronic infection: 250 million carriers!

Chronic hepatitis
Wild type virus HBeAg+
Pre-core mutant HBeAg-

Inactive carrier

Reactivation

Resolved infection
5% neonates
90% adults

Immune tolerance

Cirrhosis

Hepatocellular carcinoma

30-50 years

Seeger, Zoulim, Mason; Fields Virology; 2007-2013
The viral life cycle and HBV pathobiology

Innate responses

Adaptive immune responses

NK cells

CD8 $^{+}$ cells

CD4 $^{+}$ cells

B cells

The viral life cycle and HBV pathobiology


Slow rate of spontaneous HBsAg clearance

Chan et al, Hepatology 2010

Table 1. Clinical Characteristics of Patients at the First Visit with Respect to HBeAg Status

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>HbeAg-Positive</th>
<th>HbeAg-Negative</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>117</td>
<td>49</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>36 ± 13</td>
<td>28 ± 10</td>
<td>42 ± 11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male</td>
<td>73 (62%)</td>
<td>31 (63%)</td>
<td>42 (62%)</td>
<td>0.87</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>14.6 ± 1.4</td>
<td>14.7 ± 1.4</td>
<td>14.4 ± 1.4</td>
<td>0.33</td>
</tr>
<tr>
<td>White cell count (×10^9/L)</td>
<td>6.9 ± 1.5</td>
<td>7.1 ± 1.6</td>
<td>6.7 ± 1.4</td>
<td>0.23</td>
</tr>
<tr>
<td>Platelet (×10^5/L)</td>
<td>195 ± 50</td>
<td>198 ± 50</td>
<td>193 ± 51</td>
<td>0.57</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>79 ± 17</td>
<td>77 ± 19</td>
<td>81 ± 16</td>
<td>0.24</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>40 ± 3</td>
<td>40 ± 3</td>
<td>40 ± 3</td>
<td>0.46</td>
</tr>
<tr>
<td>Bilirubin (μmol/L)</td>
<td>9 ± 6</td>
<td>9 ± 5</td>
<td>9 ± 6</td>
<td>0.81</td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>47 (14-447)</td>
<td>64 (17-447)</td>
<td>43 (14-326)</td>
<td>0.009</td>
</tr>
<tr>
<td>Cimosis by ultrasound (%)</td>
<td>4 (3%)</td>
<td>2 (4%)</td>
<td>2 (3%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Genotype C HBV (%)</td>
<td>66 (58%)*</td>
<td>35 (71%)*</td>
<td>31 (48%)*</td>
<td>0.011</td>
</tr>
<tr>
<td>HBV DNA (log IU/mL)</td>
<td>5.31 ± 2.26</td>
<td>7.22 ± 1.26</td>
<td>3.94 ± 1.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HBsAg (log IU/mL)</td>
<td>3.27 ± 1.28</td>
<td>4.01 ± 0.91</td>
<td>2.73 ± 1.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HBsAg/HBV DNA</td>
<td>0.70 ± 0.59</td>
<td>0.56 ± 0.10</td>
<td>0.80 ± 0.59</td>
<td>0.001</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>99 ± 16</td>
<td>99 ± 20</td>
<td>98 ± 14</td>
<td>0.75</td>
</tr>
<tr>
<td>Number of visits</td>
<td>17 (8-49)</td>
<td>17 (8-37)</td>
<td>17 (8-49)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

![Graph showing HBsAg levels over visits for different groups](Graph.png)
Decreased cumulative risk of HCC after HBsAg loss

Yuen et al, Gastroenterology 2008
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 et al, Gut 2015
Can we cure the infection?
Mode of action of antivirals for CHB

**Innate responses**

- NK cells

**Adaptive immune responses**

- CD8+ cells
- CD4+ cells
- B cells

**Mode of action of antivirals for CHB**

- Nucleos(t)ide analogues
- Interferon alpha

**Hepatocyte**

- NTCP
- HBV DNA integration
- Transcription
  - pgRNA
  - mRNA
- Translation
- Encapsulation
- Reverse transcription
- Virion secretion

**Nucleos(t)ide analogues**

- Entry
- Polymerase
- cccDNA formation
- cccDNA amplification
- DNA (+)
- DNA (-)
- (+) strand synthesis

**Zoulim & Locarnini, Gastroenterology 2009; Zoulim Antiviral Research 2012; Mico et al J Hepatol 2013; Lucifora et al Science 2014**
## Current treatments: sustained virus and disease control with NUCs/IFN

