Can we eradicate chronic hepatitis B infection?

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Disclosures

- BMS: Advisory Board and invited speaker; investigator
- Gilead: Advisory Board and invited speaker; investigator
- Janssen: Advisory Board and invited speaker, investigator
- MSD: Advisory Board and invited speaker; investigator
- Roche: Consultancy; invited speaker; investigator
- Tekmira: Advisory Board

Outline

1. Concepts on HBV cure
2. HBV cccDNA as a minichromosome: HBV replication is controlled by epigenetic mechanisms
   1. modulation of cccDNA function by epigenetic drugs
   2. anti-capsid drugs also target cccDNA
HBV the concept of « cure »

HBV¹,²

- High rates of regression or halting of fibrosis/cirrhosis with TDF/ETV
- Established safety profile up to Year 7 of treatment
- Resistance with some antivirals (LAM, ADV)
- Risk of HCC persists

- Persistence of cccDNA (even after therapy or spontaneous recovery)
  - Viral reactivation
  - Hepatocarcinogenesis
- Integrated viral genome
  - Hepatocarcinogenesis

Long-term suppression of viral replication (DAA)

Methadone suppression of viral replication

Viral Suppression

Functional cure (very few)

Immune control (IFNα)

Eradication

Suppression of viral replication Immune control (IFNα)


HBV challenges

- Treat more effectively: HBV and HCC
- Develop/use tools to Predict Treatment Outcomes
  - qHBsAg
  - qHBeAg
- Aim for cure
  - Functional cure
    - off-therapy persistent HBV suppression
  - cccDNA eradication
    - surrogate endpoint HBsAg loss and anti-HBs seroconversion
- Strategies
  - Immune system
  - Viral targets
• HBV hepatic “latent” reservoirs (non-integrated functionally competent HBV genomes) is now established – OBI and more
• Extra-hepatic reservoirs described ... but never formally established .... cccDNA presence unproven

cccDNA half-life: supposedly long, not established

Antivirals do not directly target cccDNA

1 yr of monotherapy with nucleos(t)ide analogues (ADV, LAM, ETV) reduced median intrahepatic cccDNA amounts by 1 log

No data on IFN-α monotherapy
Persistence of cccDNA

- Detected in the liver of NUCs long-term suppressed patients after HBsAg to anti-HBs seroconversion [Maynard, 2005; Belloni unpublished]
- Detected in the liver of HBsAg negative patients (occult HBV infection) [Werle-Lapostolle, 2004; Pollicino unpublished]
- Present in 30 /30 patients with occult HBV infection and HCC [Pollicino, 2004]

cccDNA as a minichromosome: the cccDNA ChIP assays

A methodology to study cccDNA function

- HBV minichromosome structure
- Modifications of cccDNA bound histones
- Binding of TF and coregulators

In vitro, in animal models, ex vivo (liver samples/biopsies)

Pollicino et al. Gastroenterology 2006
Levrero et al. J Hepatol, 2009
Belloni, PNAS 2009
Belloni, JCI 2012

Persistence of cccDNA in 3 out of 4 patients with long term HBV suppression under lamivudine
In 2 out 3 patients cccDNA is inactive (no pgRNA)
Studying cccDNA back in 2004

- No reliable infection system
  - HBV transgenic mice: cccDNA (-)
  - HBV stable cell lines (2.2.15): cccDNA (+/-)
  - HBV Transfection:
    - 1.2-1.3 HBV constructs: cccDNA (+/- or -)
    - 1.0 HBV linear genome: cccDNA ++

Epigenetic marks of open and condensed chromatin

HBV cccDNA ChIP assay

- HBV replication parallels the acetylation status of HBV cccDNA-bound H3 and H4 histones in HBV replicating cells and in vivo in patients

- Transcription factors and transcriptional coactivators and repressors are recruited onto cccDNA
  - Stat1/2, Stat3, HNF1a, HNF4a, p65, YY1, FXR
  - HATs (p300, PCAF, CBP)
  - HDACs (HDAC1, hSirt1)
  - HMTs (Ezh2); PRMTs (PRMT1; PRMT5) DNA-MTs (DNMT3a)

- Viral proteins bind to and modulate cccDNA functions
  - HBx (increases/required for transcription; increase p300 and prevents HDAC1 binding)
  - Hbc

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Acetylation of cccDNA-Bound H3 and H4 Correlates to HBV Viremia Levels in Chronic Hepatitis B Patients

A. Serum HBV DNA quantification in HBsAg-positive pts with:
   - active (AcH3, AcH4 positive/HDAC1 negative, 4 cases)
   - suppressed (AcH3-AcH4 negative/ HDAC1 positive, 4 cases)
   HBV replication. P value: Wilcoxon rank sum test.

