



# Future therapies for HBV Cure

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Dipartimento di  
medicina interna e specialitA' mediche



# Disclosures

## Massimo Levrero

### Advisory Committees or Review Panels:

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

### Speaking and Teaching: - MSD

- Roche
- BMS
- Jansen
- Gilead

## Licensed drugs

Peg IFN

Nucleos(t)ide  
analogues

## Off-label use of licensed drugs

Nucleos(t)ide  
analogues

+

Peg IFN

=

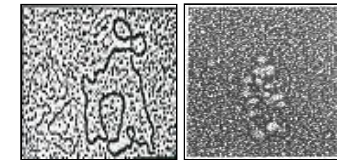
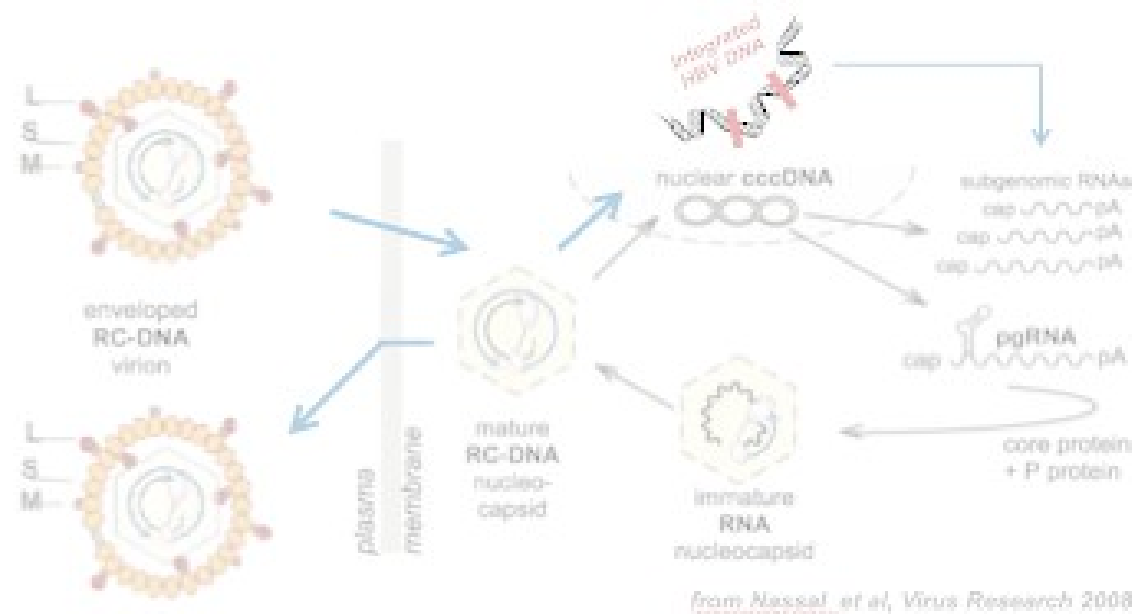
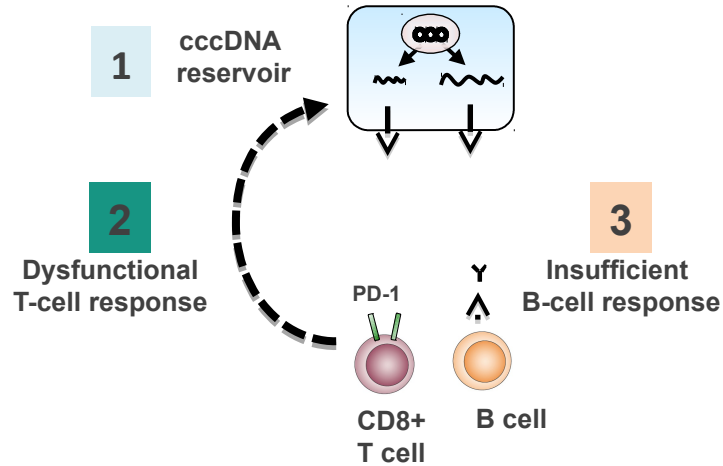
??