<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>
<tr>
<td><strong>HBeAg negative</strong></td>
<td>n = 325</td>
<td>n = 250</td>
<td>n = 177</td>
</tr>
<tr>
<td>HBV DNA undetectable</td>
<td>90%</td>
<td>93%</td>
<td>63%&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALT normalisation</td>
<td>78%</td>
<td>76%</td>
<td>38%</td>
</tr>
<tr>
<td>HBsAg loss</td>
<td>0.3%</td>
<td>0%</td>
<td>0.6%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results at 48 weeks

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

Slow kinetics of HBsAg clearance during antiviral therapy

Marcellin et al, Journal of Hepatology; 2014; 61: 1228 - 1237

HBsAg clearance mainly observed in HBeAg+, genotype A, caucasian patients

Slow kinetics of HBsAg and cccDNA during NUC therapy

Wong et al, Clin Gastroenterol Hepatol 2013
Long-term therapy is required to maintain viral suppression.

Werle et al, Gastroenterology 2004
Conclusion (1)

- HBsAg loss is rarely observed, but is achievable in some patients with long-term (current) treatments

- cccDNA eradication is not achievable with the current treatments even in HBsAg loss patients

*Maynard et al, J Hepatol 2005*

- Issue of the occult HBV infection

*Raimondo et al, J Hepatol 2008*
New treatment concepts for a functional cure of HBV infection

- Therapy
- HBVDNA
- HBsAg
- cccDNA
Targeting cccDNA

Lucifora et al, Science 2014
Belloni et al, JCI 2012
Koeniger et al, PNAS 2014
cccDNA formation: identification of TDP2

TDP2: Tyrosyl DNA Phosphodiesterase
Cortes Ledesma et al, Nature 2009

Koeniger et al, PNAS 2014
IFN alpha mediated degradation of cccDNA

A

% of HBVAg

0 10 20 30 days

B

% of cccDNA

mock LAM day 0-3 day 0-7 day 0-10

IFN-α

C

% of cccDNA

0 5 10 15 days

D

% of indicated markers

HBeAg HBV DNA cccDNA

IFN-α

E

analyses

0 7 13 days

treatment

F

analyses

0 10 12 16 days

ETV

IFN-α

J Lucifora et al. Science 2014;343:1221-1228
Model for cccDNA degradation

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

LOW-REPLICATIVE STATE

- Epigenetic regulation:
  - Histones acetylases, deacetylases, methyltransferases
  - Transcription factors
  - Binding of viral proteins: HBc & HBx

HIGH-REPLICATIVE STATE

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
Damaging cccDNA with CRISPR/Cas9

Deletions in cccDNA
Decrease in the number of cells expressing viral antigens

Seeger et al, Mol Ther Nucleic Acids. 2014
Future directions: target & drug discovery to cure HBV infection

Immune modulation
- Toll-like receptors agonists, Gilead, Roche
- Anti-PD-1 mAb, BMS, Merck
- Vaccine therapy Transgene, Gilead, Roche Innovio, Medimmune, ITS

RNA interference, Arrowhead, Tekmira, Alnylam, GSK

Targeting HBsAg Mab, Gilead Release, Replicor

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

Targeting cccDNA

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

Conclusion (2)

- Better knowledge of the key steps of the viral life cycle and antiviral immune responses to be targeted (academic effort)

- Screening of compounds on viral and immune targets in relevant pre-clinical models (industrial effort)
Can we cure the liver disease?

- Improvement of liver fibrosis
- Prevention / delay of HCC occurrence
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 (%)

No. at risk
ETV 316 316 264 185 101 44 2
Control 316 316 277 246 223 200 187

ETV
Control


HR, hazard ratio; PS, propensity score
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

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 - Chimpanzees

Similar observations in HBV infected chimpanzees and patients

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])
Conclusion (3)

- Regression of liver fibrosis is achievable with the control of viral replication and liver inflammation.
- This allows to decrease but not to eliminate the risk of HCC.
Conclusions (final)

- HBsAg clearance is the most desirable endpoint for novel treatment concepts

- Targeting cccDNA for a functional cure is a priority for drug development

- cccDNA eradication for a complete virologic cure will be challenging

- Restoration of immune responses will be needed either indirectly through viral « suppression » or directly
Conclusions (final, continued)

• Improvement of liver disease achievable with current treatments

• Further improvement in liver status with the new treatments in development?

• Specific strategies to target viral mechanisms of liver oncogenesis, i.e. integration

• A major issue is and will be the identification of patients for early treatment intervention

• Need for a concerted action from researchers, clinicians, pharma industry, and stakeholders: **plee for the establishment of an International Coalition to Eradicate Hepatitis B (ICE-HBV)**

*Revill, Testoni, Locarnini, Zoulim, Nature Reviews Gastroenterology and Hepatology*