



**SAC Meeting on
Emerging and Reemerging
Infectious Diseases**

**September 14 – 15, 2015
Fondation Mérieux
Les Pensières Conference Centre**

Moving forward HBV cure

Massimo Levrero

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IIT – Sapienza Center for Life-Nanosciences
INSERM U1052 - CRCL - Lyon*

Disclosures

Massimo Levrero

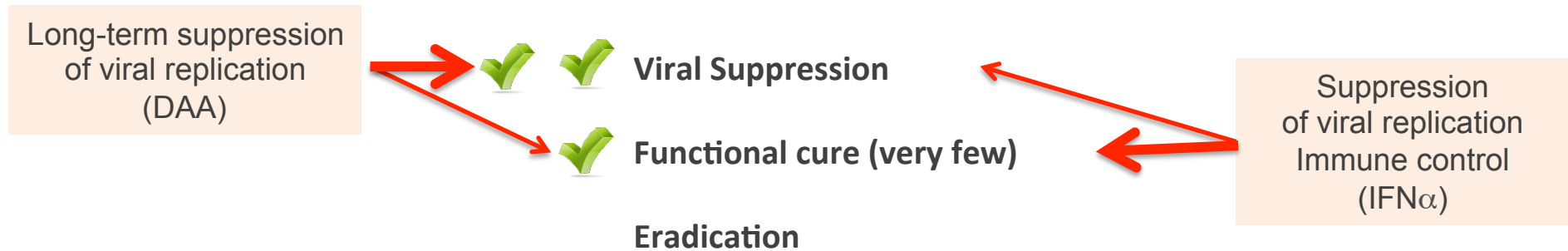
Advisory Committees or Review Panels:

- BMS
- Jansen
- Gilead
- Tekmira
- Galapagos
- Assembly Pharma
- Sanofi/Aventis

Speaking and Teaching: - MSD

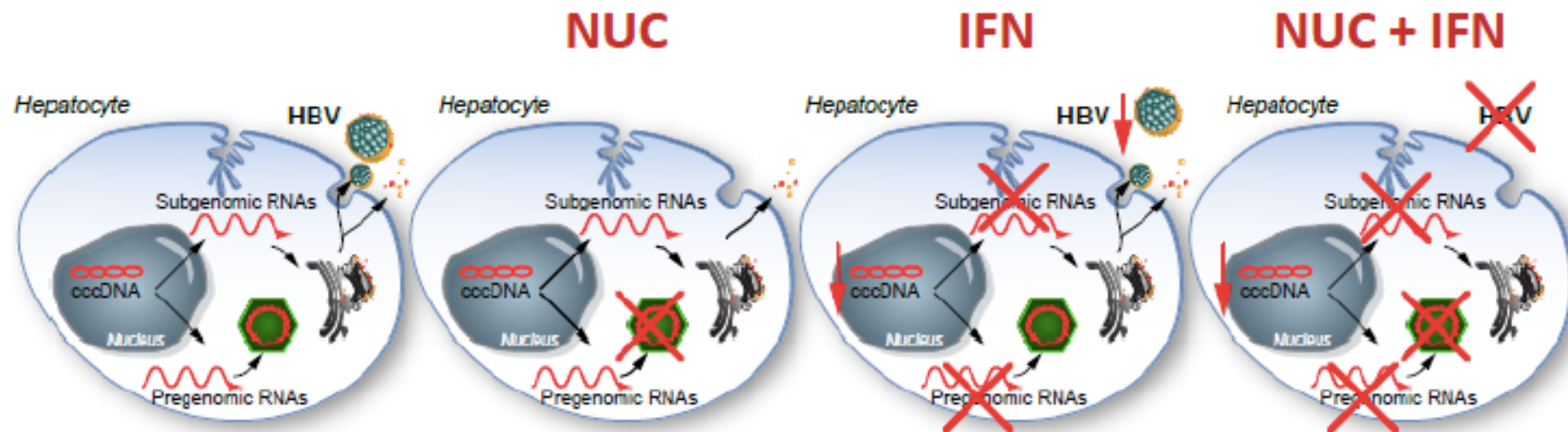
- Roche
- BMS
- Jansen
- Gilead

HBV: concepts about « cure »



NAs and PegIFN used in combination therapy

PEG and NUC have different mechanisms of action

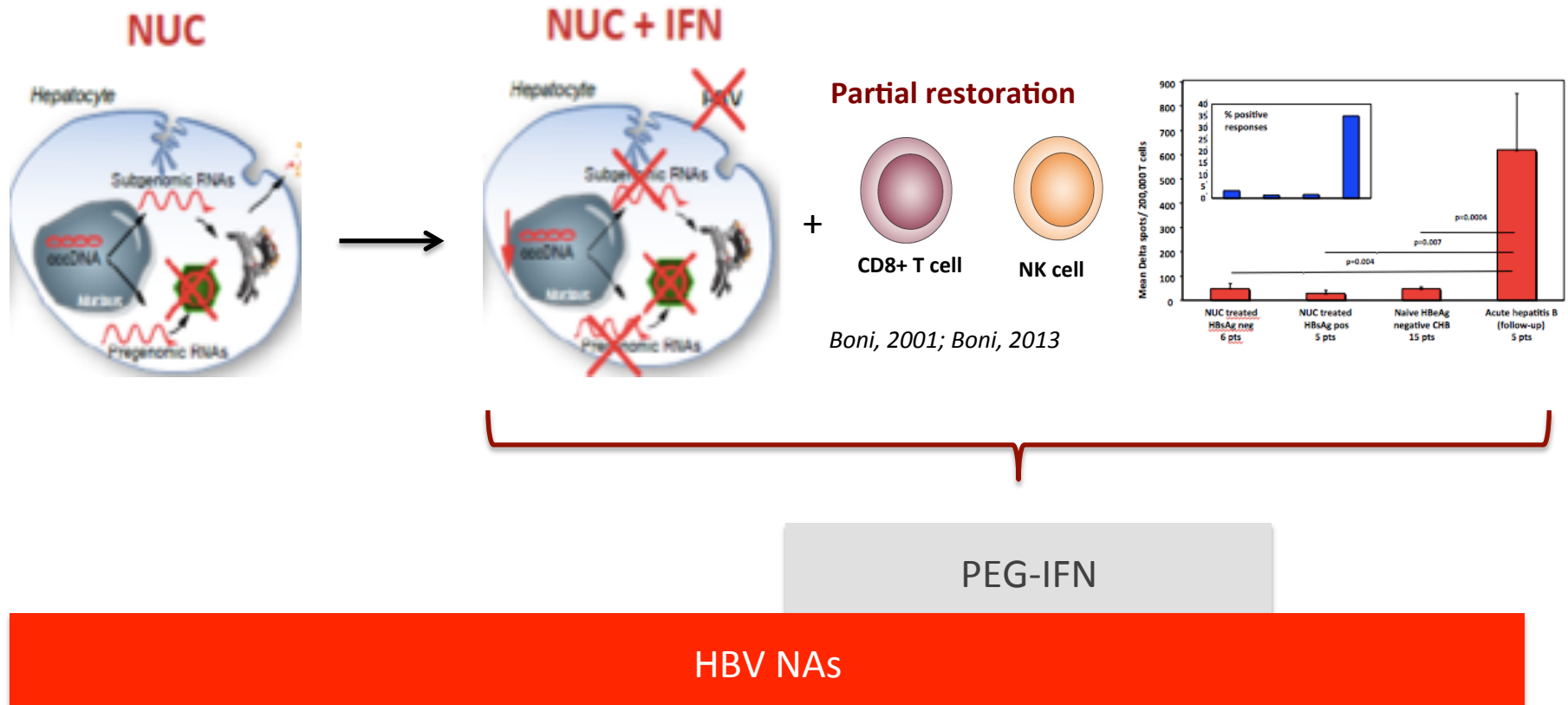


Adapted from Thimme & Dandri, J Hepatol 2012;58:205-9

Studies in patients and humanized mice indicate that combination treatments suppressing both HBV replication (NUCs) and cccDNA transcription (IFN α) may trigger significant antigen decline (HBe and HBs) – combination needs to be done in a smart way

- should have additive or synergistic activity against HBV
- should have no added toxicity
- may induce cccDNA loss or control and higher rates of HBsAg loss

Pre-treatment with NAs



PEG-IFN *add-on*

HBV NAs

PEG-IFN

ARES (HBe+, ETV) [Brouwer, Hepatology 2014]
Increased HBs decline

HBV NAs

PEG-IFN

PEGON (HBe+, NA) [Chi, AASLD 2014]
Increased HBe loss, and HBs decline

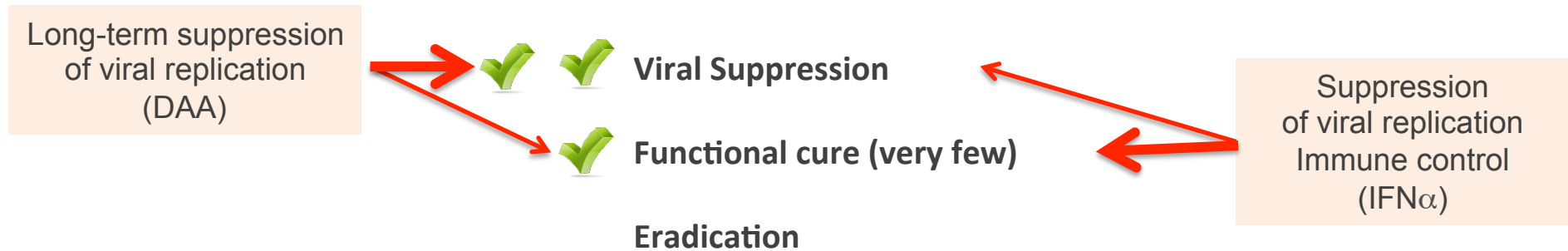
HBV NAs

PEGAN (HBe -, NA) [Bourliere, EASL 2015]
Low HBs loss (7.7% IT; 10.7% PP + full IFN)
Low BL HBs predictive

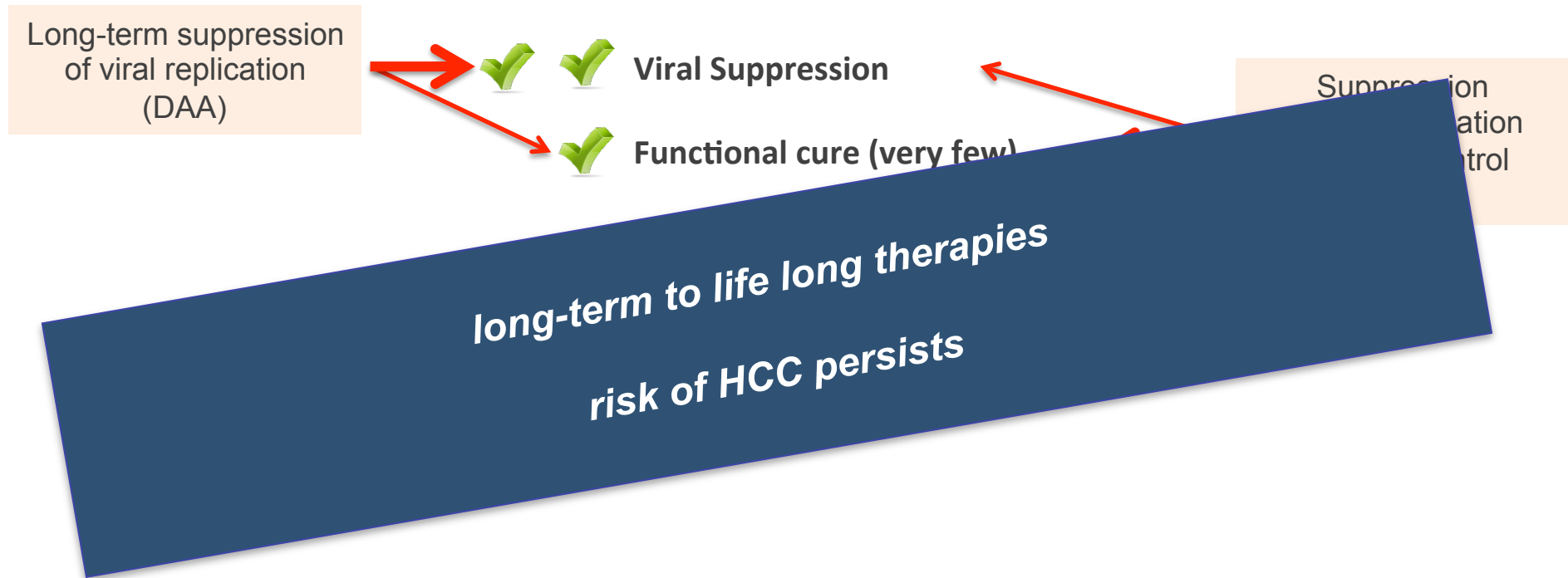
HBV NAs

HERMES (HBe -, NA) [Lampertico, AASLD 2014]
Increased HBs decline,
1 pt HBs loss; 2 pts qHBs < 10 UI

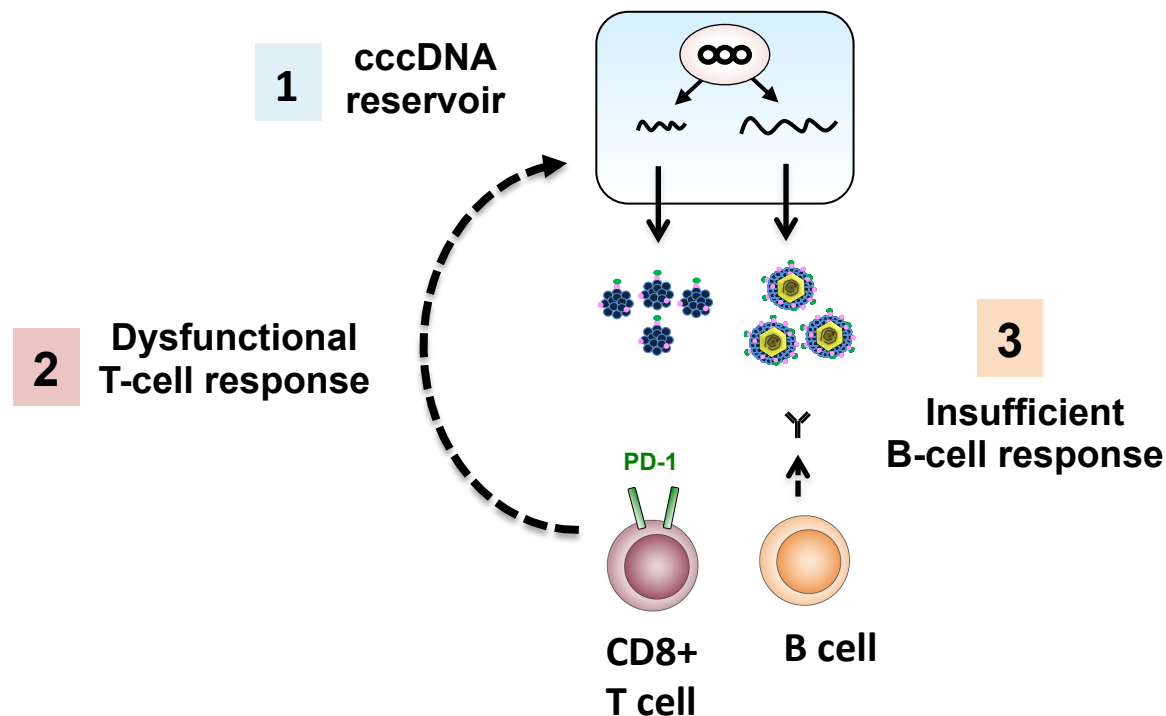
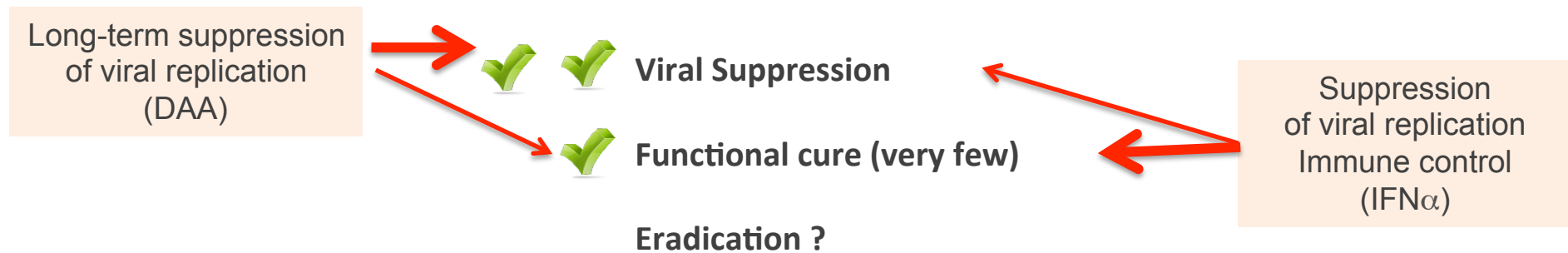
HBV: concepts about « cure »



