New Targets for HBV Therapy

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Virus suppression but persistence of intrahepatic viral DNA synthesis during Tenofovir therapy

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

Boyd et al, J Hepatol 2016
Major virologic discoveries for HBV cure research programs

• Better knowledge of the viral life cycle
  Receptor – cccDNA - HBx

• Improvement of cell culture for target identification and drug screening
  Hepatoma cell lines – receptor and cccDNA formation
  Primary Human Hepatocytes and other culture systems

• Improvement of animal models for target identification and drug screening
  Liver humanized mouse models

• Identification & characterization of novel targets

New treatment concepts for HBV cure

- Therapy
- Functional Cure
- Complete Cure

- HBVDNA
- HBsAg
- Anti-HBsAb
- cccDNA

SERUM

LIVER
Mechanisms of viral persistence

cccDNA reservoir
Antigenic load
Liver tolerance
HBV persistence

Defective CD8+ response
Defective B cell response
Inefficient innate response

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

Testoni et al, Hepatology 2015; Liver International 2016
The main targets

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

Testoni et al, Hepatology 2015; Liver International 2016
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

Ezetimib
Cyclosporin

Li et al, elife 2012; Urban et al, Gastroenterology 2014
Targeting cccDNA, the viral minichromosome

- cccDNA replenishment
- cccDNA formation
- cccDNA degradation
- cccDNA loss
- cccDNA silencing

Lucifora et al, Science 2014
Belloni et al, JCI 2012
Koeniger et al, PNAS 2014
Durantel&Zoulim, J Hepatol 2016
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
Challenges in targeting cccDNA

Further knowledge required

Specificity for cccDNA?
Delivery?

Partial effect?
Efficacy in vivo?

Off-target effect?
Delivery?

RC-DNA > cccDNA conversion?
anti-host DNA repair factors

Further knowledge required

Modified from Nassal, Gut 2015

Targeting the HBV capsid with capsid assembly modulators

BAY-41-4109  Core + pgRNA  AT-130

Tyrosine  NVR 3-778

AAG pol  Assembly  (cf. Campagna et al. J. Virol. 2013)

GLS-4  Assembly  rcDNA-containing nucleocapsid

Retrotranscription + DNA replication

Winne et al., Mol. Cell 1999
Phase 1b clinical trial: CpAM NVR 3-778 reduces serum HBV DNA and RNA

Pre-clinical evaluation in hepatocyte culture and chimeric mouse models

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

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

Cohort I: 600 mg BID
Decrease of circulating HBV RNA

Yuen M-F, et al. AASLD 2015, San Francisco. #LB-10
HBsAg targeting strategies

- HBsAg clearance an **endpoint of therapy**
- Decline in HBsAg levels may **restore the antiviral activity of exhausted T cells**
- **Several strategies** in evaluation
  - RNA interference (SiRNA): « gene silencing »
  - Nucleic acid polymers (NAPs): HBsAg release
  - HBs antibodies
SiRNA ARC-520 produces deep and durable knockdown of viral antigens and DNA in a phase II study

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

Impact of integrated sequences on siRNA efficacy

Will this result in restoration of immune responses?

Yuen M-F, et al. AASLD 2015, San Francisco. #LB-9
Towards combination therapy

Effect of a triple combination therapy on viral antigen load in a humanized mouse model
Restoration of antiviral immunity

Repression of intrahepatic expression of innate immunity genes in CHB patients

Lebossé, Testoni et al, J Hepatol 2017
Recovery of T cell response is possible after resolution of chronic HBV

Rehermann B, J Clin Invest. 1996; 97: 1655

Boni C, Gastroentrol 2012; 143: 963
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
Clinical Evaluation of Immunotherapeutics

• **Innate Immunity**
  - TLR-7 agonists (other TLR agonists?): inducing endogenous type I IFN responses
  - Targeting RIG-I: Restoration of endogenous IFN production & interference on Polymerase/pgRNA interaction
  - Restoring innate responses: blocking virus specific functions

• **Adaptive immunity**
  - Therapeutic vaccines: stimulating HBV specific CD4 and CD8 T cells
  - Check-point inhibitors: restoration of specific CD4 and CD8 T cells
  - T Cell engineering: redirecting T cells to infected hepatocytes
HBV cure - New treatment concepts – Will we need combination of DAA and immune therapy?
HBV cure - Where are we going?

- Towards improved therapies & cure within the next decade!
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INSERM U1052

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HBV cure - A highly dynamic drug discovery effort

Testoni & Zoulim, Hepatology 2015; Durantel & Zoulim, J Hepatol 2016
Definition of Cure

Durantel & Zoulim, J Hepatol 2016;
### Realistic definition of HBV cure

<table>
<thead>
<tr>
<th></th>
<th>Complete cure</th>
<th>Idealistic functional cure</th>
<th>Realistic functional cure</th>
<th>Partial “cure”</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical scenario</strong></td>
<td>Never infected</td>
<td>Recovery after acute HBV</td>
<td>Chronic HBV with HBsAg loss</td>
<td>Inactive carrier off treatment</td>
</tr>
<tr>
<td>HBsAg</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>Positive/negative</td>
<td>Positive</td>
<td>Positive/negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Serum HBV DNA</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Low level or not detected</td>
</tr>
<tr>
<td>Hepatic cccDNA, transcription</td>
<td>Not detected</td>
<td>Detected</td>
<td>Detected</td>
<td>Detected</td>
</tr>
<tr>
<td>Integrated HBV DNA</td>
<td>Not detected</td>
<td>Detected?</td>
<td>Detected</td>
<td>Detected</td>
</tr>
<tr>
<td>Liver disease</td>
<td>None</td>
<td>None</td>
<td>Inactive, fibrosis regression over time</td>
<td>Inactive</td>
</tr>
<tr>
<td>Risk of HCC</td>
<td>Not increased</td>
<td>Not increased</td>
<td>Declines with time</td>
<td>Risk lower vs. immune active phases</td>
</tr>
</tbody>
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The main targets