Can HBV be cured by antiviral therapy alone?

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# Disclosure

<table>
<thead>
<tr>
<th>Relations that could be relevant for the meeting</th>
<th>Company names</th>
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<tr>
<td>Sponsorship or refund funds</td>
<td>Jansen, Gilead, MSD, Roche, Intercept</td>
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<td>Payment or other financial remuneration</td>
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<tr>
<td>(Advisory Committees or Review Panels)</td>
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HBV cure and HIV cure

similarities and differences

believes and knowledge

hopes and realities
Can HBV be cured by antiviral therapy alone?
Can HBV be **cured** by antiviral therapy alone?

+ infection + disease + cured
Can HBV be **cured** by antiviral therapy alone?

cure in HBV requires more « words » and more specifications to recapitulate the reality
Can HBV be *cured* by antiviral therapy alone?

cure in HBV requires more « words » and more specifications to recapitulate the reality

rc-DNA (replicative intermediate -- > virions)

cccDNA (replicative intermediate -- > transcription)  
(« reservoir », can reinitiate replication)

integrated HBV DNA (-- > transcripts and proteins)  
(« reservoir », *cannot* sustain replication)
Can HBV be cured by antiviral therapy alone? needs for definitions

From viral suppression to cure

UNTREATED

NUCs

Risk of HCC reduced (after 5 yrs) but not eliminated
Life-long / ptolomined

Levrero & Zoulim, Current Opinion Virology 2016
Barriers to eradicating HBV

cccDNA reservoir
- Long t1/2
- Continuous replenishment
- Not affected by NAs and IFN

Integrated forms

HBV persistence

Defective CD8+ responses

Defective B cell responses

Inefficient innate response

Defective immune responses

Revill et al, Lancet Gastroenterol and Hepatol, 2019

www.iasociety.org
Curative approaches: Targeting the pool of cccDNA

Entry inhibitors

Controlling viral replication: Pre- & Post-cccDNA targets

Entry inhibitors

Antiviral approaches

Immunomodulatory approaches

Curing hepatocytes

Specific hepatocyte killing

Virus neutralization

Entry inhibitors

Enveloped RC-DNA virion

NAs “Polymerase inhibitors”

CpAMs “Capsid inhibitors”

Targeting cccDNA

Targeting HBx

RNA interference

RNA destabilizers

Inhibitors of HBsAg release

Inhibitors of HBsAg release

Adaptive immunity modulation

Specific hepatocyte killing

Virus neutralization

Innate immunity modulation

• Toll-like receptor agonist
• RIG-I
• STINGs

Insufficient B-cell response

Dysfunctional T-cell response

CpAM: core protein allosteric modulators; HBx: hepatitis B X protein; IFN: interferon; IL: interleukin; KC: Kupffer cells; mAb: monoclonal antibody; NA: nucleos(t)ide analogue; NK: natural killer; NKT: natural killer T cell; pDC: plasmacytoid dendritic cell; PD-1: programmed cell death-1; TCR: T cell receptor


www.iasociety.org
Can HBV be cured by antiviral therapy alone?
Do we need anti-viral immunity to cure HBV

Chronic HBV Infection

Resolved HBV
“Functional Cure”

Adapted from: Bertoletti A SHC 2019
Do we need anti-viral immunity to cure HBV

If we have an antiviral that will completely eliminate the cccDNA

Adapted from: Bertoletti A SHC 2019
Do we need anti-viral immunity to cure HBV

If we have an antiviral that will completely eliminate the cccDNA

We do not discuss integrated HBV DNA yet

Adapted from: Bertoletti  A SHC 2019
cccDNA is the key molecule of HBV lifecycle and is responsible of viral persistence

cccDNA does not undergo semiquantitative replication

HBV replicates via a RNA intermediate (pgRNA)

Modified after Testoni, EASL 2019
cccDNA is the key molecule of HBV lifecycle and is responsible of viral persistence

cccDNA does not undergo semiquantitative replication

HBV replicates via a RNA intermediate (pgRNA)

Current available treatments, Nucleos(t)ide Analogues, control viral replication but do not eliminate cccDNA

Therapies must be lifelong to avoid viral rebound

One single cccDNA molecule can give rise to a full-blown infection

Modified after Testoni, EASL 2019
cccDNA is a minichromosome

16-18 nucleosomes with a spacing ~ 180-200bp

Bock et al, 1994; Newbold et al, 1995

Constraints due to the circular nature of HBV genome induce supercoiling

Chromatin
complex structure composed of DNA and proteins and localized in the nucleus of eucaryotes
cccDNA is the key molecule of HBV lifecycle

“If you know the enemy and know yourself, you need not fear the result of a hundred battles.