- PEG-IFN + NAs
- PEG-IFN “add-on” on NAs

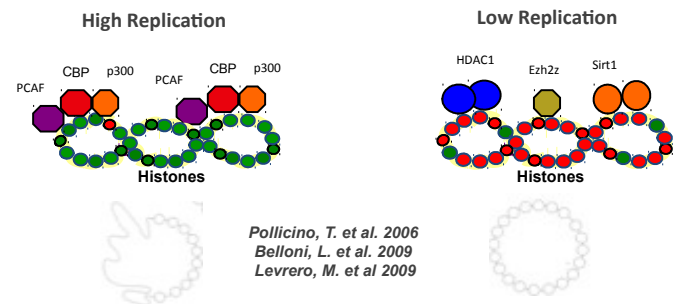
## New approaches

- early clinical development
- pre-clinical studies
- target discovery

# HBV: concepts about « persistence »



Bock, T. et al 1994  
Newbold, J. et al. 1995  
Bock, T. et al 2001



## Concepts about « cure »

### **Eradication**

- Equates to driving the virus to extinction from the earth [e.g. small pox (vaccination)]

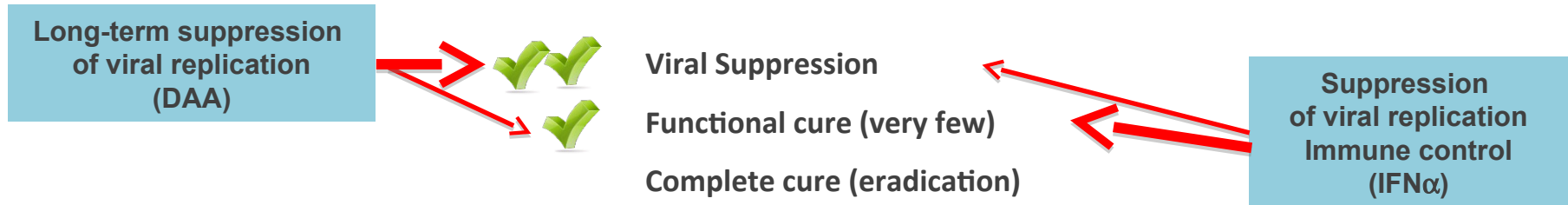
**versus**

### **Cure**

- Equates to eliminating the virus from the infected host [e.g. HCV (treatment)]

**HEPATITIS B can theoretically be eradicated (vaccine)  
AND  
“maybe cured”**

# HBV: concepts about « cure »



- **Sustained suppression of viral replication**

undetectable viremia with sensitive HBV-DNA assays

- **Functional cure**

“off therapy” persistent HBV suppression [*make all patients true “inactive carriers”*]