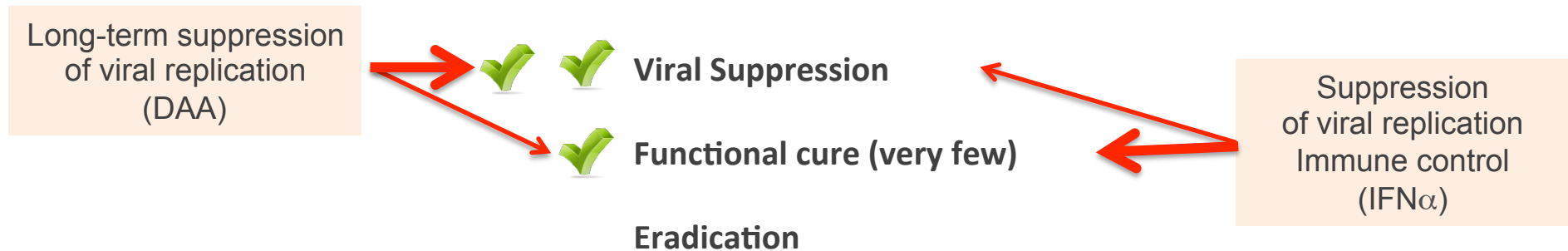
HBV: concepts about « cure »



Barriers to Resolution of Chronic HBV Infection



HBV: concepts about « cure »



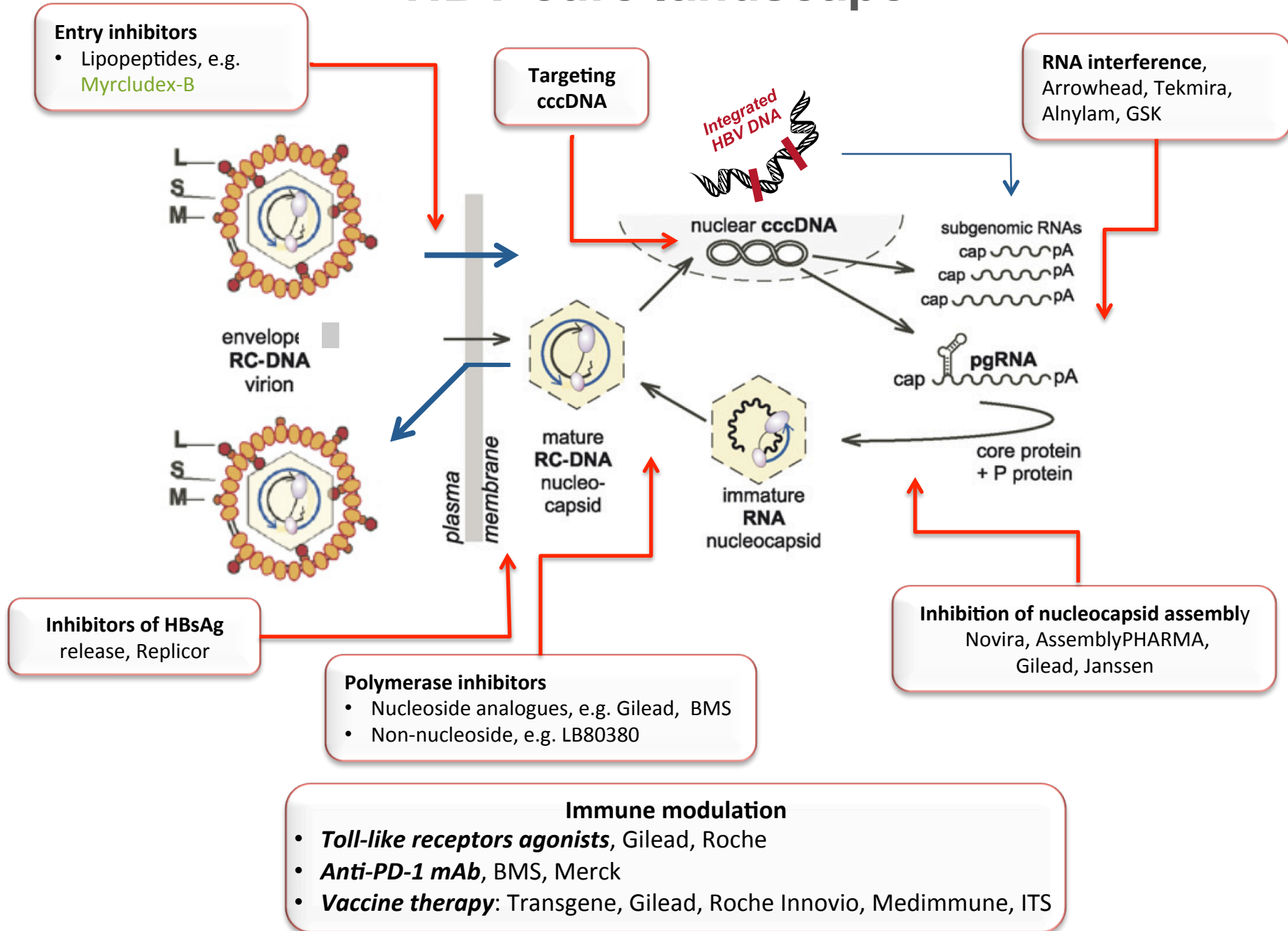
Strategies

Immune system

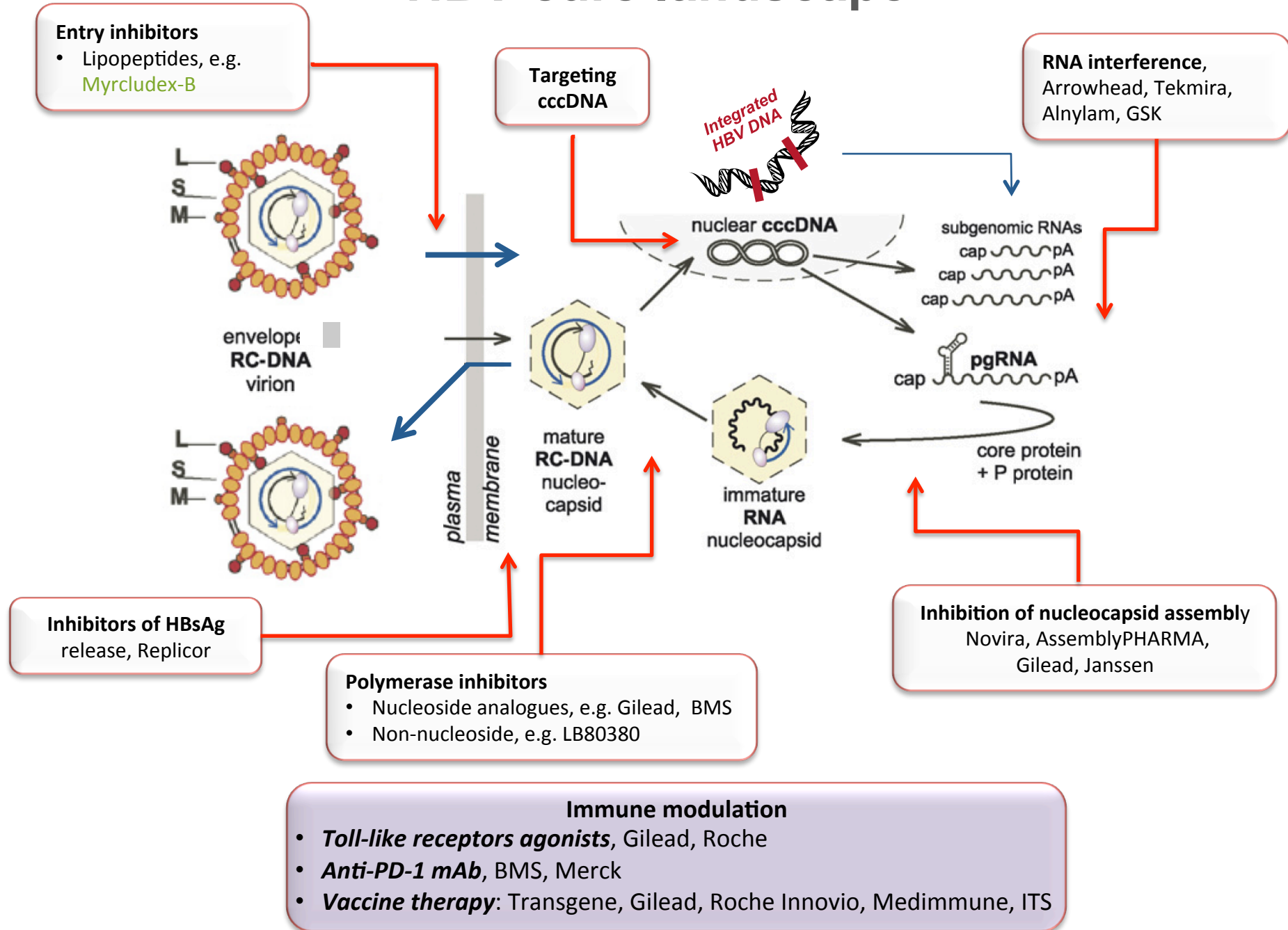


Viral targets

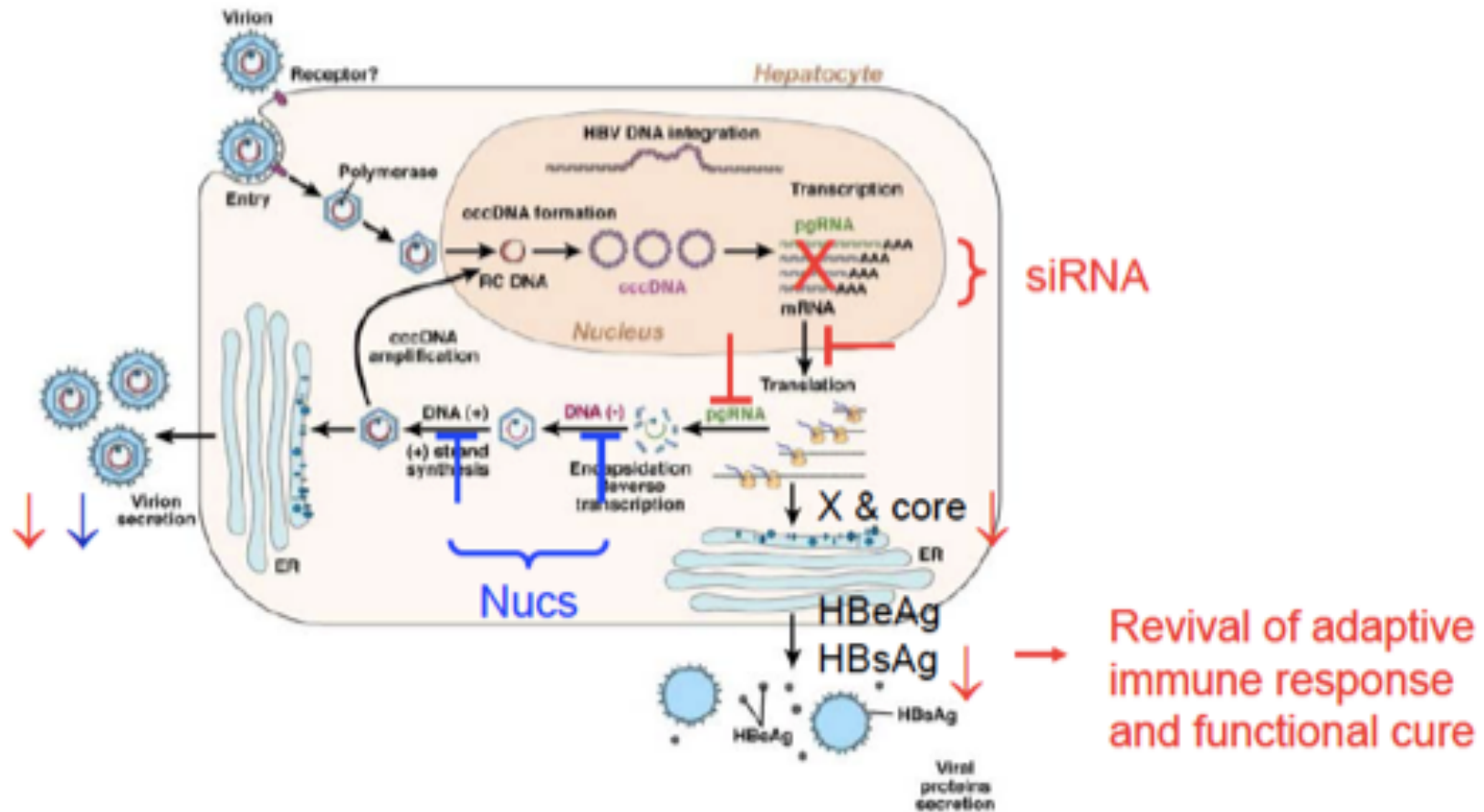
HBV cure landscape



HBV cure landscape

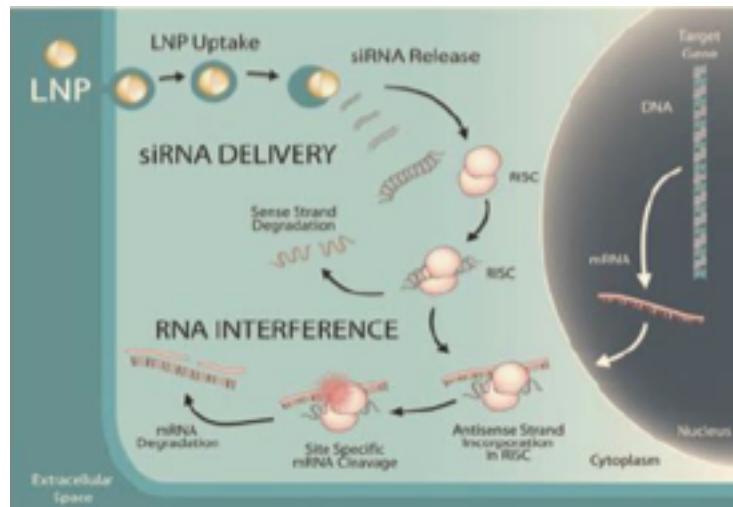


RNAi treatment for chronic hepatitis B



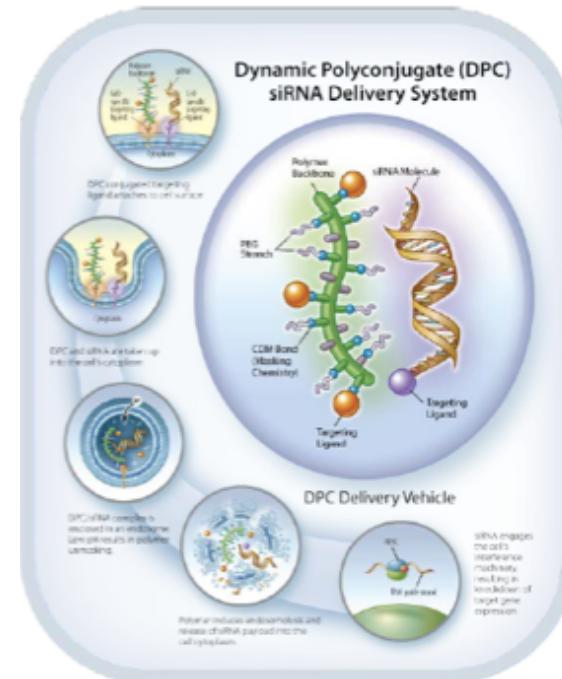
RNA-interference (RNAi) mechanism: role of delivery

Tekmira ONCORE
BIOPHARMA, INC.