B. ChIP of liver nuclear extracts from 10 HBsAg-positive pts using specific antibodies to AcH3, AcH4, HDAC1 or control IgG

Pollicino et al., Gastroenterology, 2006
cccDNA status in HBV patients

High Replication
Low Replication
Occult HBV

Low-replicative to latent infection
Epigenetic control

cccDNA ChIP assay in vitro and in vivo
HBV replication models

Bac-HBV transduction of HepaRG cells
[Lucifora, 2008]
• cccDNA formed from nucleocapsid recycling
• AcH3/H4 ChIP positive

HBV infection of PHH or HepaRG cells
[Lucifora, 2011; Sonnabend & Belloni, unpublished]
• cccDNA detected
• H3 and AcH3 ChIP performed
• infection efficiency as limitation

HBV infection of NTCP-HepG2 cells
[Sonnabend & Belloni, unpublished]
• cccDNA detected
• H3 and AcH3 ChIP performed

Human Hepatocytes in uPA mice
[Belloni, 2012; Belloni & Allweiss, unpublished]
• cccDNA detected
• H3 and AcH3 ChIP performed
• infection efficiency: cccDNA levels required 0.5-1 cp/cell

Human Hepatocytes
[Pollicino, 2006; Belloni, Pollicino, Guerrieri, unpublished]
• cccDNA detected
• H3, AcH3, HDACs, HMTs ChIP performed

0.2 cccDNA/cell = cccDNA ChIP LOD
0.5-1.0 cccDNA/cell = multiple parameters cccDNA ChIP
HBV models for cccDNA

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<th>cccDNA formation</th>
<th>cccDNA chromatin assembly</th>
<th>cccDNA chromatin function</th>
<th>cccDNA stability</th>
<th>cccDNA transcription</th>
<th>Core particles recycling</th>
<th>HBV replication</th>
<th>Chronic infection</th>
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*Inferred, direct study limited by cccDNA levels

HBV cure

• Aim for cure
  – Functional cure
    Off-therapy persistent HBV suppression
  – cccDNA eradication

• Strategies
  - Immune system
  - Viral targets
Emerging antiviral approaches

- cccDNA formation inhibitors
- cccDNA transcription inhibitors
- cccDNA destabilization/degradation
- anti-HBV siRNAs
- Nucleocapsid assembly inhibitors

- cccDNA - silencing - degradation
- pgRNA - siRNAs
- sub-genomic RNAs - siRNAs
- RNAse H inhibitors
- "capsid" - Core protein Assembly Modulators (CpAMs)
- Entry inhibitors (HBV and HDV)
- Prenylation inhibition (HDV)
- HBsAg release inhibitors
- Cyclophilin inhibitors
- TLR agonists
- Therapeutic vaccination

Silencing cccDNA
"functional cure" – off therapy control


**Interferon-α (IFNα) inhibits HBV transcription and replication in vitro and in vivo by favoring the long term recruitment to the nuclear cccDNA mini-chromosome of the class III HDAC hSirt1 and of the PRC2 repressive complex, including the transcriptional co-repressors HDAC1 and Ezh2**

Effects of IFNα treatment on HBV replication and transcription confirmed in humanized uPA/SCID mice

The HBV ISRE mediates IFNα transcriptional repression

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**Histone acetylation/methylation affects the regulation of gene expression**

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**Histone acetylation/methylation affects the regulation of gene expression**
IFNα targets the HBV cccDNA

Our results indicate that IFNα induces a condition of “active epigenetic control” of HBV cccDNA transcription that likely contributes to the persistent, yet reversible, “off therapy” inhibition of HBV replication (30-35% of HBeAg+ patients and 20-25% of HBeAg- patients) and suggest that HBV cccDNA function can be modulated by epigenetic drugs.

Modulation of Ezh2 histone methyltransferase activity mimics IFNα-induced repression of cccDNA transcription

Palumbo GA et al. AASLD 2013 Abs 928
Proof of concept of modulation of cccDNA function by “epigenetic” compounds

- Modulation of Ezh2 activity by MC3119 mimics IFNa-induced transcriptional repression of the cccDNA.

- PERSPECTIVE:
  - explore sequential treatments as a model for IFN sparing regimens
  - increase the subset of IFN SVRs

Make active carriers “true” inactive and, eventually, over time “occult” carriers by “locking” the cccDNA

Inhibitors of PCAF/p300 and activators of hSirt1/2 display the most significant effects on cccDNA transcription and HBV replication

Inhibition of cccDNA bound HATs activity leads to detachment of (PCAF) and p300

The hSirt1/2 agonist induce a shift in hSirt1/ hSirt2 occupancy and global cccDNA-bound H4 deacetylation

These results provide a proof of concept that small molecules / drugs that affect cccDNA bound chromatin modifying enzymes can modulate HBV transcription and replication

PERSPECTIVE
- possible synergisms ??