immune control / silencing of cccDNA

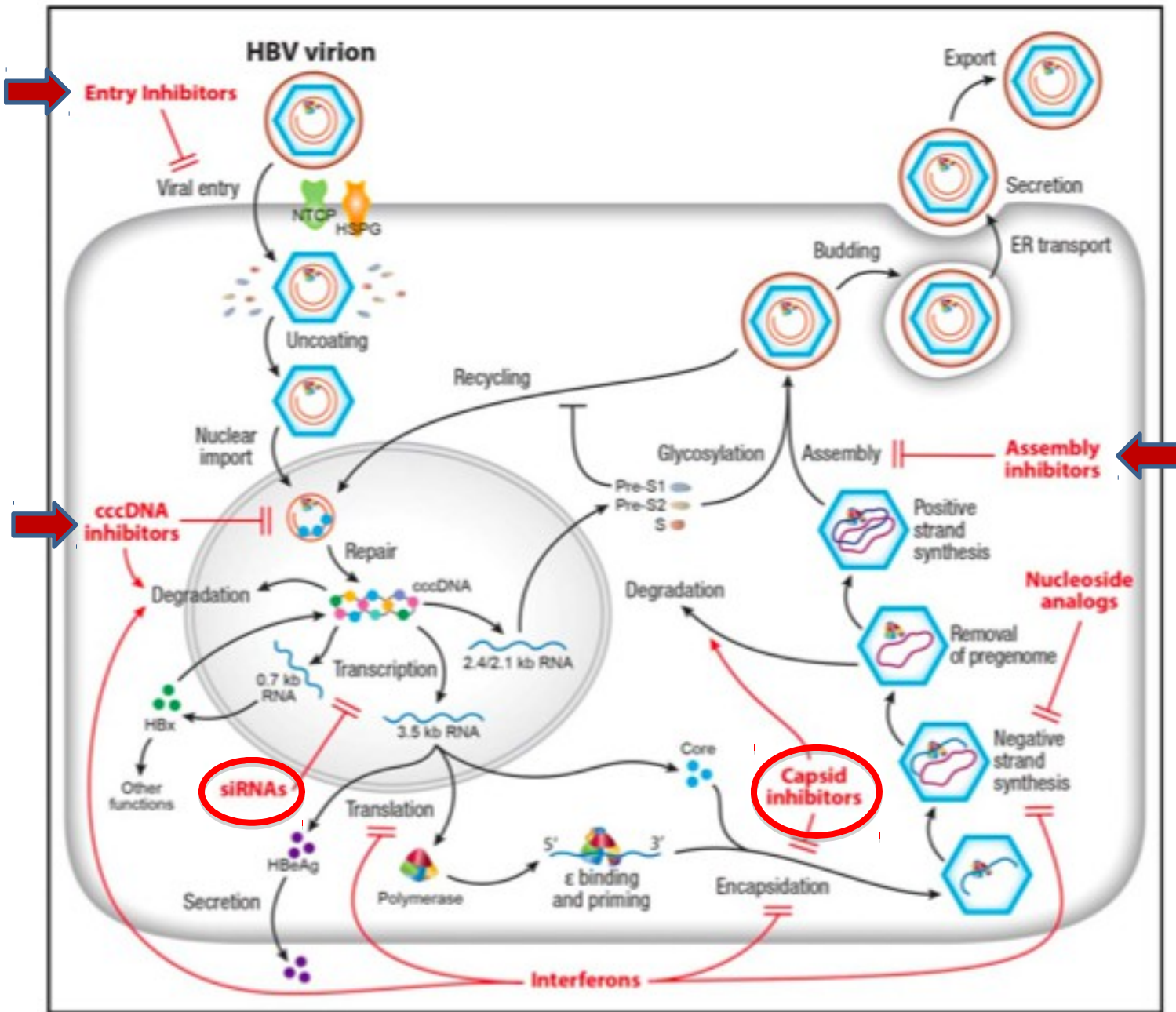
**HBsAg loss** as preferred endpoint

- **Complete / sterilizing cure**

elimination of cccDNA

elimination of infected hepatocytes, including cells with integrated HBV DNA