1. Shield siRNA from serum nuclease to avoid degradation
2. Induce cellular uptake by target organ/cells
3. Promote endosomal escape
4. Potent delivery of payload to RISC machinery

Arrowhead Research
CORPORATION



- **DPC polymer composition and physical characteristics**
 - Amphipathic peptide
 - peptide amines reversibly "masked" with CDM
 - Slightly negatively charged
- **Cellular uptake of peptide is ligand-driven (N-acetyl galactosamine (NAG)) for hepatocytes**
- **siRNA is made liver tropic by attachment of lipophilic ligand (e.g. cholesterol)**
- **↓ pH in endosomes drives peptide unmasking**
- **Unmasked peptide disrupts endosomal membrane**

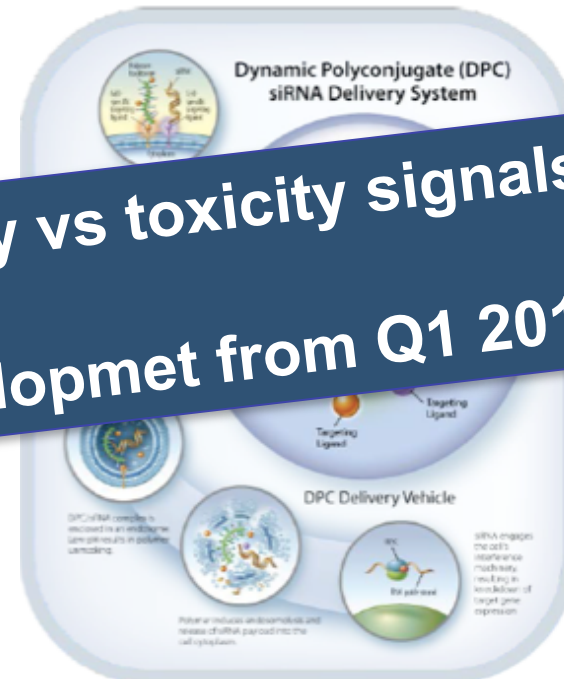
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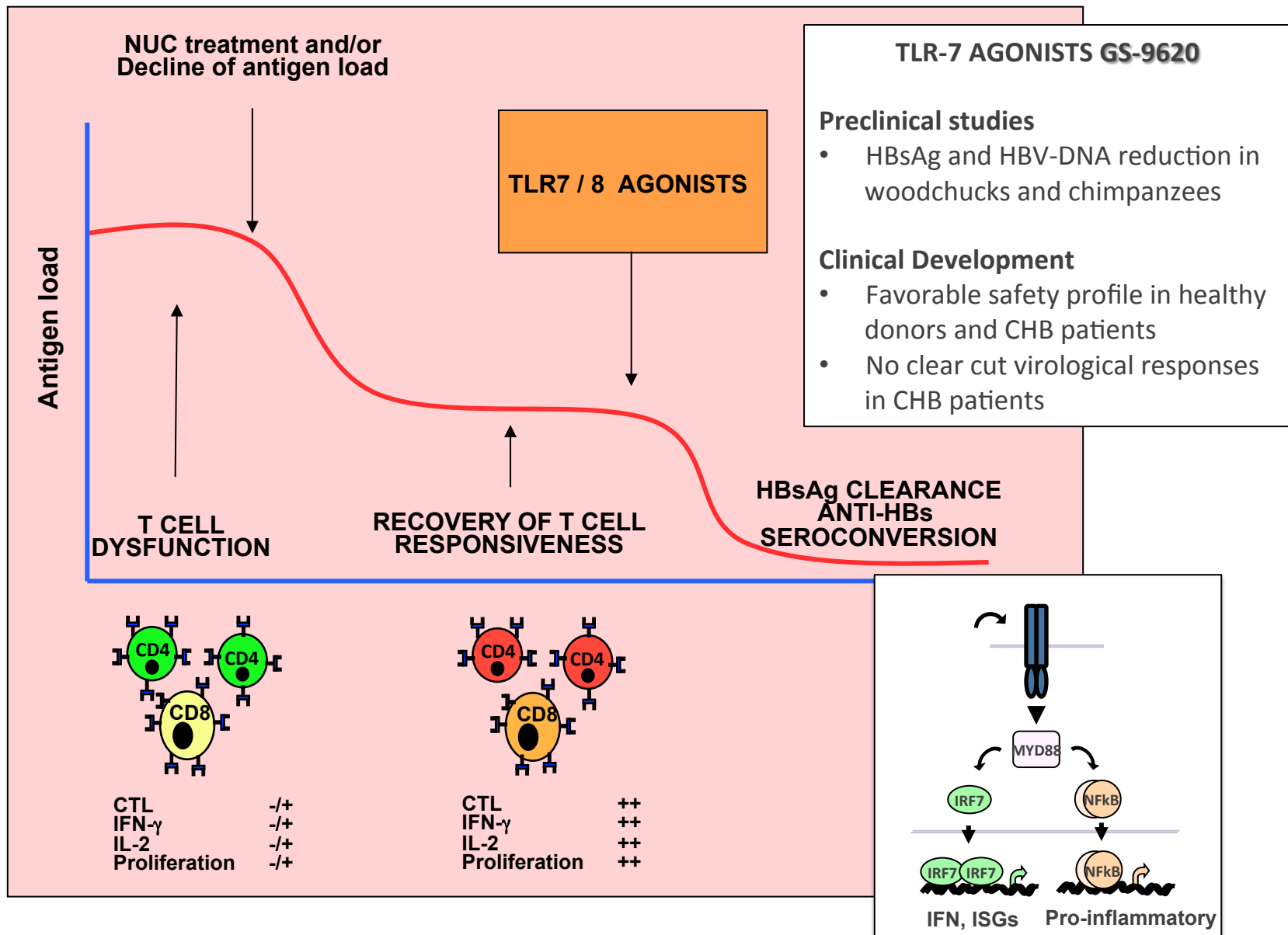


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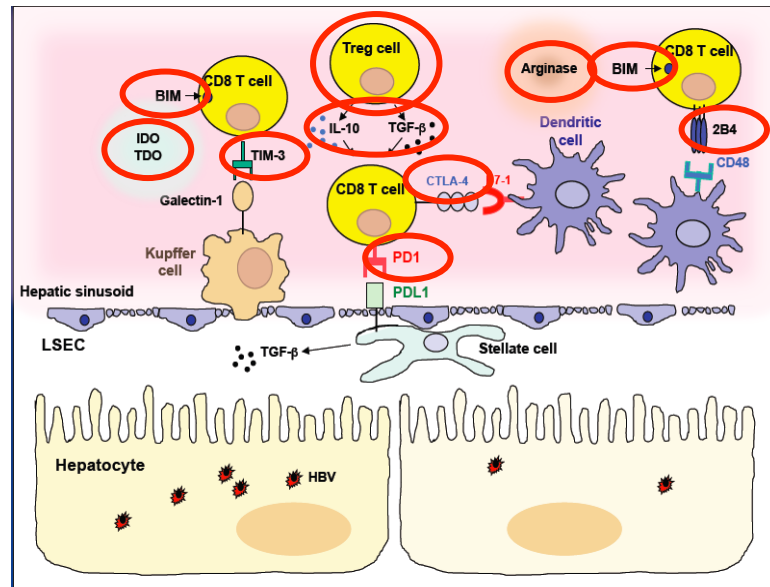


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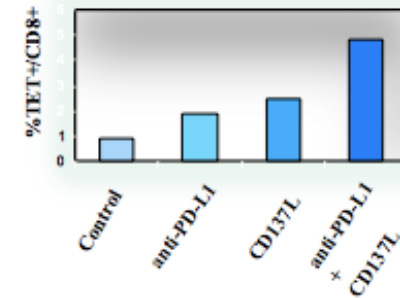
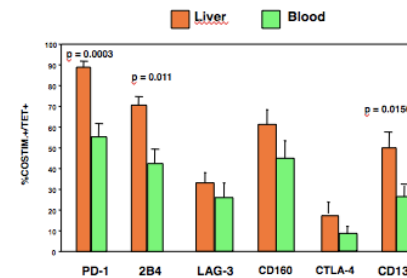
Modified from: Ferrari C. Gastroenterology 2008

BLOCKING INHIBITORY RECEPTORS ON T CELLS



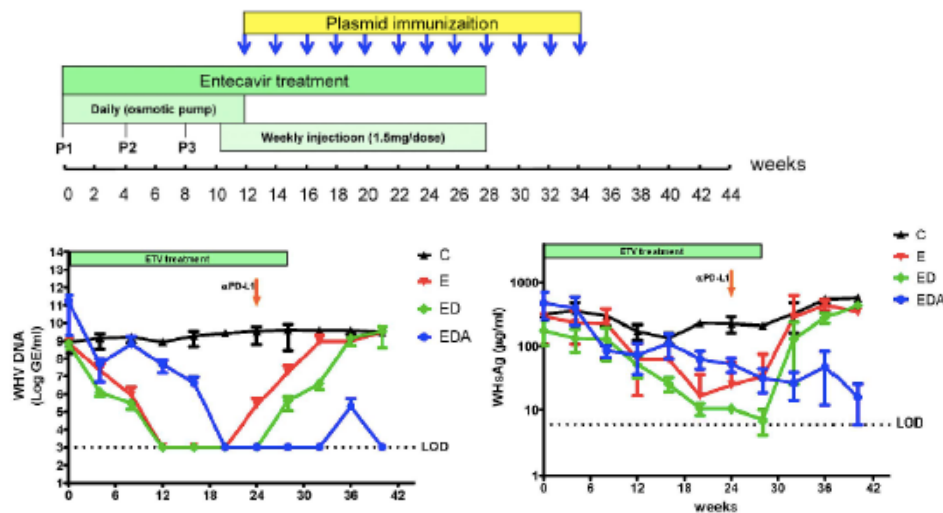
Modified from U. Protzer et al. Nature Reviews in Immunology 2012

RESTORATION OF THE T CELL FUNCTION BY COMBINED MANIPULATION OF PD-1/PD-L1 AND CD137/CD137L PATHWAYS

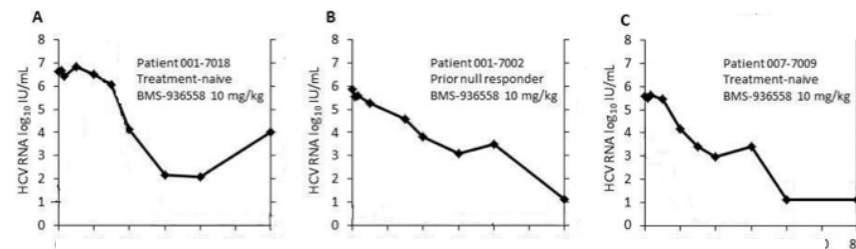


Fisicaro P et al Gastroenterology 2012

PD-1 PATHWAY BLOCKADE Proof of concept of α -PD-1 in Chronic HCV



Liu et al. PLoS Pathog. 2014 Jan 2;10(1):e1003856.

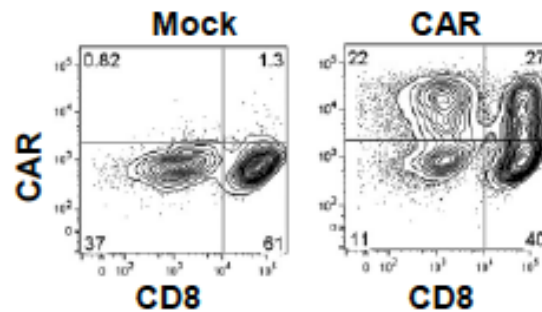


- Blinded, PBO controlled, SAD study
- α -PD-1 in 54 HCV infected patients, IFN failures and treatment naive
- 0.03mg/kg -10mg/kg
- 3 subjects w/ > 4 log HCV RNA decline: All 3 received 10mg/kg dose
 - 1 subject (A) had isolated, transient Grade 4 ALT increase to ~17x ULN
 - 1 subject (B) undetectable > 1 year post treatment

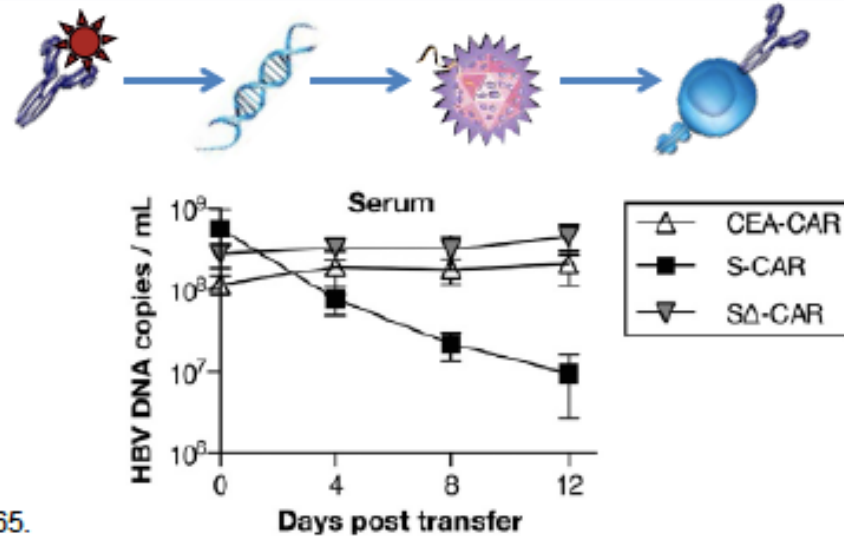
Gardiner et al. 2013. PLoS ONE 8(5): e63818.

Engineering anti-HBV Immunity

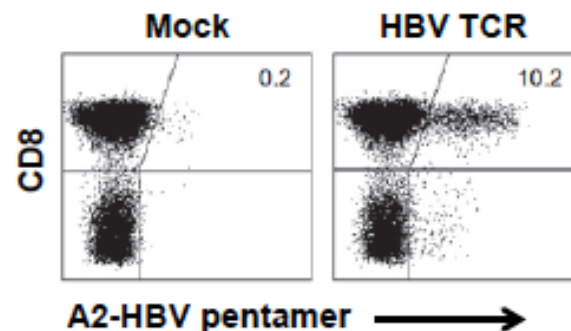
Chimeric antigen receptor (CAR)



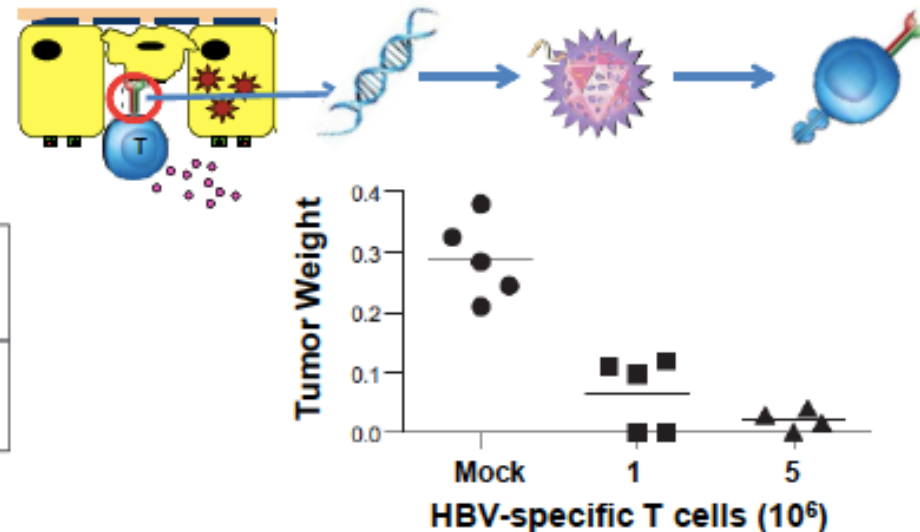
Krebs et. al. Gastroenterology. 2013 Aug;145(2):456–65.



T cell receptor (TCR) gene therapy



Gehring et. al. J Hepatol. 2011 Jul 1;55(1):103–10.