Palumbo GA et al. AASLD 2013 Abs 928
HBc protein / capsid

- HBc binds the cccDNA and regulates its function

HBc protein / capsid

- HBc binds the cccDNA and regulates its function
- HBc binds to cellular promoters and regulates gene expression
The Hap12 Core Protein Assembly Modulator (CpAM)
- inhibit HBV replication
- target the cccDNA

Palumbo (unpublished)
The Hap12 Core Protein Assembly Modulator (CpAM)

- Inhibit HBV replication
- Target the cccDNA
- Affect HBc occupancy on both the cccDNA and cellular promoters

HEPATITIS B CORE (HBC) PROTEIN IS A KEY AND VERY EARLY NEGATIVE REGULATOR OF THE INTERFERON RESPONSE

HBc affects the epigenetic state of the host genome to control innate immunity

- Very early inhibition of the hepatocyte IFN response by core protein through the recruitment of epigenome-modifying enzymes (Ezh2 and G9 HMTs) that leads to repressive marks (H3K27me3 and H3K9me3)

Gruffaz M, Testoni B et al AASLD 2013 Abs 136
The Hap12 Core Protein Assembly Modulator (CpAM)
- Inhibit HBV replication
- Target the cccDNA
- Affect HBc occupancy on both the cccDNA and cellular promoters

Anti-capsid drugs may have the potential to target the HBV cccDNA and to restore innate immunity/affect HBV pathogenesis

Different CpAMs – Different Results?

The effects of different anti-capsid drugs may vary according to their mode of action
- [HBc trap vs capsid disassembly]
- [free dimers availability]
- [targeting capsid vs dimers]

Adapted from Zlotnick, ICAR 2014
CpAMs Conclusions

- HAP12 "early treatment" inhibits cccDNA accumulation and HBV replication
  [direct effect on cccDNA or NUC-like effect on capsid recycling ?]

- HAP12 treatment affects significantly an existing cccDNA pool
  [experiments in HepG2-NTCP stable infection needed]

- HAP12 treatment apparently does not change HBc nuclear levels
  [how it works ?]

- HAP12 treatment effect on cccDNA transcription "possible" but not "proven"

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Specific and Nonhepatotoxic Degradation of Nuclear Hepatitis B Virus cccDNA

- Interferon-α and lymphotoxin-β-receptor activation up-regulated APOBEC3A and 3B cytidine-deaminases, respectively, in HBV-infected cells, primary hepatocytes and human liver-needle biopsies.
- HBV-core protein mediates the interaction with nuclear cccDNA resulting in cytidine-deamination, apurinic/apyrimidinic site formation and finally cccDNA degradation

Lucifora et al. Science 343, 1221-8, 2014
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**Targeted gene disruption strategies**

- Zinc Finger
- Talen
- CRISP/Cas

**Recognized challenge: delivery vehicle**
Targeted gene disruption strategies

- Zinc Finger
- Talen
- CRISP / Cas

Recognized challenge: delivery vehicle

- Inhibition of HBV replication with ZNF in Hep AD38 cells
  Weber, Plos1, 2014

- TALENs treatment reduces cccDNA level in vitro and and HBV pgRNA in vivo
  Chen, Mol Therapies, 2014

Conclusions

- Still striving for finite therapy ("functional cure")
  "...where the old and the new ways meet";

- In theory, the ideal goal of antiviral therapy for CHB would be complete HBV elimination including complete eradication of cccDNA from infected hepatocytes
  "...a new beginning" ... and keep open mind and a combinatorial attitude (HAPs+NUCs; HAPs+Mycludex ...)

- Lack of adequate in vitro replication models to study cccDNA
  "...one model does not fit all ... yet"
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 Innovo, Medimmune, ITS

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

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

What Might HBV Cure Will Look Like?

Potent NA → to prevent viral spread and cccDNA re-amplification

cccDNA Inhibitor → safe and selective agent to reduce or silence cccDNA

Immune Activator → agent(s) to activate specific antiviral immune responses or relieve repression/exhaustion of the immune system

HBV Antigen Inhibitor → agent(s) to block/inhibit the HBV life-cycle [entry, cell-spread, capsid assembly, HBx, HBeAg, HBsAg]

Modified from S. Locarnini 6.2014

Development stage: preclinical, clinical
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