**HBsAg loss** and **anti-HBs seroconversion**: surrogate endpoint



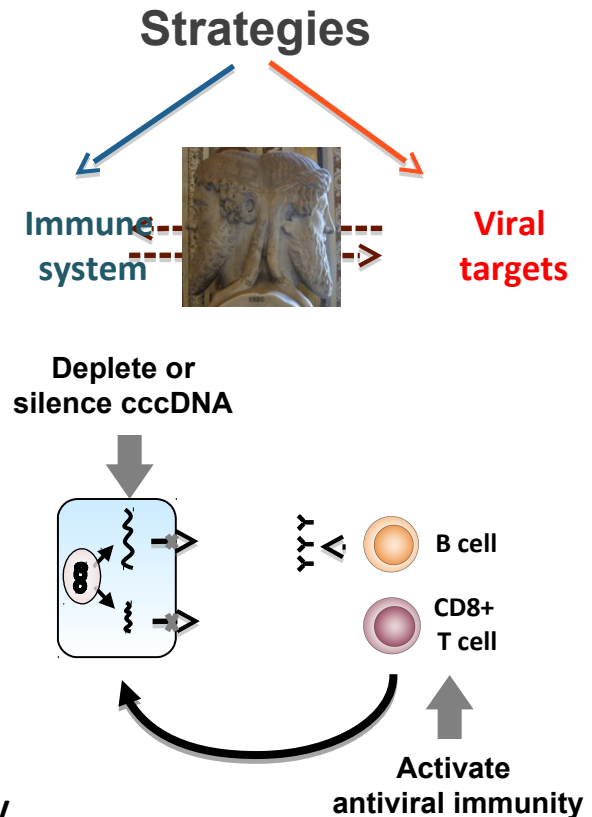
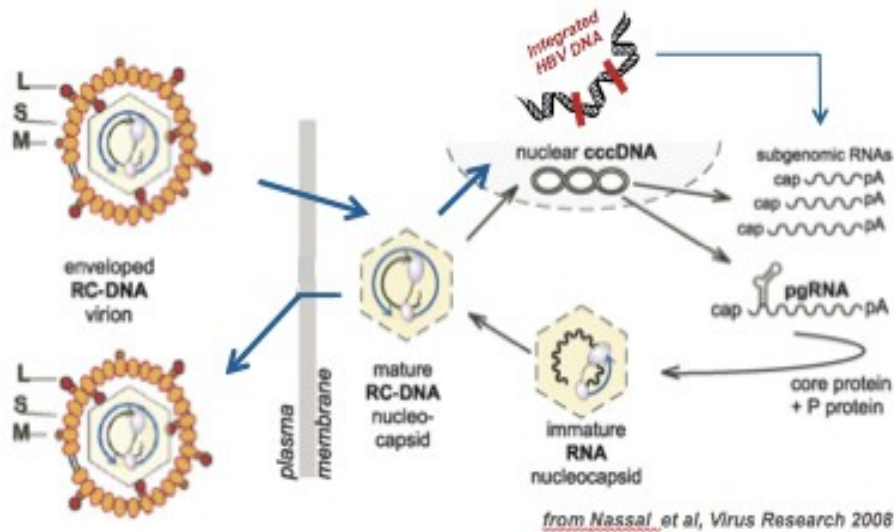
Adapted from Liang et al, Hepatology 2015