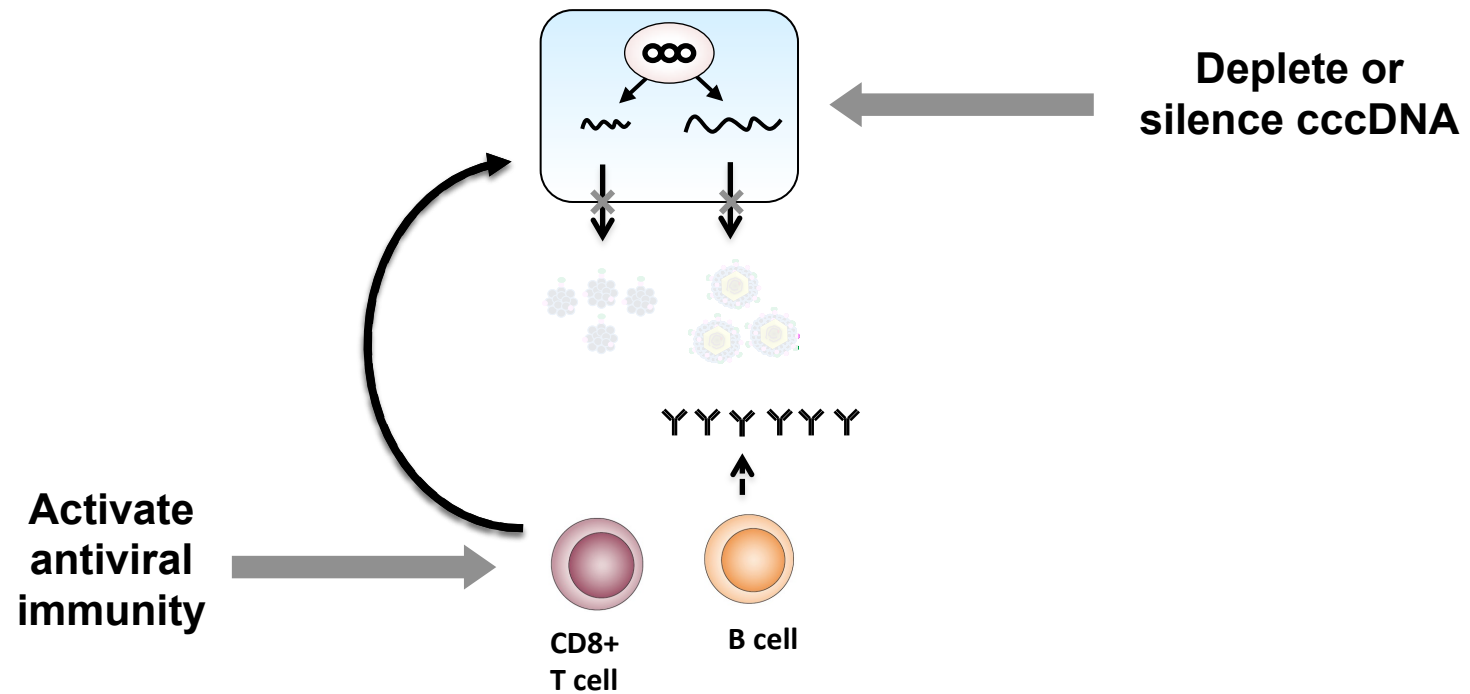


HBV Cure

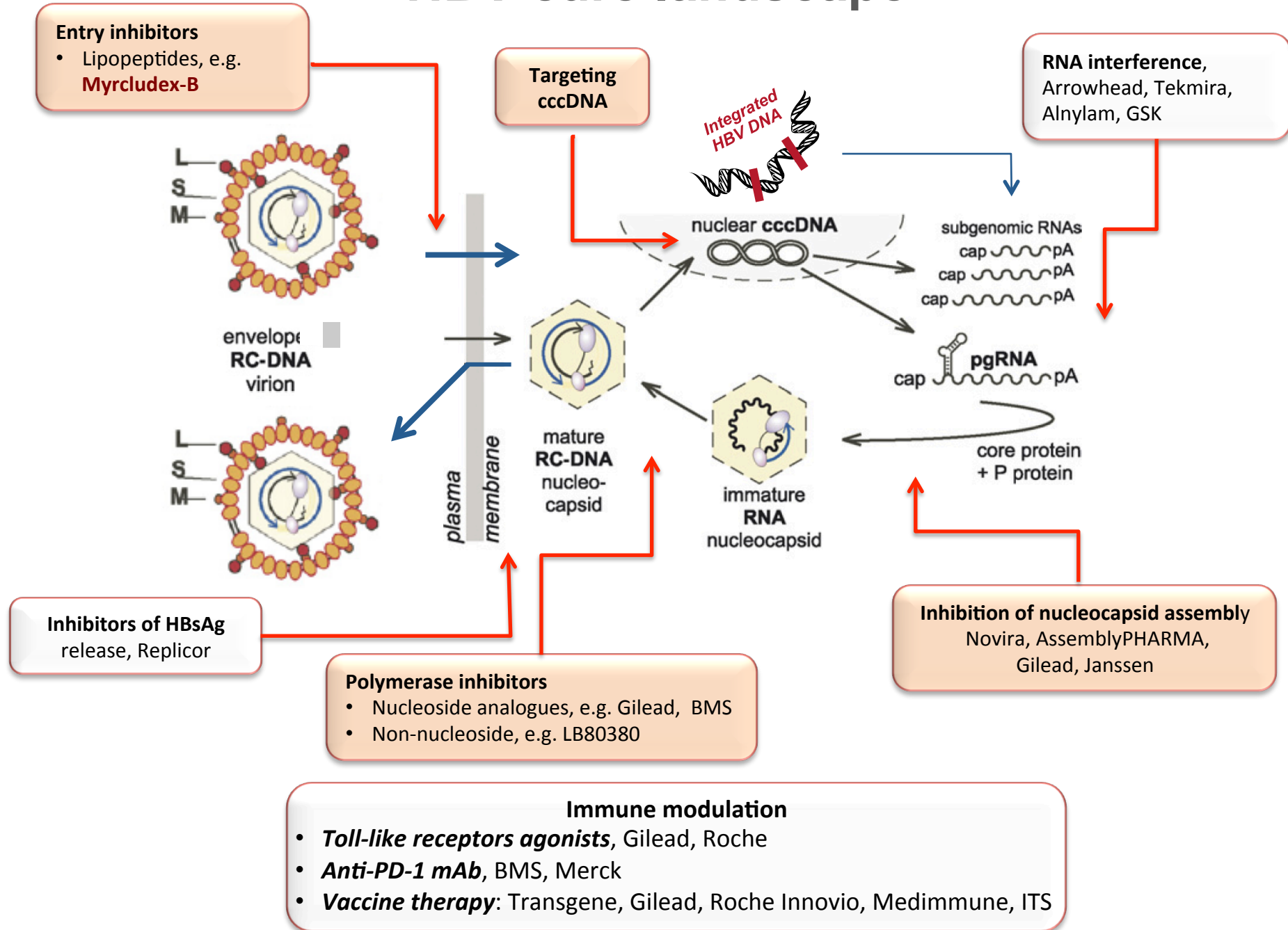
Strategies

Immune system

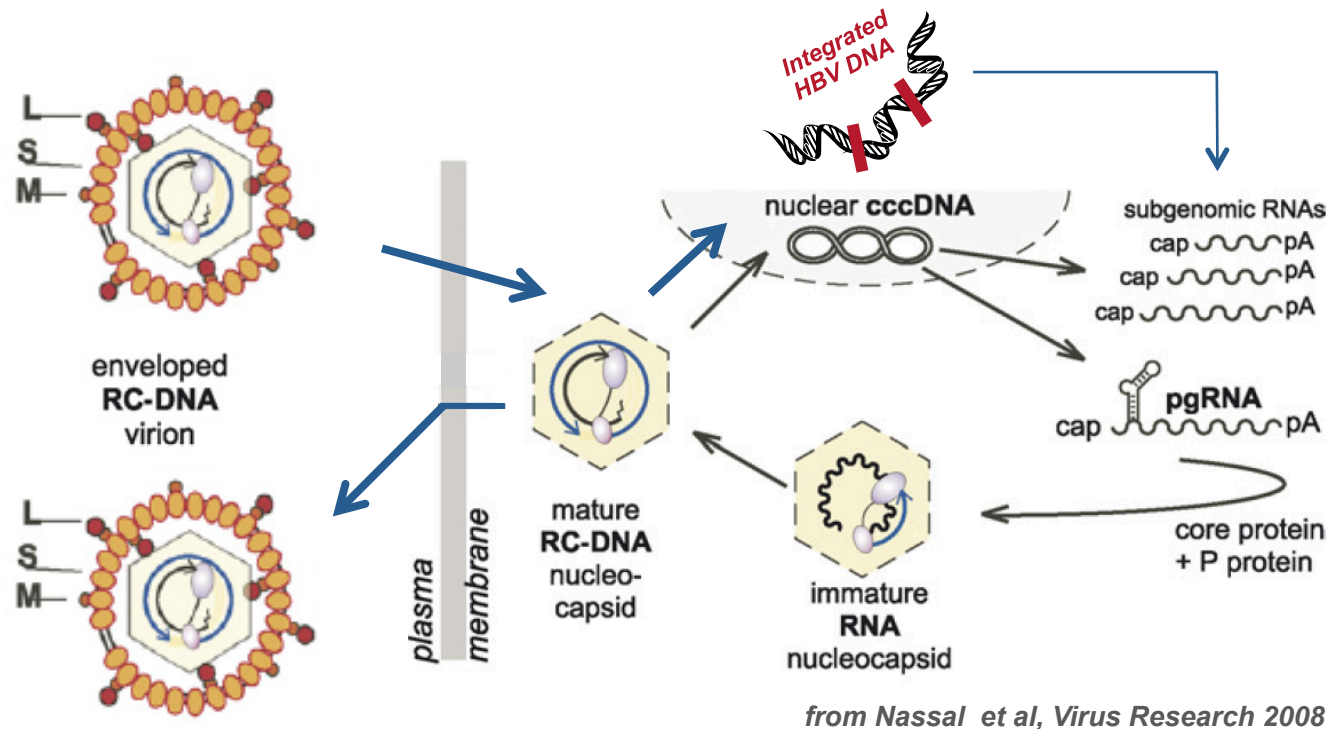
Viral targets



HBV cure landscape

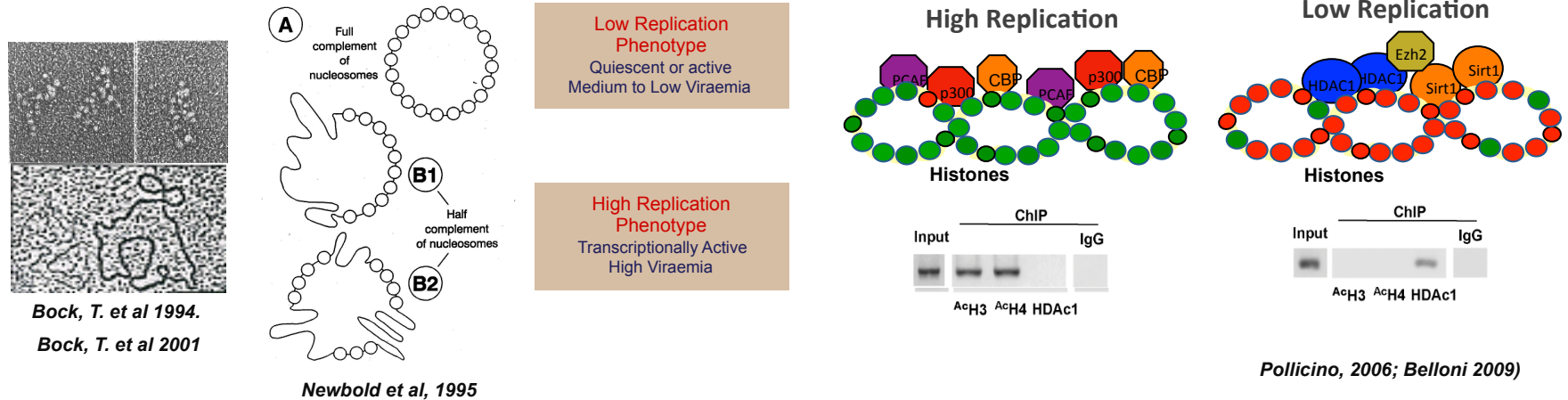


HBV cccDNA



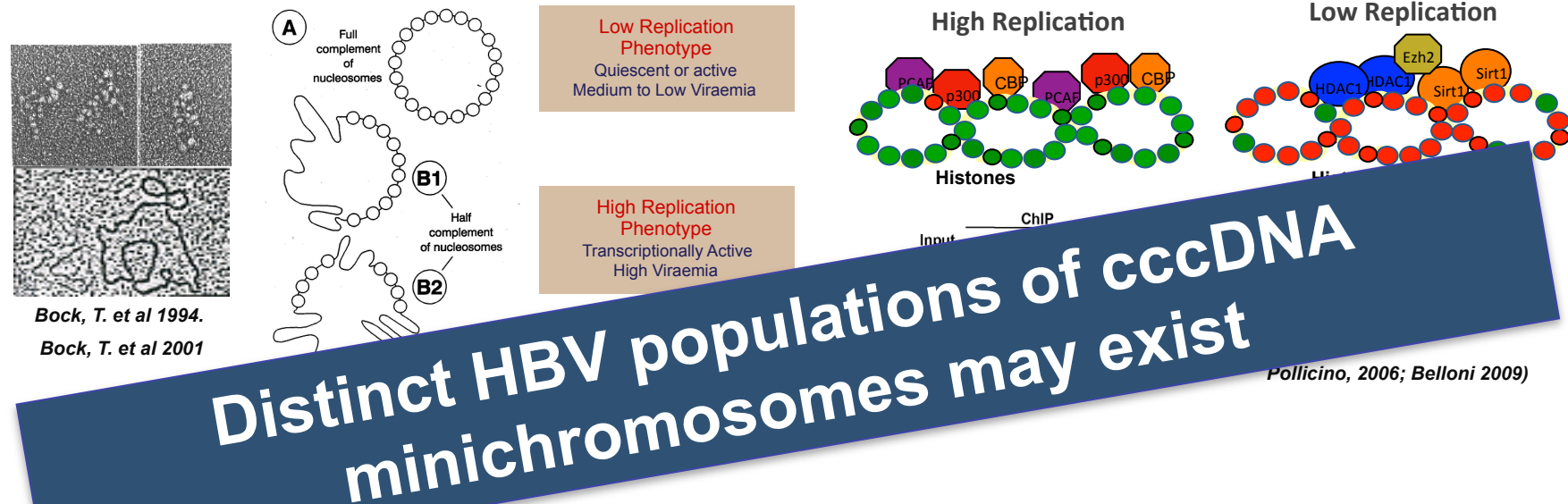
- template for all HBV mRNAs and the HBV pgRNA
- not directly targeted by NUCs
- may exist hepatic “latent” reservoir (non integrated functionally competent HBV genomes): OBI and inactive carriers

The HBV cccDNA as a “minichromosome”



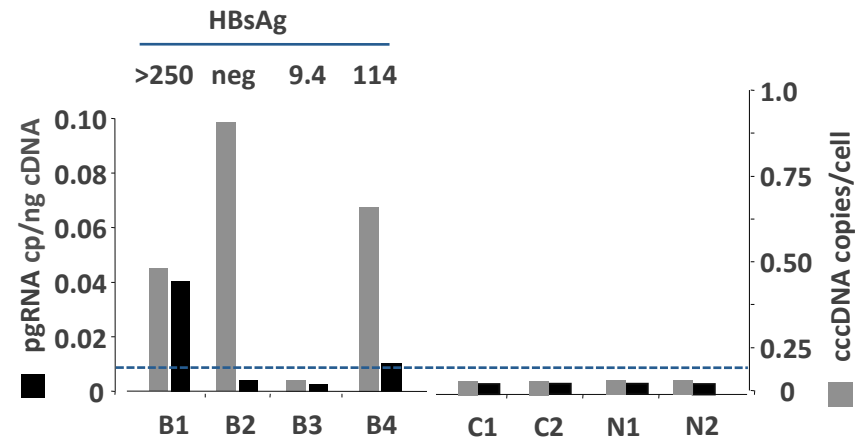
- HBV cccDNA is organized as a minichromosome in the nucleus of infected cells by histone and non-histone proteins (Newbold 1995, Bock 2001, Pollicino 2006).