If you know yourself but not the enemy, for every victory gained you will also suffer a defeat.

If you know neither the enemy nor yourself, you will succumb in every battle.”
cccDNA is the key molecule of HBV lifecycle

Gaps in cccDNA knowledge

- **What are the kinetics of cccDNA during the infection and therapy?**
  - Guidance for the design of future antiviral strategies
  - Standardization of quantification methodologies
  - Visualization of cccDNA (kinetics of infected cells)

- **Knowledge on cccDNA formation and chromatinisation**
  - Establishment of cccDNA
  - Maintenance in infected cells, (stability, recycling)

- **How is cccDNA transcriptional activity regulated?**
  - Host and viral proteins
  - Can we interfere with this process to silence cccDNA?

- **Can we degrade and eradicate cccDNA within already infected cells?**
  - Cure of infected cells?

“If you know the enemy and know yourself, you need not fear the result of a hundred battles.

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cccDNA is the key molecule of HBV lifecycle

- How many cccDNA molecules are found in an infected liver?
- How long is cccDNA half-life in infected livers?
- How is the steady-state of cccDNA pool maintained?
- Is the cccDNA pool homogenous?

“If you know the enemy and know yourself, you need not fear the result of a hundred battles.

If you know yourself but not the enemy, for every victory gained you will also suffer a defeat.

If you know neither the enemy nor yourself, you will succumb in every battle.”
How many cccDNA molecules are found in an infected liver?

Laras, 2006

1-10 cccDNA/cell HBeAg(+) patients

1-2 logs less in HBeAg(-) patients

Laras, 2006; Voiz, 2007; Lebossé, Testoni, 2017

Much less than in animal viruses!

Host and virus-specific control of cccDNA synthesis rate

Kock, 2010

Simultaneous quantification of cccDNA, cccDNA transcriptional activity (and integration) burden in patients in different phases of the disease
How long is cccDNA half-life in infected livers?

It appears to survive for the lifespan of the cell (in vitro studies) (Moraleda, 1997; Dandri, 2000)

Hepatocytes have a very long half-life (6 to 12 months or more). Maybe shorter in CHB patients with active liver disease?

Long-term (>3 years) NUCs-treated patients

Approaching the LLoD of cccDNA qPCR! (half of the samples scored negative for cccDNA)

ddPCR as a solution ??

Estimated cccDNA half-life

9 months for HBeAg(+)
How long is cccDNA half-life in infected livers?

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Long-term (>3 years) NUCs-treated patients

Longitudinal studies conducted on samples from patients with emerging resistance to NUCs (liver HBV DNA, pgRNA, cccDNA and serum)

cccDNA half-life could be shorter than previously suggested?
Rapid turnover of HBV cccDNA in NUC CHB patients during drug resistance emergence and breakthrough

**Aim:** Determine turnover rates of cccDNA reservoirs in HBeAg positive HBV patients (by monitoring emergence and disappearance of NUC\textsuperscript{res})

- Retrospective analysis of serum and liver samples from patients of the EFFORT and ML 18376 trials: cccDNA in biopsy, pgRNA and rcDNA in serum and biopsy; Sequencing of Pol RNA; detection and quantification of the percentage of LDV and TBV resistance mutations
- Correlation of directly obtained cccDNA sequence and pgRNA sequences.
- Use of pgRNA sequence dynamics as surrogate marker for cccDNA population turnover

**Results:** Almost complete “switch” of WT-cccDNA to Res-cccDNA within only 3–4 month (although impaired fitness may influence transition time)

**Study ML 18376**

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<th>Treatment</th>
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<td>LVD\textsuperscript{a}</td>
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<tr>
<td>LDV+ADV</td>
<td>12 wk</td>
<td>ADV 36 wk</td>
</tr>
<tr>
<td>LDV+IFN</td>
<td>12 wk</td>
<td>IFN\textsubscript{a} 36 wk</td>
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At BL LDV resistance; W24 switch to IFN; fast rebound of WT HBV cccDNA and pgRNA

**Study EFFORT: Mono group arm**

Viral breakthrough Switch to ADV

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<th>ADV</th>
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TBV patients during breakthrough; rapid emergence of resistant viral DNA, followed by mutant pgRNA (5–28-week delay)

- Turnover rates of cccDNA populations in CHB patients are much higher than previously calculated based on NUC treatment (3–4 months instead of 14y)
- Results compatible with replenishment of cccDNA by intra- and extra-cellular (de novo virus entry) routes
- cccDNA elimination may be therapeutically achievable
How is the steady-state of cccDNA pool maintained?