Table 1. Experimental HBV Therapeutics in Late Preclinical or Clinical Stage\*

Compound	Mechanism/ Target <sup>†</sup>	Stage of Development	Sponsor	Reference
<b>Direct-acting antivirals:</b>				
GS-7340 (tenofovir alafenamide fumarate)	Polymerase (produg of tenofovir)	Phase 2/3	Gilead Sciences	47; NCT0194047, NCT01940341 <sup>‡</sup>
CMX157	Polymerase (produg of tenofovir)	Phase 1/2 <sup>§</sup>	Contrain (Chimerix)	146; NCT01080820 <sup>‡</sup>
NWR1221/3778	Capsid	Phase 1/2	Novus	84; NCT02112799 <sup>‡</sup>
Sulfamoylbenzamides	Capsid	Animal	Oncare	147
GLS4	Capsid	Phase 1	HEC Pharm Group, China	148
Bay41-4109	Capsid	Phase 1	AiCure	83
REP 2139-Ca	Assembly/HBcAg	Phase 1/2	Replacor	NCT02233075 <sup>‡</sup>
ARC-520	RNAi	Phase 1/2	Arrowhead	94; sponsor's website; NCT02065336 <sup>‡</sup>
TKM-HBV	RNAi	Phase 1	Telesira	Sponsor's website; NCT02041715 <sup>‡</sup>
ALN-HBV	RNAi	Animal	Ailyam	Sponsor's website
DNA-directed RNAi	RNAi	Animal	Bentec	Sponsor's website
ISS-HBV	Antisense	Phase 1	Isis	Sponsor's website
<b>Host targeting agents:</b>				
Myxindex B	Entry/NTCP	Phase 1/2	Myr-GmbH/Hepatera	75
Birapart	Apoptosis/second mitochondrial activator of caspases	Phase 1	Tetralogic	Sponsor's website; NCT02288208 <sup>‡</sup>
Flavonoids	STING agonist (pattern recognition receptor)	Animal	Oncare	149
NVP018	Cyclophilins, IRF-9	Animal	Oncare (NeuroViv)	Sponsor's website
Epitope HBV	Glycosidase/therapeutic vaccine	Animal	Blumberg Institute	150
<b>Immune modulatory agents:</b>				
GS-9620	TLR-7 agonist	Phase 2	Gilead Sciences	122; NCT02166047 <sup>‡</sup>
Nivolumab	PD-1 blockade	Phase 1 <sup>  </sup>	BMS	151; Sponsor's website; NCT01658878 <sup>‡</sup>
SB 9200HBV	RIG-I and NOD2 activation	Phase 1/2	INC/Springbank	152; NCT01803308 <sup>‡</sup>
GS-4774	Therapeutic vaccine	Phase 2/3	Gilead Sciences/GlobeImmune	144; NCT02174276 <sup>‡</sup>
ANRS HB02	Therapeutic vaccine	Phase 1/2	French National Agency for Research on AIDS and Viral Hepatitis	141; NCT02166047 <sup>‡</sup>
Hepslav B Dynavax 601	Therapeutic vaccine	Phase 1	Dynavax	153; NCT01023230 <sup>‡</sup>
Nasvac	Therapeutic vaccine	Phase 2/3	CGEB, Cuba	154
YG1050	Therapeutic vaccine	Phase 1/1b	Yanogene	NCT02428400
HBIG + GM-CSF + HBV vaccine	Therapeutic vaccine	Phase 1/2	Beijing 302 Hospital	NCT01878565
HBV vaccine + FN-α2b + IL-2	Therapeutic vaccine	Phase 2/3	Tongji Hospital	NCT02360592 (labeled as Phase 4)
HBV vaccine-activated dendritic cells	Therapeutic vaccine	Phase 1/2	Third Affiliated Hospital, Sun Yat-Sen University	NCT01935635
Evax + PEG-FN-α	Therapeutic vaccine	Phase 2/3	Seoul National University	NCT02097004 (labeled as Phase 4)
PD-1 monoclonal antibody	PD1 blockade	Animal	AcadSin	155
Altravax HBV	Therapeutic vaccine	Animal	Altravax	Sponsor's website
INO-1800	Therapeutic vaccine	Animal	Innovio	Sponsor's website



# Towards HBV cure

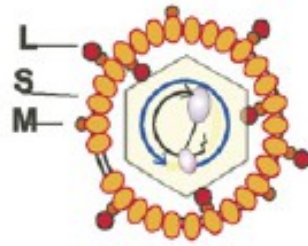


- Complete inhibition of HBV replication and [including entry inhibitors and capsid inhibitor]
  - to avoid new hepatocytes infection and low level core particles recycling
- Restoration of host innate/adaptive antiviral immunity against HBV
  - reduce HBs load [si/shRNA approaches; NAPs]
  - checkpoint inhibitors [anti-PD1/PDL1; others]
  - TCR engineering
  - TLRs agonists [TLR7 and others]
- Direct targeting of cccDNA
  - inhibit cccDNA formation
  - target cccDNA with endonucleases
  - transcriptional silencing of cccDNA [FUNCTIONAL CURE]
  - cccDNA bound viral proteins: HBc and HBx

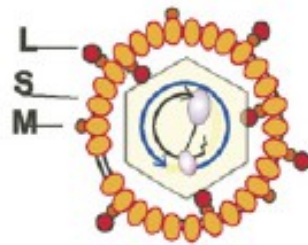
# HBV cure landscape

## Entry inhibitors

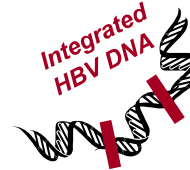
- Lipopeptides, e.g. Myrcludex-B
- Cyclosporin derivatives