The HBV cccDNA as a “minichromosome”



- HBV cccDNA is organized as a minichromosome in the nucleus of infected cells by histone and non-histone proteins
(Newbold 1995, Bock 2001, Pollicino 2006)

Persistence of cccDNA

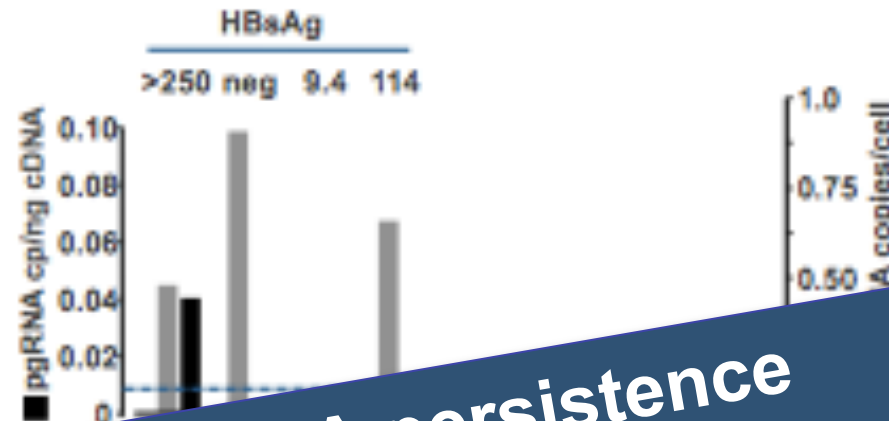


Persistence of cccDNA in 3 out of 4 patients with long term HBV suppression under lamivudine
In 2 out 3 patients cccDNA is inactive (no pgRNA)

Belloni, Levrero, Gaeta HBV meeting 2010

- 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]

Persistence of cccDNA



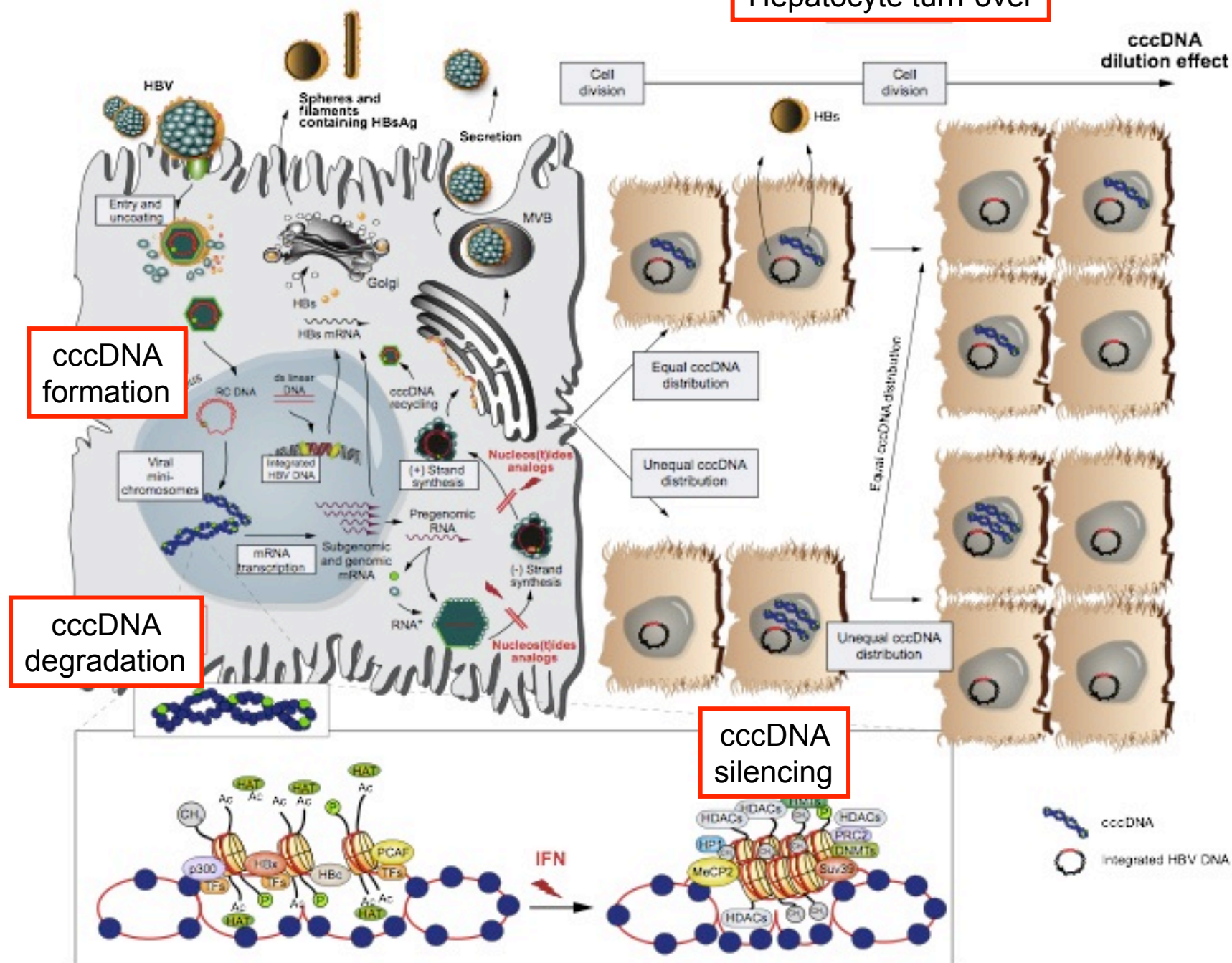
cccDNA persistence

active cccDNA (pgRNA pos)
inactive cccDNA (pgRNA neg)

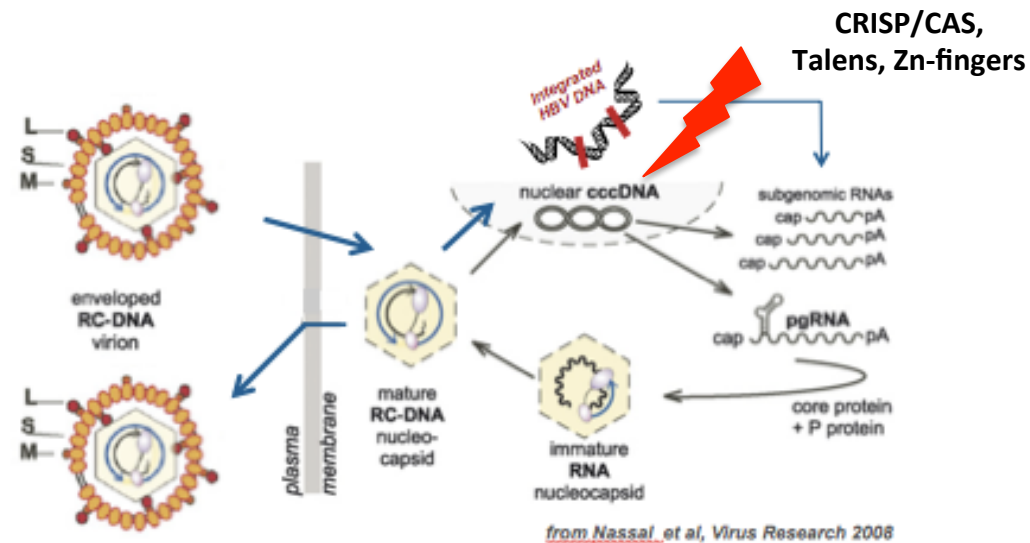
directly target cccDNA
persists in NUC long-term suppressed patients, occult HBV infection and subjects recovered from AVH

(Werle-Lapostolle 2004; Pollicino 2004; Maynard, 2005; Belloni, 2010)

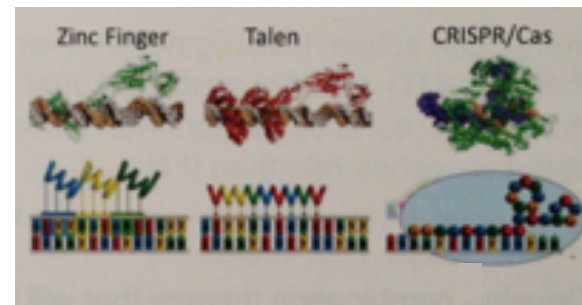
Hepatocyte turn-over



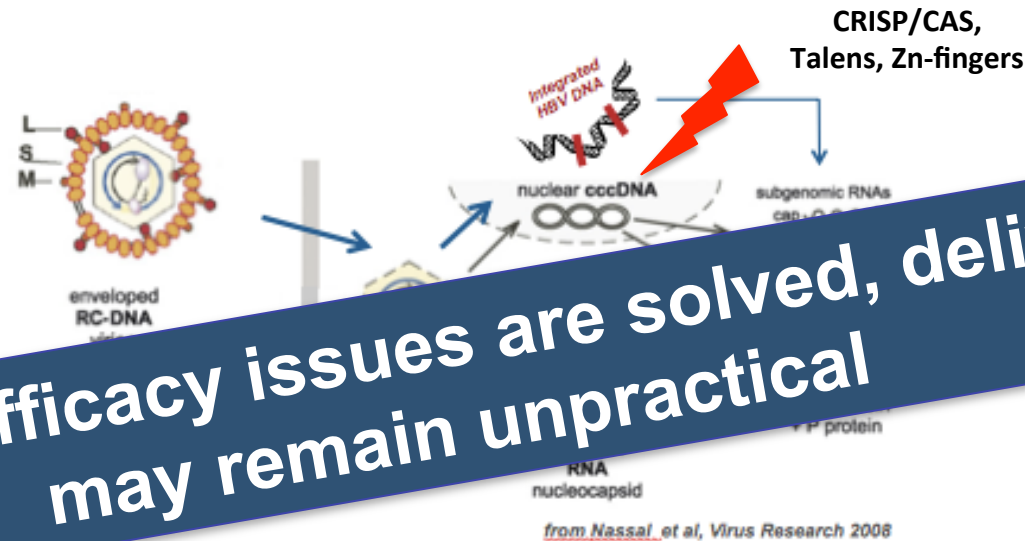
Cleavage of cccDNA by targeted gene disruption strategies



- ◆ **Zinc Finger** [Weber, PlosOne, 2014]
- ◆ **Talens** [Chen, Mol Therapies, 2014]
- ◆ **Bacterial CRISP / Cas**
RNA-guided DNA endonucleases
[Seeger, Mol Therapy Nucl Acids, 2014;
[Lin, Mol Therapy Nucl Acids, 2014;
[Kennedy, Virology, 2015]

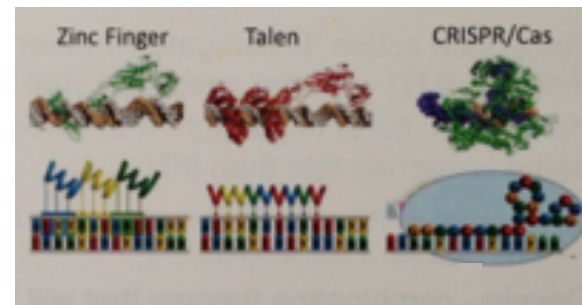


Cleavage of cccDNA by targeted gene disruption strategies

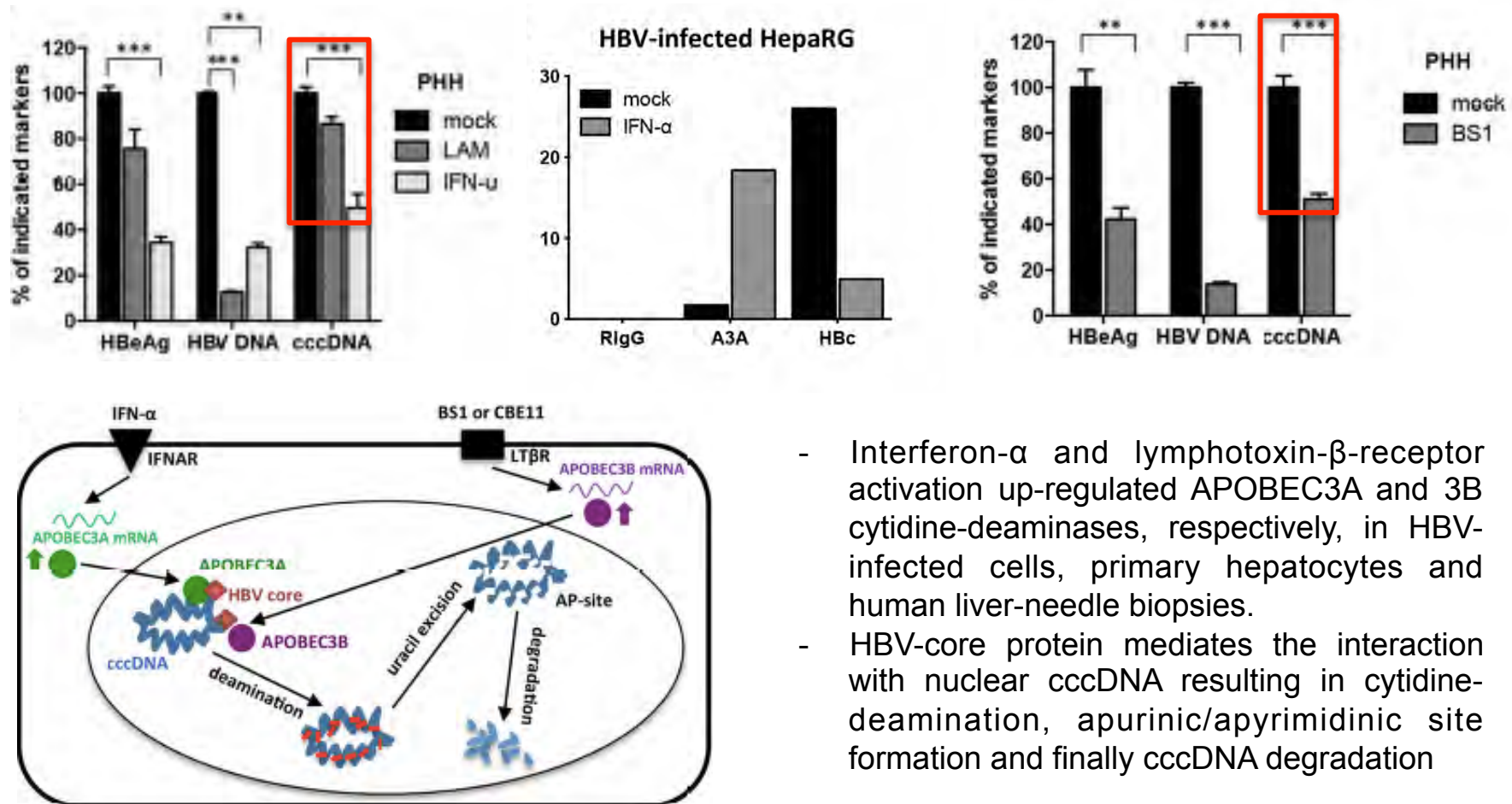


Even if efficacy issues are solved, delivery may remain impractical

- ◆ Zinc Finger [Weber, PlosOne, 2014]
- ◆ Talens [Chen, Mol Therapies, 2014]
- ◆ Bacterial CRISP / Cas
RNA-guided DNA endonucleases
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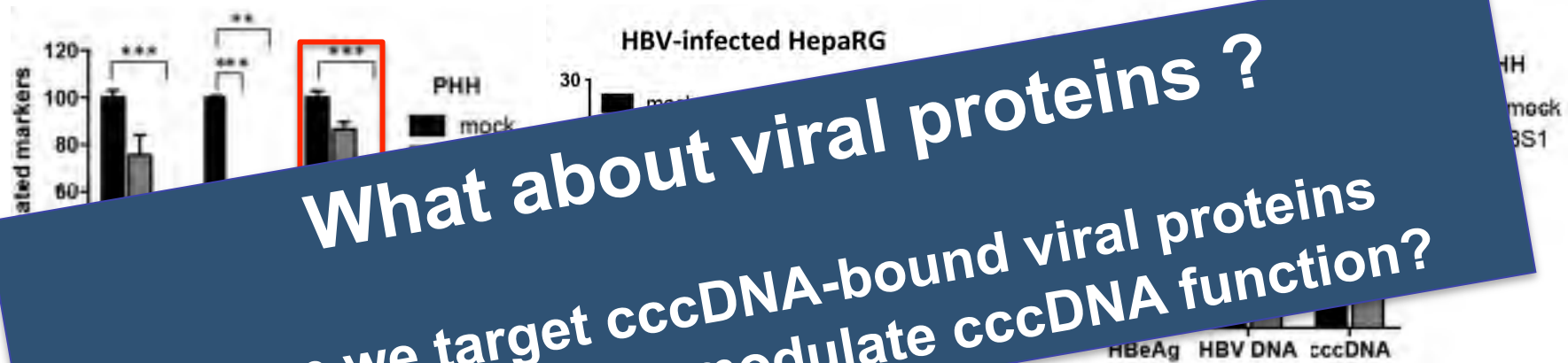


Specific and Nonhepatotoxic Degradation of Nuclear Hepatitis B Virus cccDNA

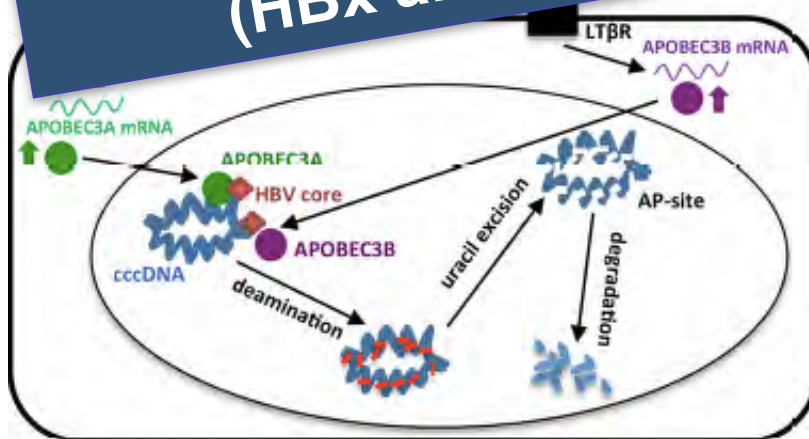


- Interferon-α and lymphotoxin-β-receptor activation up-regulated APOBEC3A and 3B cytidine-deaminases, respectively, in HBV-infected cells, primary hepatocytes and human liver-needle biopsies.
- HBV-core protein mediates the interaction with nuclear cccDNA resulting in cytidine-deamination, apurinic/apyrimidinic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

Specific and Nonhepatotoxic Degradation of Nuclear Hepatitis B Virus cccDNA



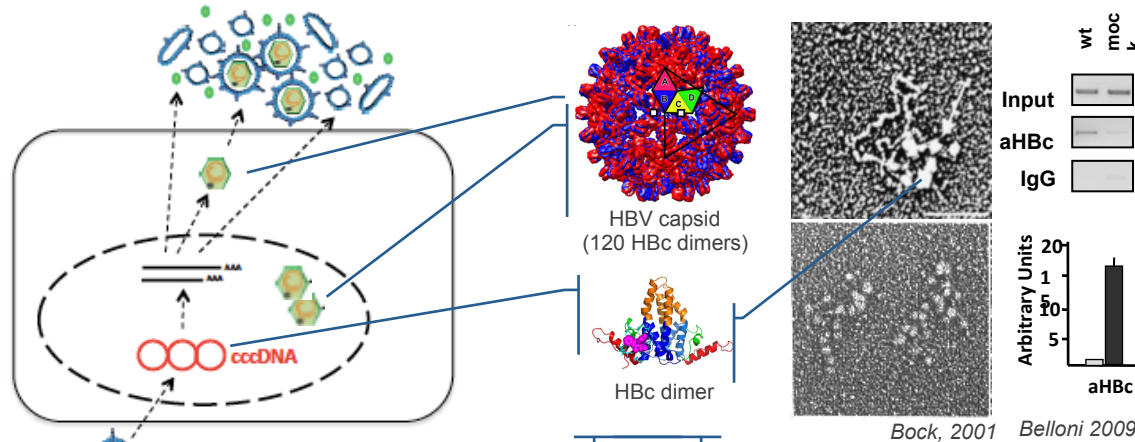
What about viral proteins ?
can we target cccDNA-bound viral proteins (HBx and HBc) to modulate cccDNA function?