Modified after Testoni, EASL 2019
How is the steady-state of cccDNA pool maintained?

Contribution of recycling seems to be limited in human hepatocytes, compared to duck and woodchuck models.

- Non-cytolytic cccDNA elimination
- Hepatocyte turnover

Modified after Testoni, EASL 2019
Decrease cccDNA synthesis

Entry inhibitors

- Only approved drug: HBlg

Pre-S1 peptide mimetics: Myrcludex-B

Capsid allosteric modulators (CAMs)

Disubstituted sulfonamides

Blockade of virions release

- rcDNA nuclear delivery
- rcDNA to cccDNA conversion
- viral RNAs
- capsids
- secretion

NUCs (and other HBV Pol inhibitors?)

Alpha-IFN

Capsid allosteric modulators (CAMs)

Nucleic acid polymers (NAPs)
Decrease cccDNA synthesis

Entry inhibitors

- Only approved drug: HBlg
- Pre-S1 peptide mimetics: Myrcludex-B
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Blockade of virions release

- rcDNA nuclear delivery
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- viral RNAs
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- secretion

- NUCs (and other HBV Pol inhibitors?)
- Alpha-IFN
- Capsid allosteric modulators (CAMs)
- RNAi, RNA destabilizers

Efficiency vs duration of therapy
Increase cccDNA loss

Non-cytolytic curing of the infected hepatocyte

cccDNA clearance can occur in vivo:
- Acute self-limiting infections
- Non replicating cells with HBV integration
  (Guidotti, 1999; Mason, 2005; 2010)

Cytokines
Guidotti, 1999
Lucifora, 2014
Xia, 2016
Qiao, 2016
Bockmann, 2019

Genome editing
CRISPR/Cas9
Ramanan, 2015
Seeger, 2016
Jiang, 2017
and others…

Small molecules
cccDNA destabilizers
Gao, 2019

We have no « numbers »

Efficiency vs Safety

Delivery
Efficiency (ann cccDNA targeted ?)
Degradation vs mutation
Selection of resistant species
Non-cytolytic curing of the infected hepatocyte

cccDNA clearance can occur in vivo:
- Acute self-limiting infections
- Non replicating cells with HBV integration
  (Guidotti, 1999; Mason, 2005; 2010)

**Cytokines**
- Guidotti, 1999
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**Genome editing**
- CRISPR/Cas9
  - Ramanan, 2015
  - Seeger, 2016
  - Jiang, 2017
  - and others...

**Small molecules**
- cccDNA destabilizers
  - Gao, 2019

**DHBV**
- Bock et al, J Mol Biol, 2001

**HBV**
- Condensed: Low transcriptional activity
- Open: High transcriptional activity

- Host factors: histones, TFs, Regulators...
- Viral factors: HBc, HBx

**Increase cccDNA loss**
Non-cytolytic curing of the infected hepatocyte

cccDNA clearance can occur in vivo:
- Acute self-limiting infections
- Non replicating cells with HBV integration
  (Guidotti, 1999; Mason, 2005; 2010)

Immune-mediated killing of the infected hepatocyte

Increased hepatocyte turnover

Liver regeneration

Inverse relationship between Hepatocyte turnover and cccDNA loads
  Mason, 2007; 2010
  Addison, 2002
  Reaiche-Miller 2013
  Allweiss, 2018

Efficiency vs liver reserve
Selection of resistant hepatocytes

Cytokines
Guidotti, 1999
Lucifora, 2014
Xia, 2016
Qiao, 2016
Bockmann, 2019

Genome editing
CRISPR/Cas9
Ramanan, 2015
Seeger, 2016
Jiang, 2017
and others...