- 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.
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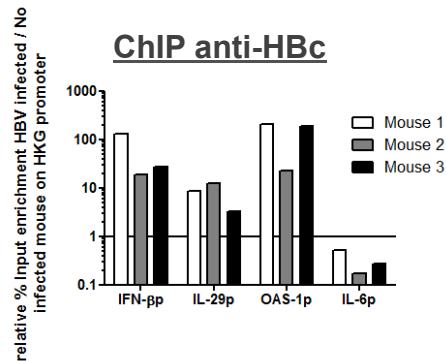
HBc protein / capsid

- ◆ HBc binds the cccDNA and modifies cccDNA nucleosome spacing



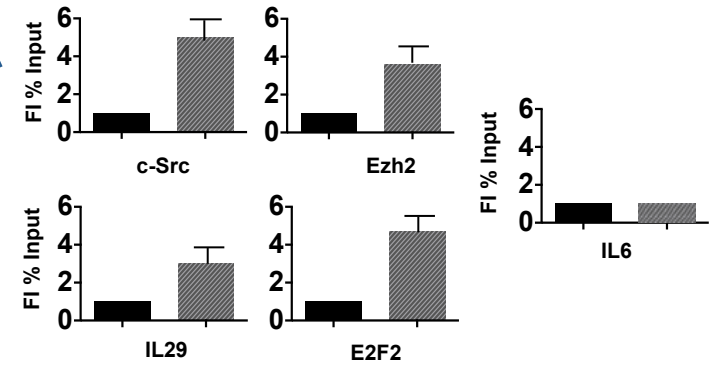
- ◆ HBc binds to (and represses) the IFN- β , IL-29 and OAS1 cellular promoters

(Durantel D, AASLD 2013)



- ◆ HBc binds to cellular promoters and regulates gene expression

(Guo, BMC genomics, 2013)



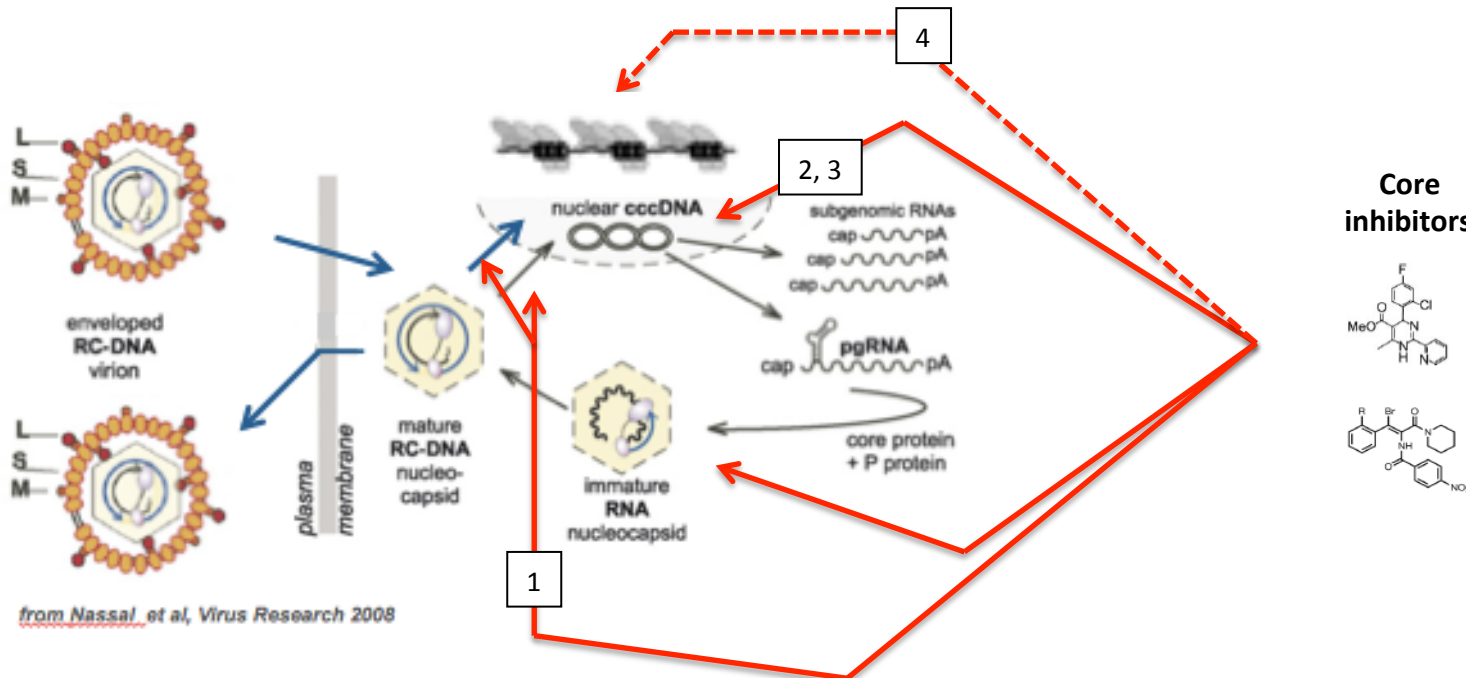
Lupacchini (unpublished)

Anti-capsid drugs

- Core inhibitors (Hap12 and AT130) impact on Cp nuclear functions at multiple levels:

- block new cccDNA accumulation (Rc-DNA delivery and/or core particles recycling) 1
- reduce the size of an *established* cccDNA pool 2
- inhibit HBc recruitment on the cccDNA 3

The effects on HBc recruitment on cellular genes remains to be determined 4

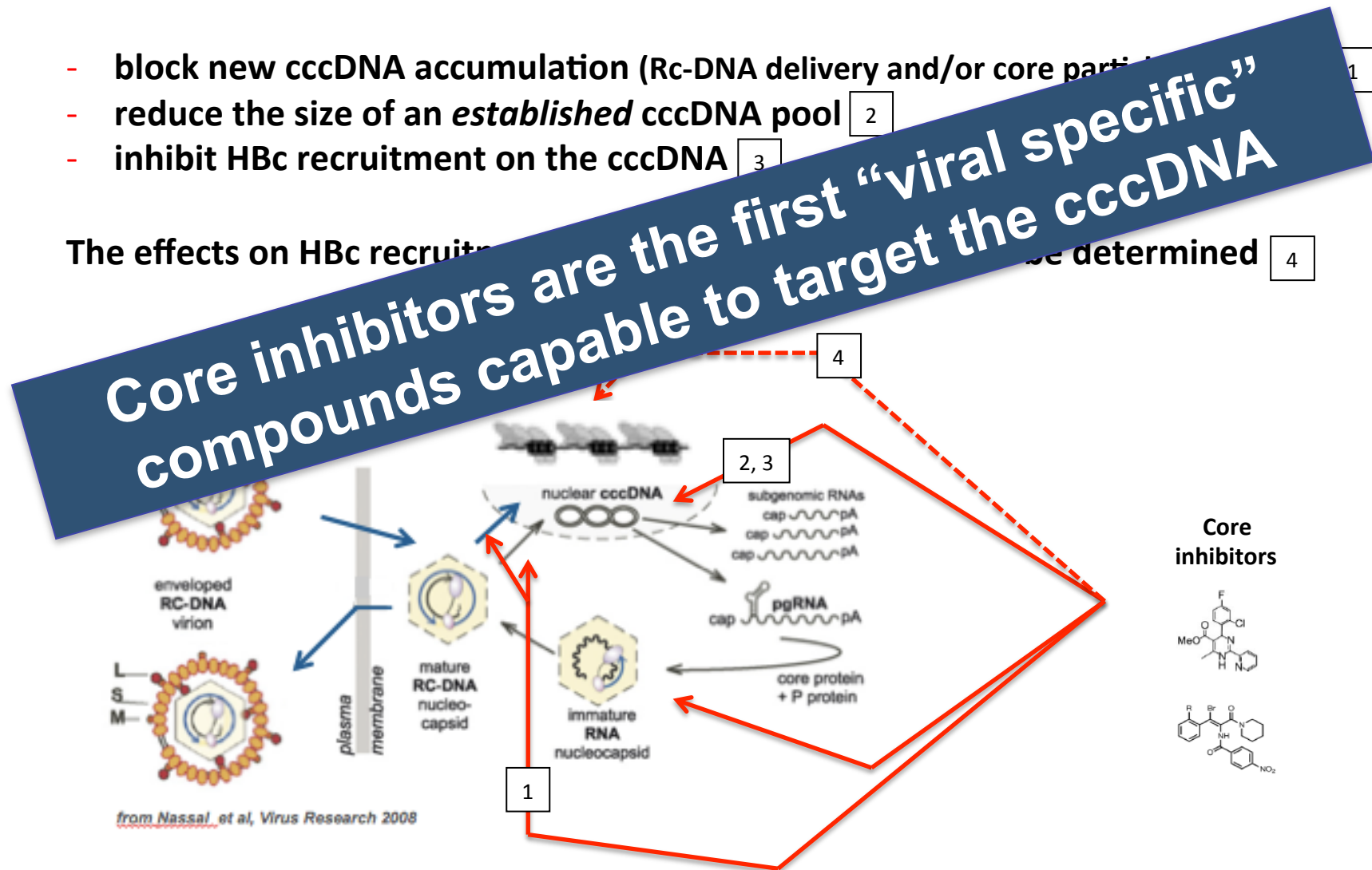


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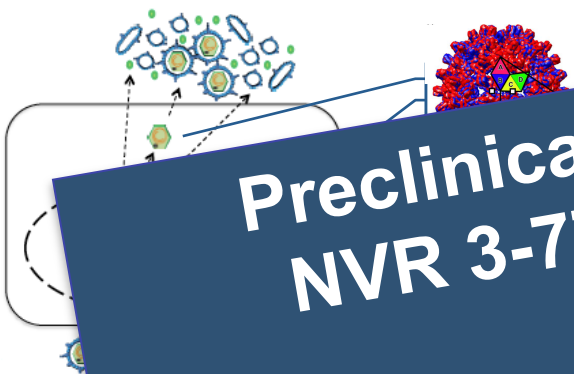
- block new cccDNA accumulation (Rc-DNA delivery and/or core particle formation) [1]
- reduce the size of an *established* cccDNA pool [2]
- inhibit HBc recruitment on the cccDNA [3]

The effects on HBc recruitment are the first “viral specific” compounds capable to target the cccDNA [4]



Core inhibitors / Anti-capsid

A growing family



The diagram on the left illustrates the HBV life cycle. It shows a cell with a nucleus where viral DNA is replicated. Arrows indicate the movement of viral components between the nucleus and the cytoplasm. In the cytoplasm, viral particles are shown, including a 3D model of a capsid (a red and blue sphere) and a diagram of a viral particle with a core and surface. The 3D model of the capsid is a red and blue sphere with a yellow triangle in the center.

Phenylpropenamide derivatives (A7)

Hetero

**Preclinical and Early Clinical Profile of
NVR 3-778, a Potential First-In-Class
HBV Core Inhibitor**

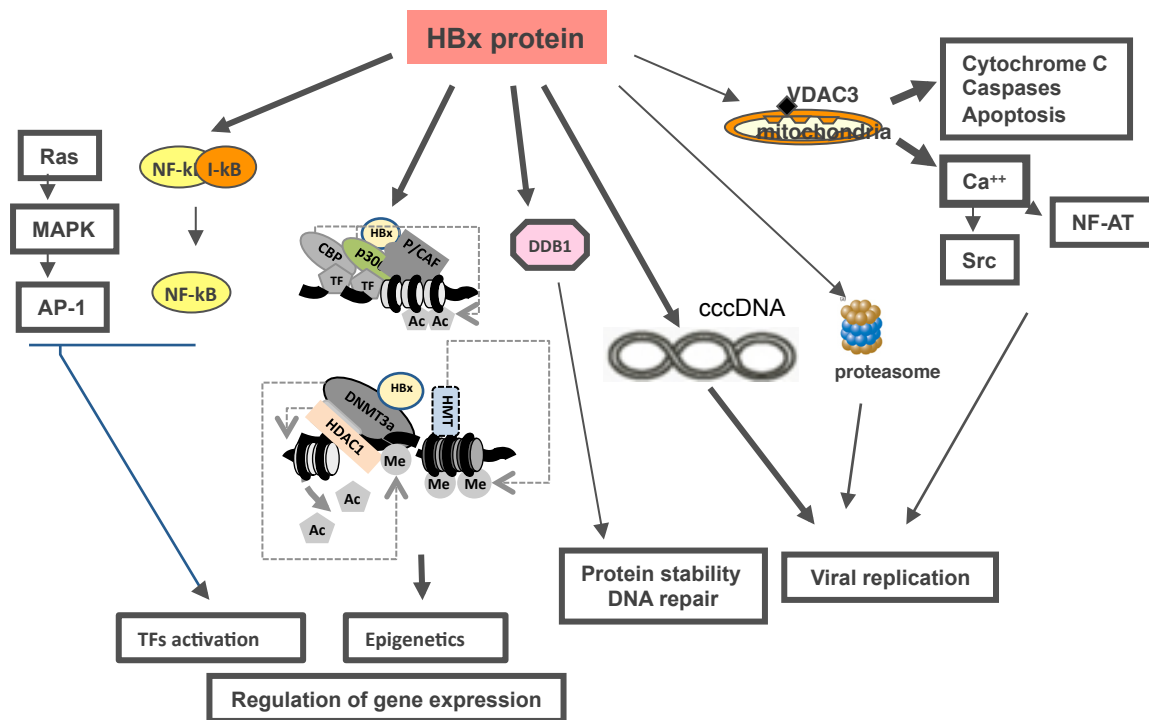
Gane, AASLD 2014

... [acetamide family]

... RNA packaging)

HBx protein

- ◆ HBx binds to and is required for cccDNA transcription and viral replication
- ◆ HBx binds to cellular promoters and modulates the epigenome by relocating chromatin regulators
- ◆ HBx contributes to hepato-carcinogenesis