Small molecules
cccDNA destabilizers
Gao, 2019

in vitro/mice models
A first-in-class orally available HBV cccDNA destabilizer ccc_R08 achieved sustainable HBsAg and cccDNA reduction in the HBVcircle mouse model.

ccc_R08 Reduces HBsAg, HBeAg, HBV DNA, HBV RNA, and cccDNA in HBV infected primary human hepatocyte (PHH)

Orally available ccc_R08 achieved sustained HBsAg and cccDNA reduction in the HBVcircle mouse

ccc_R08 has excellent oral availability and favorable liver enrichment in rodents.
Targeted *Silencing* of cccDNA transcription

*convert active carriers into true inactive carriers – functional cure*

Epigenetic modulators

cccDNA

High transcriptional activity

Low transcriptional activity
Targeted *Silencing* of cccDNA transcription

*convert active carriers into true inactive carriers – functional cure*

Modulation of cccDNA epigenetic control: small molecules

Epigenetic modulators

**cccDNA**

**High transcriptional activity**

**IFN**

*Belloni et al JCI 2012*

*Liu et al PlosPath 2013*

*Tropberger et al PNAS 2015*

**HATs Inhibitors**

*Tropberger et al PNAS 2015*

*Palumbo et al ACS submitted*

**Sirt3 agonist**

*Palumbo et al ACS submitted*

**HDMs Inhibitors**

Low transcriptional activity
Targeted Silencing of cccDNA transcription convert active carriers into true inactive carriers – functional cure

Modulation of cccDNA epigenetic control: small molecules

Epigenetic modulators

cccDNA

High transcriptional activity

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Tropberger et al PNAS 2015
Belloni et al JCI 2012
Liu et al PlosPath 2013

Sirt3 agonist

Palumbo et al ACS submitted

HDMs Inhibitors

Low transcriptional activity

Sirt1/3 stimulator

1,4-dihydropyridine

Sirt1/3 stimulator

MC2791

B

A

Days p.i.

0 1 10

Days p.i.

0 10 20

Capsid associated HBV DNA

3.5 Kb RNA

Capsid associated HBV DNA

3.5 Kb RNA

Actin

% of Input

1

0.02

NT T

ctrl HBV

AcH3

Arbitrary Unit

Arbitrary Unit

Arbitrary Unit

Arbitrary Unit

* ** ***

Sirt1/3 agonists acting as epidrugs:
• inhibit cccDNA transcription
• modulate acetylation of cccDNA-bound histones

Palumbo ACS 2018 submitted
Targeted Silencing of cccDNA transcription
convert active carriers into true inactive carriers – functional cure

Modulation of cccDNA epigenetic control: small molecules

Epigenetic modulators

cccDNA

High transcriptional activity

HATs Inhibitors
Tropberger et al PNAS 2015
Palumbo et al ACS submitted

Sirt3 agonist
Palumbo et al ACS submitted

HDMs Inhibitors

Low transcriptional activity

IFN

Belloni et al JCI 2012
Liu et al PlosPath 2013
Tropberger et al PNAS 2015

Funtional redundance
Off-target effects expected
Generation of a protected pool ??!!
Targeted Silencing of cccDNA transcription
convert active carriers into true inactive carriers – functional cure

Modulation of cccDNA epigenetic control: small molecules

Epigenetic modulators

cccDNA

High transcriptional activity

IFN
Belloni et al JCI 2012
Liu et al PlosPath 2013
Tropberger et al PNAS 2015

HATs Inhibitors

Tropberger et al PNAS 2015
Palumbo et al ACS submitted

Sirt3 agonist
Palumbo et al ACS submitted

HDMs Inhibitors

HDAC1
PRMT5
Suv39
SETDB1

Low transcriptional activity

HBx Inhibitors

cccDNA silencing

HBx degrades the Smc5/6 restriction factor (DDB1-Cul4 E3 ligase complex)

Decorsiere, Nature 2016
Murphy, Cell Rep 2016
Kim, JVI 2016

HBx prevents the epigenetic silencing of cccDNA

Attractive target
Virus specific
Off-cccDNA effects beneficial

Transcription +++

Belloni, PNAS 2009;
Benhenda, JVI 2013;
Ducroux, PlosPath 2014;
Riviere, J Hepatol 2015;
Decorsiere, Nature, 2015;
Alarcon, SciRep 2016;
Yang, Hepatology 2017
Should we change the paradigm

Should HBV virologist become bolder and learn from the HIV community

Neither “shock” without “Kill” nor “Kill” without “Shock” will work
cccDNA clearance vs cccDNA silencing
Implications for clinical endpoints and diagnostic tools

Possible dissociation between cccDNA levels and cccDNA transcriptional activity!