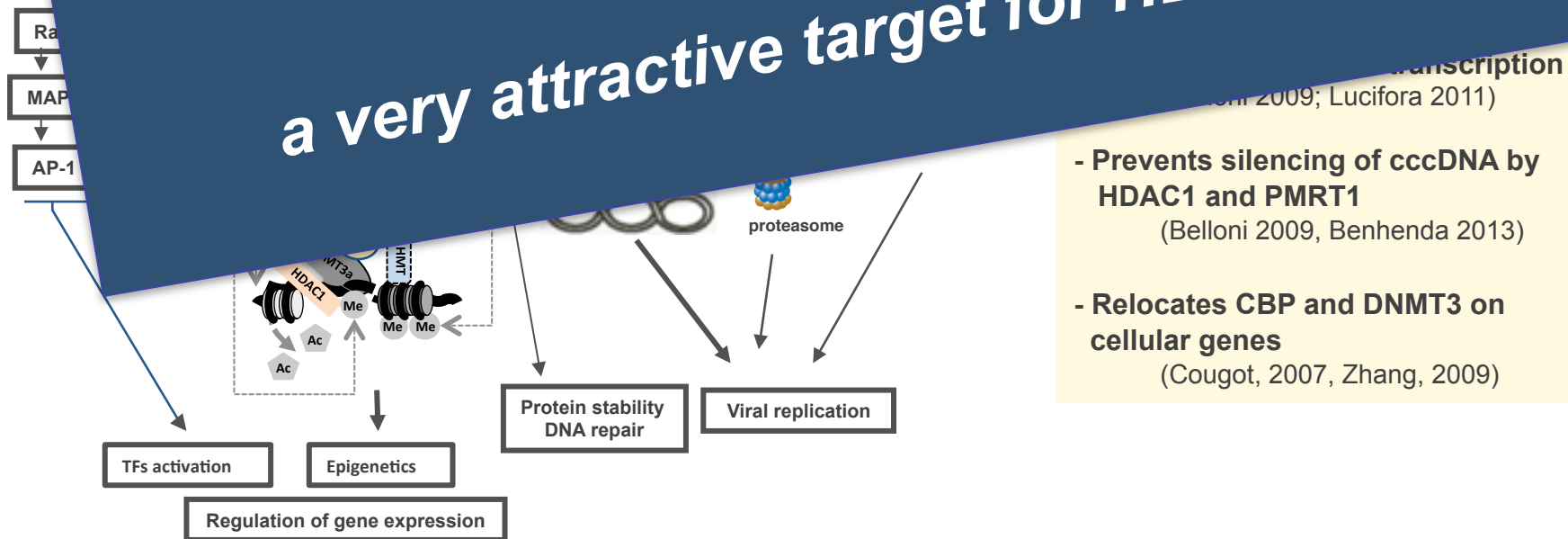


- Required for cccDNA transcription
(Belloni 2009; Lucifora 2011)
- Prevents silencing of cccDNA by HDAC1 and PMRT1
(Belloni 2009, Benhenda 2013)
- Relocates CBP and DNMT3 on cellular genes
(Cougot, 2007, Zhang, 2009)

HBx protein

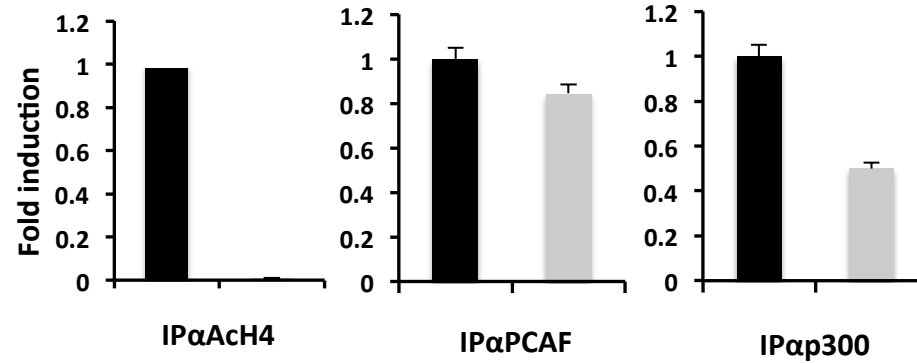
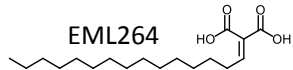
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HBx
a very attractive target for HBV cure

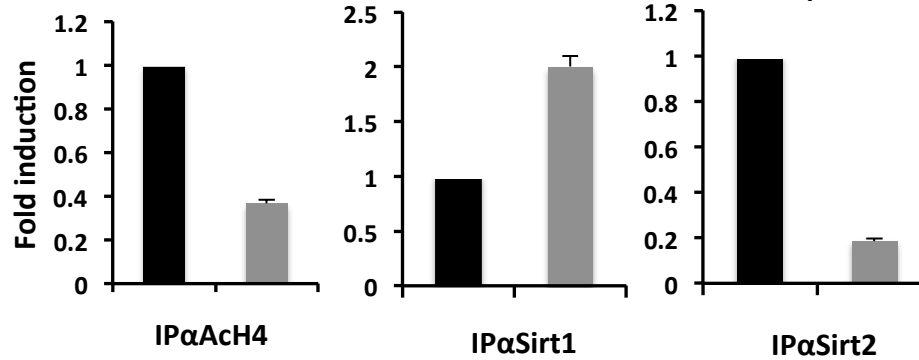
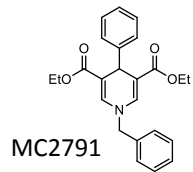


Targeting cccDNA-bound HATs and HDACs by “epigenetic” compounds

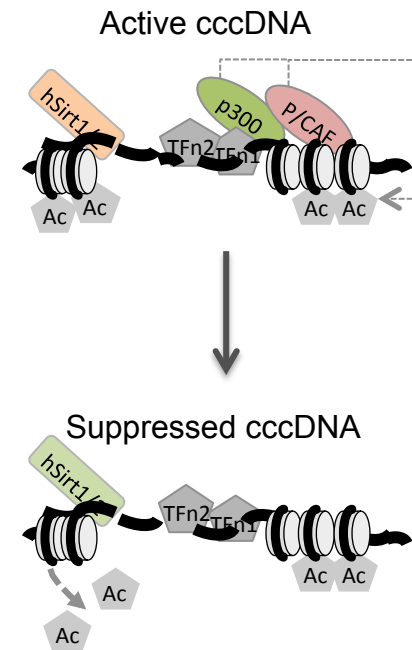
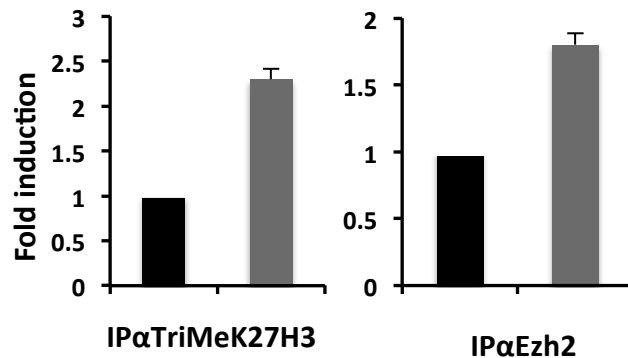
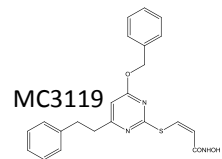
PCAF/p300 inhibitor



Sirt1/2 stimulator



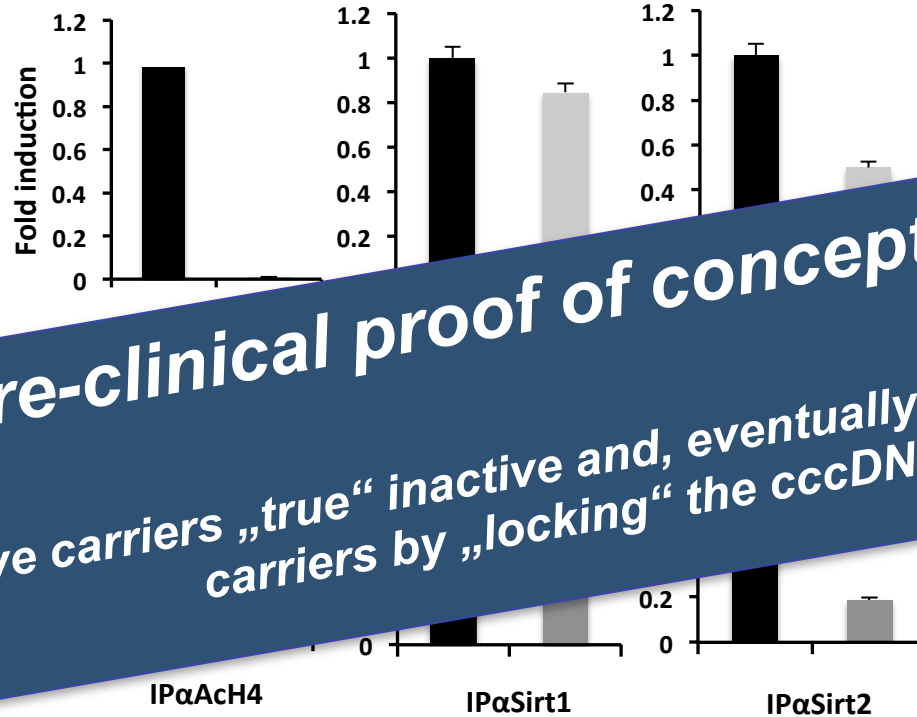
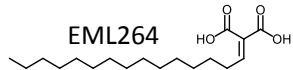
JMD3 inhibitor



EMs modify chromatin remodelling enzymes recruitment onto HBV minichromosome

Targeting cccDNA-bound HATs and HDACs by “epigenetic” compounds

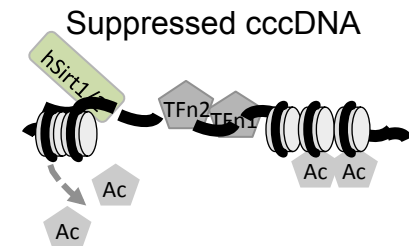
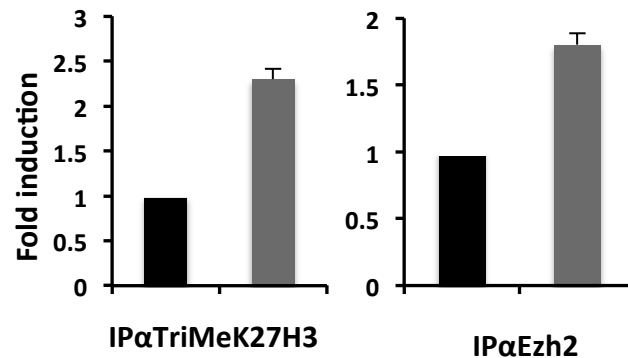
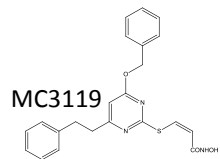
PCAF/p300 inhibitor



Pre-clinical proof of concept stage

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

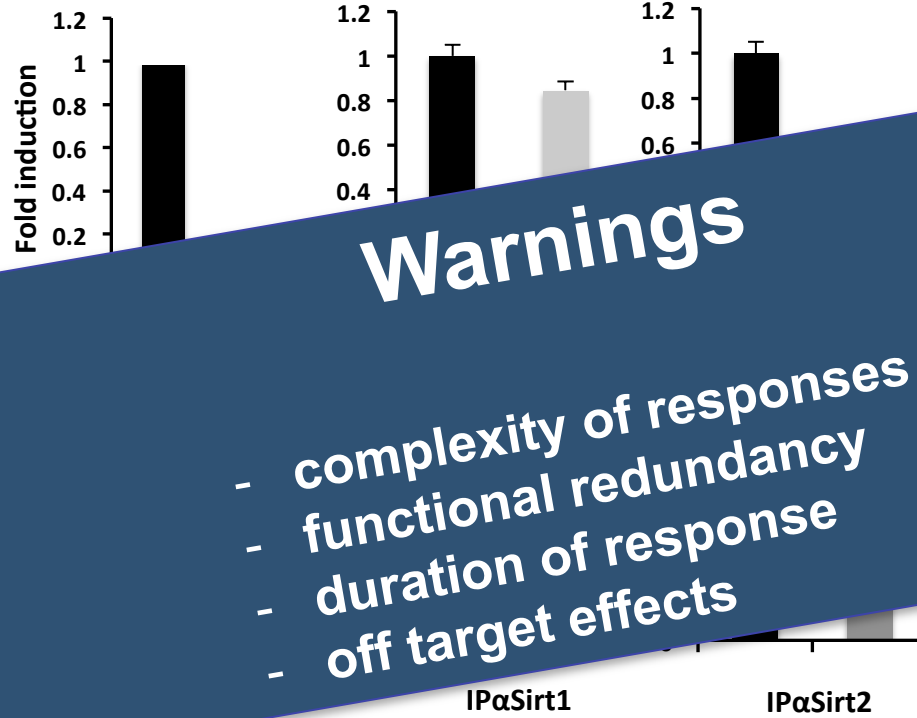
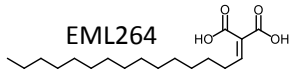
JMD3 inhibitor



EMs modify chromatin remodelling enzymes recruitment onto HBV minichromosome

Targeting cccDNA-bound HATs and HDACs by “epigenetic” compounds

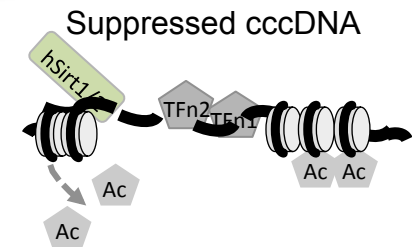
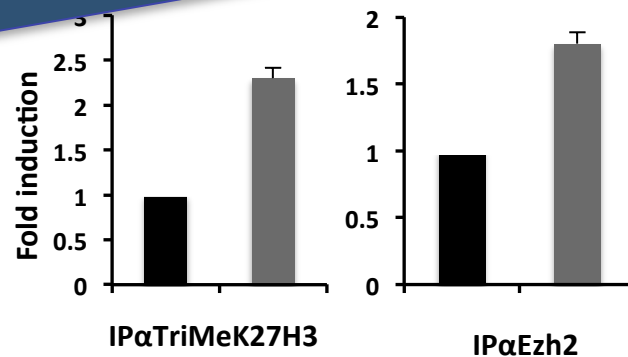
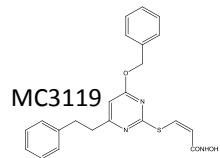
PCAF/p300 inhibitor



Warnings

- complexity of responses
- functional redundancy
- duration of response
- off target effects

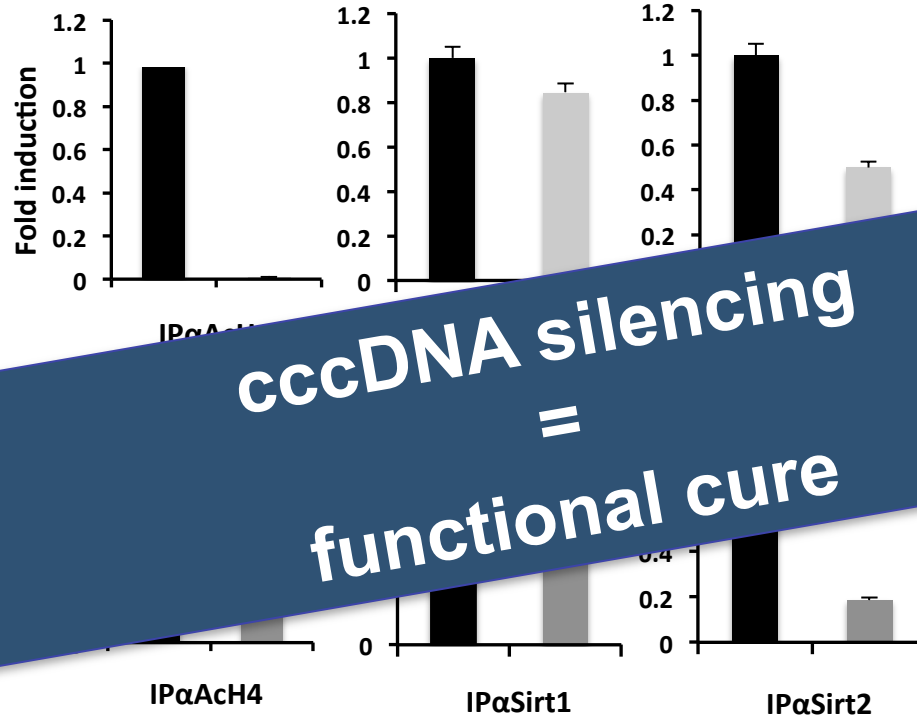
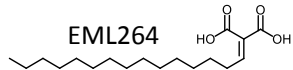
JMD3 inhibitor



EMs modify chromatin remodelling enzymes recruitment onto HBV minichromosome

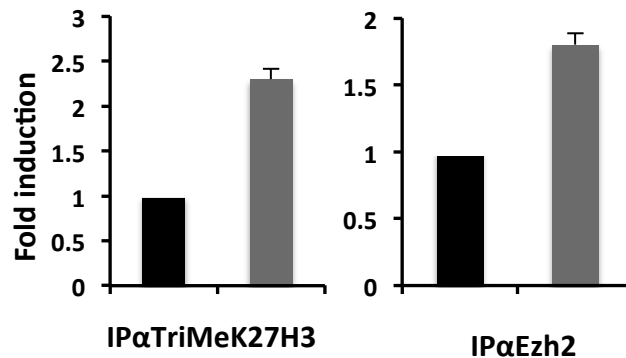
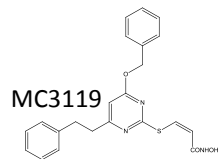
Targeting cccDNA-bound HATs and HDACs by “epigenetic” compounds

PCAF/p300 inhibitor

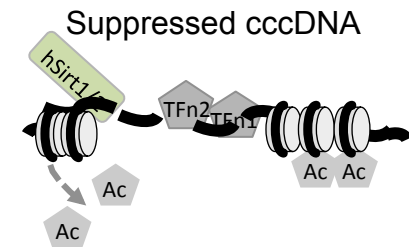


Sirt1/2 inhibitor

JMD3 inhibitor

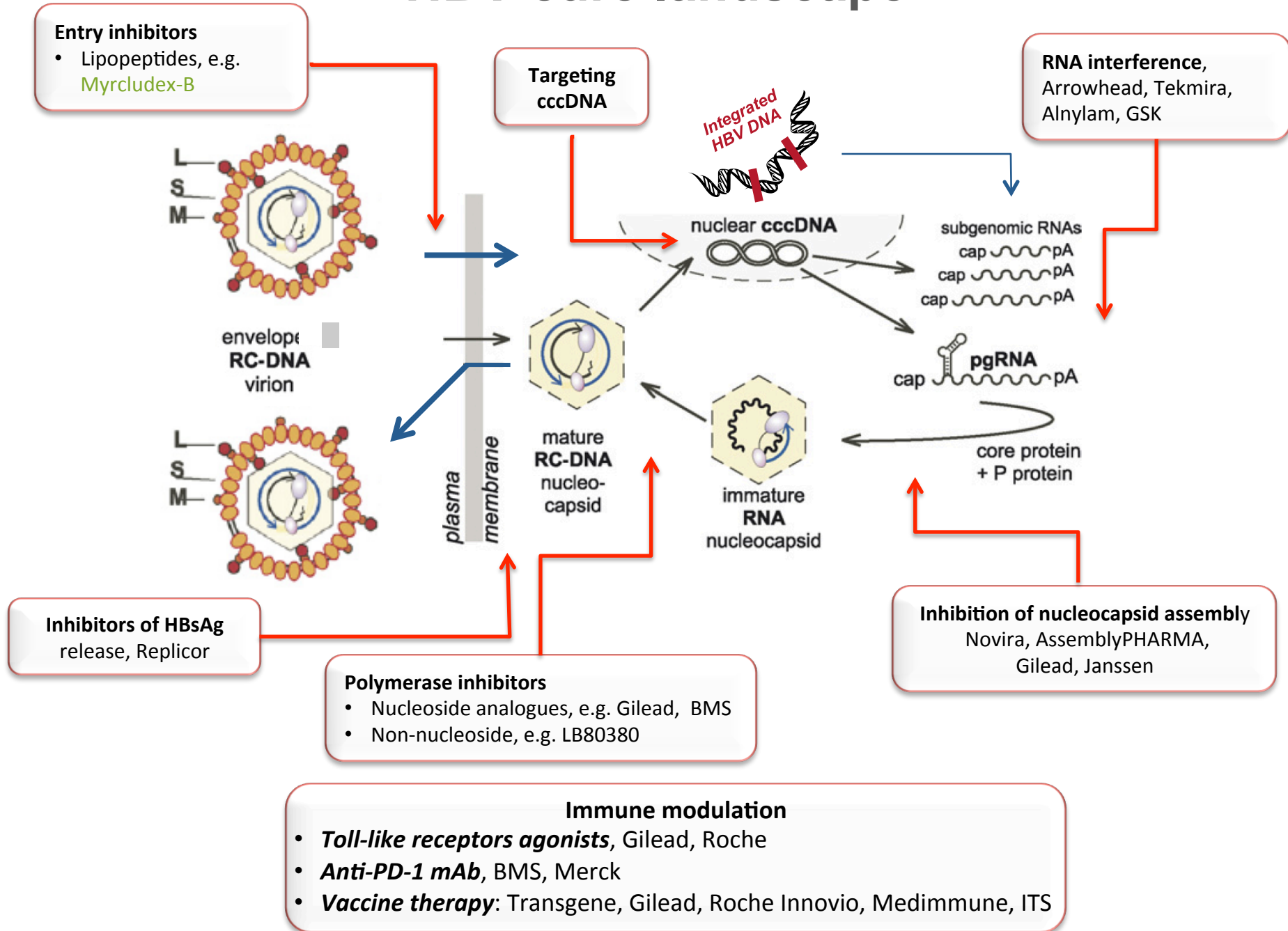


cccDNA silencing
=
functional cure

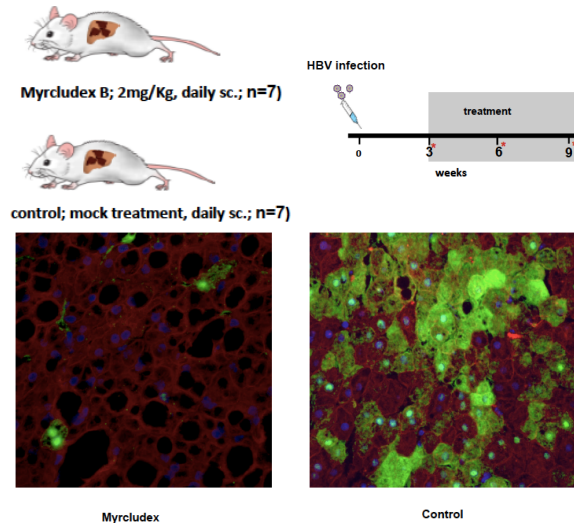
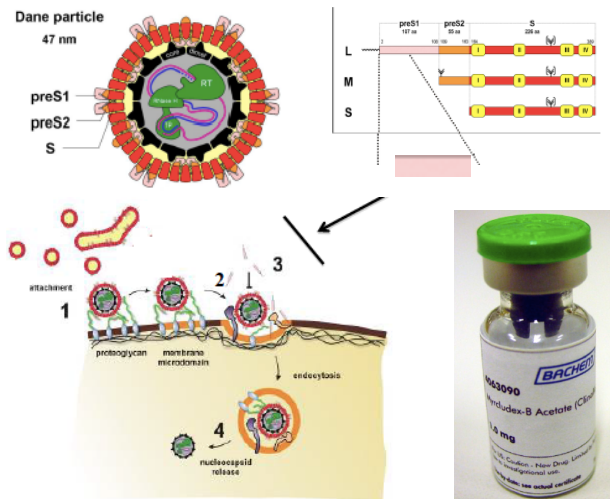


EMs modify chromatin remodelling enzymes recruitment onto HBV minichromosome

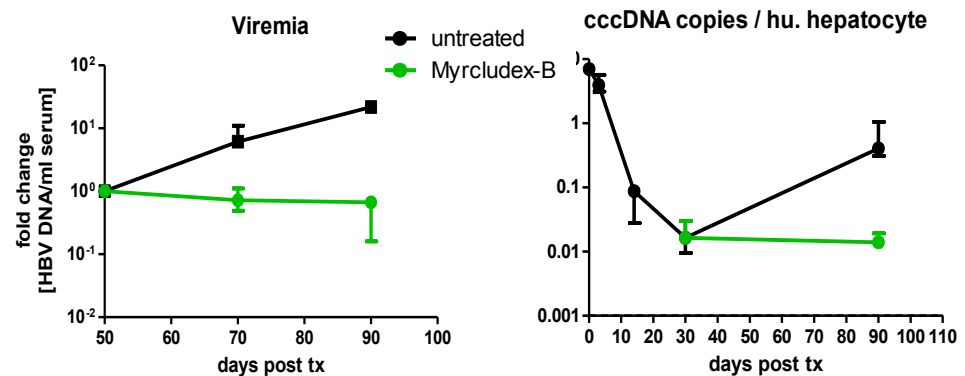
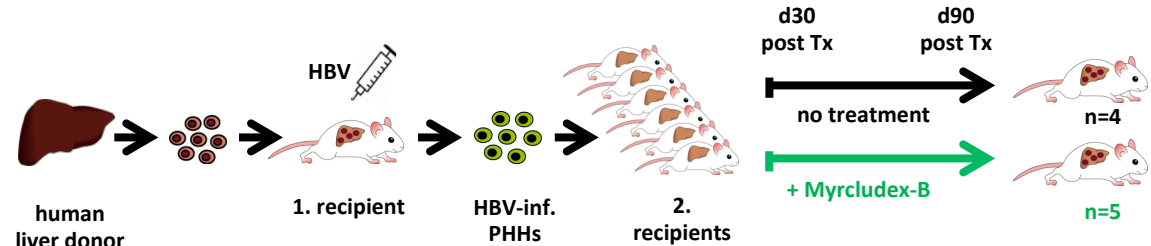
HBV cure landscape



Myrcludex B: Targeting Entry of HBV into Hepatocytes

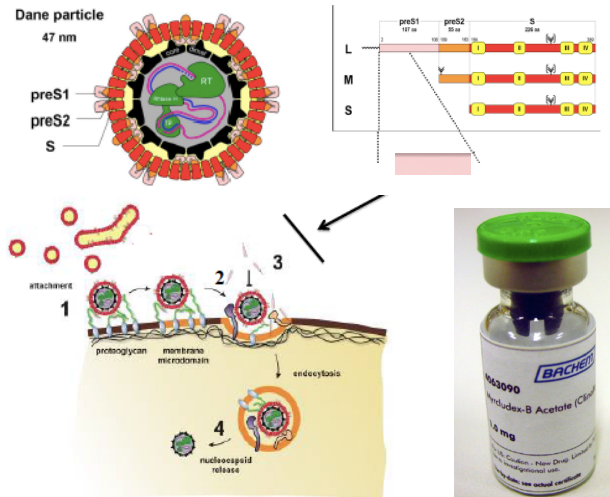


Entry inhibitor plus cell proliferation support loss of cccDNA and HBsAg

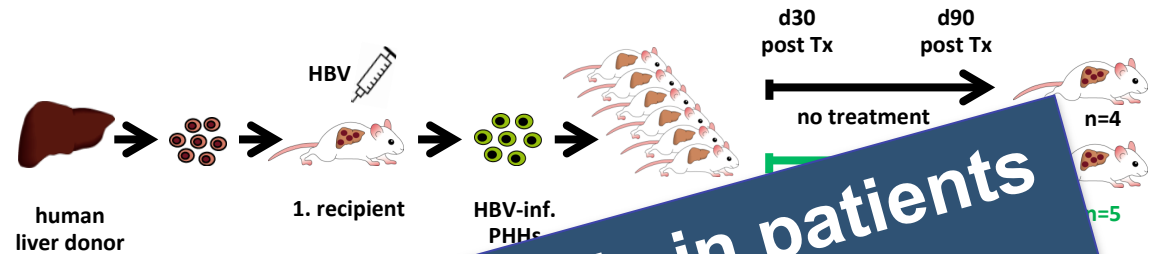


Cell proliferation combined with antiviral treatment to block re-infection (Myrcludex B) promoted cccDNA clearance in the majority of the human hepatocytes.

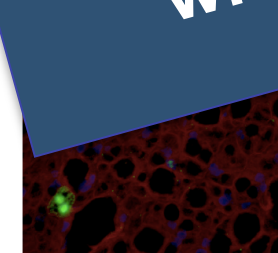
Myrcludex B: Targeting Entry of HBV into Hepatocytes



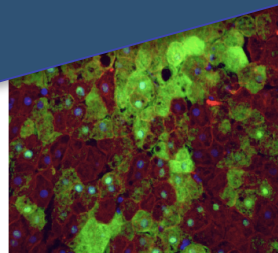
Entry inhibitor plus cell proliferation support loss of cccDNA and HBsAg



Myrcludex B

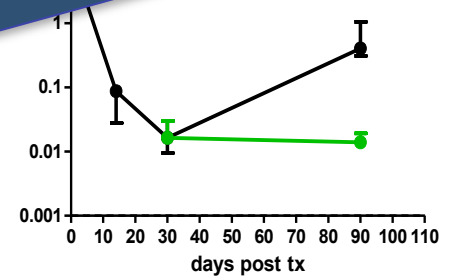
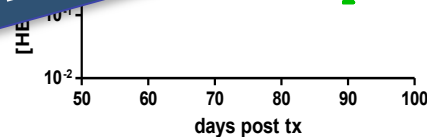


Myrcludex



Control

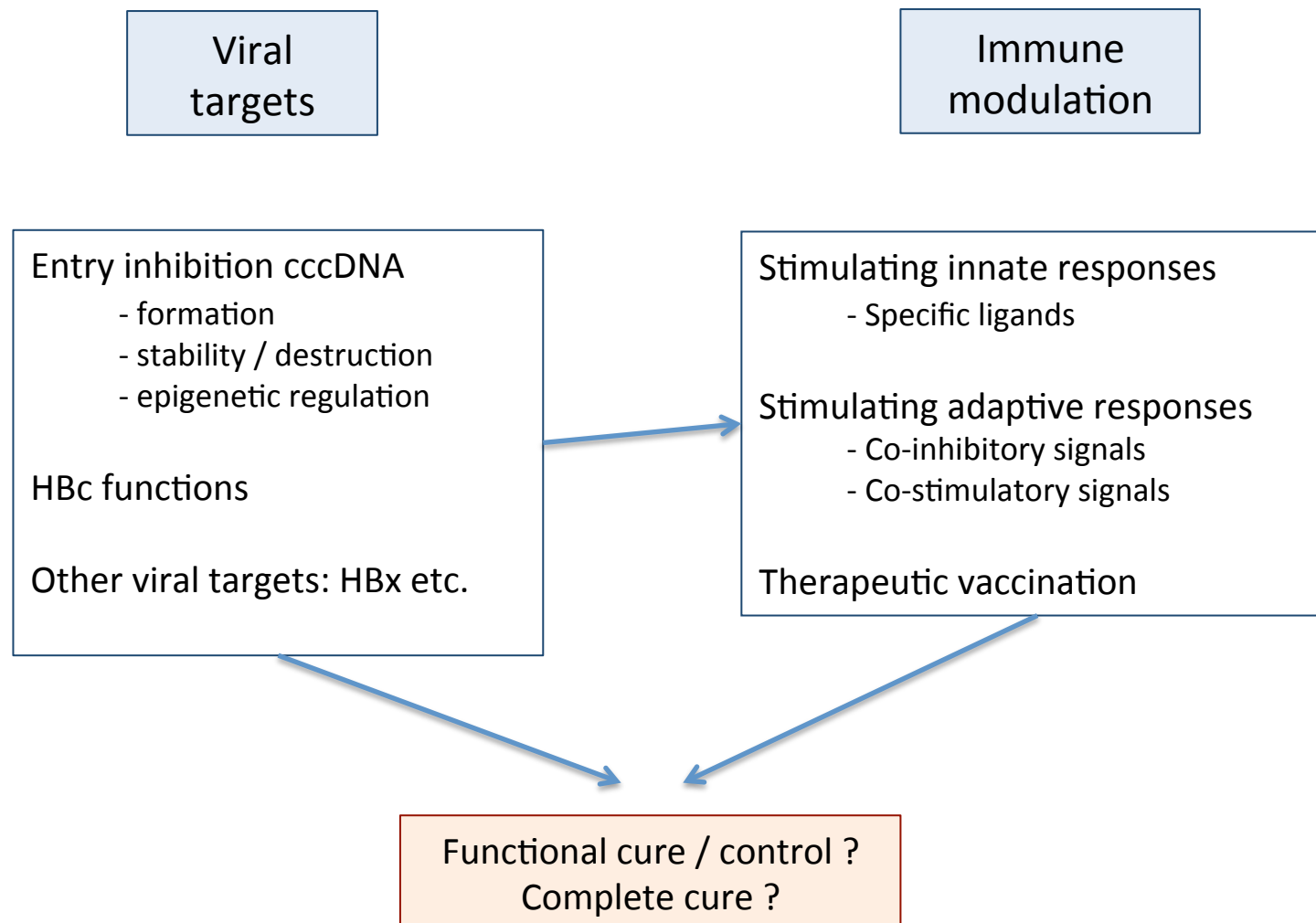
Evidence for ongoing low level viremia in patients with CHB receiving long term NUC therapy
Marcellin, AASLD 2014



Cell proliferation combined with antiviral treatment to block re-infection (Myrcludex B) promoted cccDNA clearance in the majority of the human hepatocytes.

What Might HBV Cure Will Look Like?

let's keep an open mind



ANRS HBV Cure Meeting

Hepatitis B virus pathobiology: state of the art
and unresolved questions



Agence autonome de l'Inserm

June 17, 2014
UIC
Paris, France



PDF

**Towards an HBV cure: state-of-the-art and unresolved questions—report
of the ANRS workshop on HBV cure**

Gut 2015;64:8 1314-1326 Published Online First: 10 February 2015



Second ANRS-HBV Cure Workshop

Paris
Tuesday, May 19th, 2015



Agence autonome de l'Inserm

www.anrs-hbvcure2015.com

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