Modified from Testoni et al. 2019
Methods to investigate cccDNA activity
the cccDNA-ChIP assays the cccDNA-ChIP-Seq assay

cccDNA-ChIP assays

- HuH7 or HepG2 cells
- liver tissue
- transient transfection of linear full-length HBV monomers

cccDNA-ATAC-Seq assay

- Tropberger, PNAS 2015

Pollicino et al. Gastroenterology 2006
Belloni, JCI 2012

Floriot et al., unpublished
cccDNA clearance vs cccDNA silencing
Implications for clinical endpoints and diagnostic tools

Possible dissociation between cccDNA levels and cccDNA transcriptional activity!

HBV cure
cccDNA clearance

Measure cccDNA in liver

Modified from Testoni et al. 2019
cccDNA clearance vs cccDNA silencing
Implications for clinical endpoints and diagnostic tools

Possible dissociation between cccDNA levels and cccDNA transcriptional activity!

HBV cure cccDNA clearance

Measure cccDNA in liver

HBV functional cure cccDNA clearance

Measure cccDNA transcriptional activity in liver (pgRNA/cccDNA ratio)

Modified from Testoni et al. 2019
cccDNA clearance vs cccDNA silencing
Implications for clinical endpoints and diagnostic tools

Possible dissociation between cccDNA levels and cccDNA transcriptional activity!

HBV cure
cccDNA clearance

HBV functional cure
cccDNA clearance

Measure cccDNA in liver

qHBsAg poorly reflects cccDNA transcriptional activity
HBcrAg reflects cccDNA transcriptional activity
clinical utility limited by:
- the sensitivity of the assay
- HBeAg as a confounding

Serum
- qHBsAg
- HBcrAg
- cirHBV RNAs

Modified from Testoni et al. 2019
Can HBV be cured by antiviral therapy alone?
Can HBV be cured by antiviral therapy alone?

If cccDNA half life is shorter than presumed

You may think of it as « intensification »

If lowering HBsAg is sufficient to restore immune responses

YES
Can HBV be cured by antiviral therapy alone?

**YES**

If cccDNA half life is shorter than presumed,

*You may think of it as « intensification »*

If lowering HBsAg is sufficient to restore immune responses

**Definition of immune control**

Immune control vs epigenetic control

Remember the occult HBV infection

---

Adapted from: Bertoletti  A SHC 2019
Open questions

**HBV cure / cccDNA clearance is better than HBV functional cure / cccDNA silencing**

<table>
<thead>
<tr>
<th>The risk of reactivation is eliminated</th>
<th>strong biological basis</th>
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<tr>
<td>The risk of HCC is lower</td>
<td>unproven but likely</td>
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</table>

- Risk of cancer is not increased in « occult HBV » infection in the absence of liver co-morbidities
- Stochastic risk of « highly oncogenic integrants « (i.e. in HCC driver genes) is not reduced
- Integrants rearrangement is reduced (but the same might be true in HBsAg +ve NUC suppressed patients of persistently HBV inactive infection)
Open questions relevant for research efforts

What distinguish a persistent HBV inactive infection (HBsAg positive) from

- patients who recovered from acute infection
- patients who lost HBsAg spontaneously after becoming chronic carriers
- patients who lost HBsAg after treatment
- patients with occult HBV infection

Integrated view of clinical, virological, liver microenvironment and immunological parameters in well characterized patients

Generate new knowledge on:

- cccDNA pool across CHB phases and under therapy
- cccDNA epigenetic regulation in vivo
- HBV integration burden

Validated diagnostics for cccDNA and HBV RNAs detection and quantitation needed
Keep you informed about HBV cure

Come to Paris !!!

Follow on the web !!!

6th ANRS HBV Cure Workshop
Paris, Monday May 13th, 2019

5th ANRS HBV cure workshop
Paris, Tuesday April 10th, 2018

7th ANRS HBV Cure Workshop

International Coalition to Eliminate HBV
Collaborations:
Jane Mc Keating – Oxford, UK
Ourania Andrisani – Purdue IN, USA
Maura Dandri - Hamburg, Germany
Carlo Ferrari - Parma, IT
Letizia Chiodo, Giancarlo Ruocco – Roma, IT
Sabrina Strano, Giovanni Blandino – Roma, IT

Financial support

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Ludovica Calvo
Debora Salerno

Lab of Gene Expression

Fabien Zoulim
Barbara Testoni
Fanny Lebossé
Caroline Scholtes
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modified from Levrero et al, CoViRo, 2018