envelope  
RC-DNA  
virion



## Targeting cccDNA



nuclear cccDNA

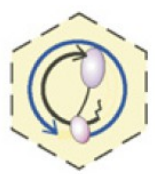
## RNA interference, Arrowhead, Tekmira, Alynlam, GSK

subgenomic RNAs  
cap ~~~~~ pA  
cap ~~~~~ pA  
cap ~~~~~ pA

cap ~~~~~ pgRNA ~~~~~ pA

core protein  
+ P protein

plasma  
membrane



mature  
RC-DNA  
nucleo-  
capsid



immature  
RNA  
nucleocapsid

## Inhibitors of HBsAg release Replicor

## Polymerase inhibitors

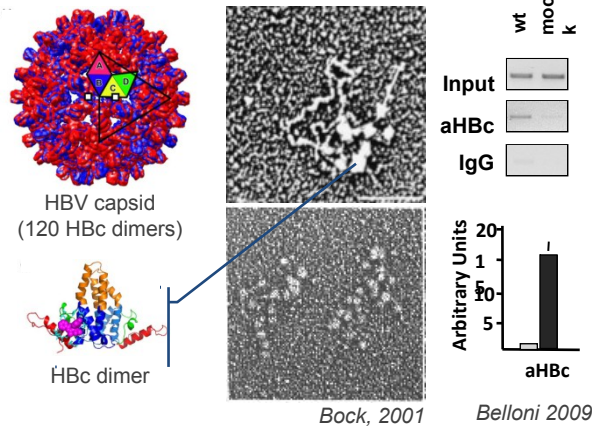
- Nucleoside analogues, e.g. Gilead, BMS
- Non-nucleoside, e.g. LB80380

## Inhibition of nucleocapsid assembly Novira, AssemblyPHARMA, Gilead, Janssen

## Immune modulation

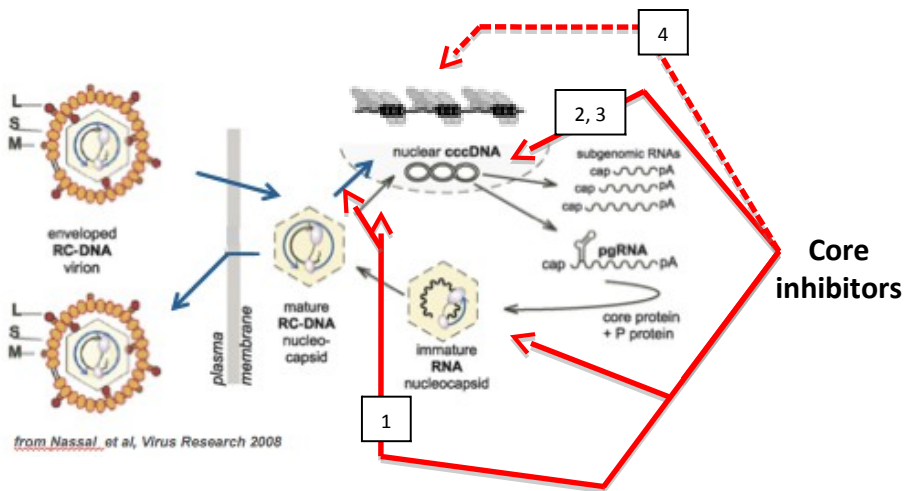
- **Toll-like receptors agonists**, Gilead, Roche
- **Anti-PD-1 mAb**, BMS, Merck
- **Vaccine therapy**: Transgene, Gilead, Roche Innovio, Medimmune, ITS

# Core inhibitors drugs



- ◆ Hbc binds the cccDNA and modifies cccDNA nucleosome spacing
- ◆ Hbc binds to cellular promoters and regulates gene expression
- ◆ Hbc binds to (and represses) the IFN- $\beta$ , IL-29 and OAS1 cellular promoters

1. Bock T. et al., JMB 2001; 2. Belloni L. et al. PNAS USA 2009; 3. Guo, BMC genomics, 2013; 4. Durantel D. et al., AASLD 2013



## Core inhibitors are the first “viral specific” compounds capable to target the cccDNA

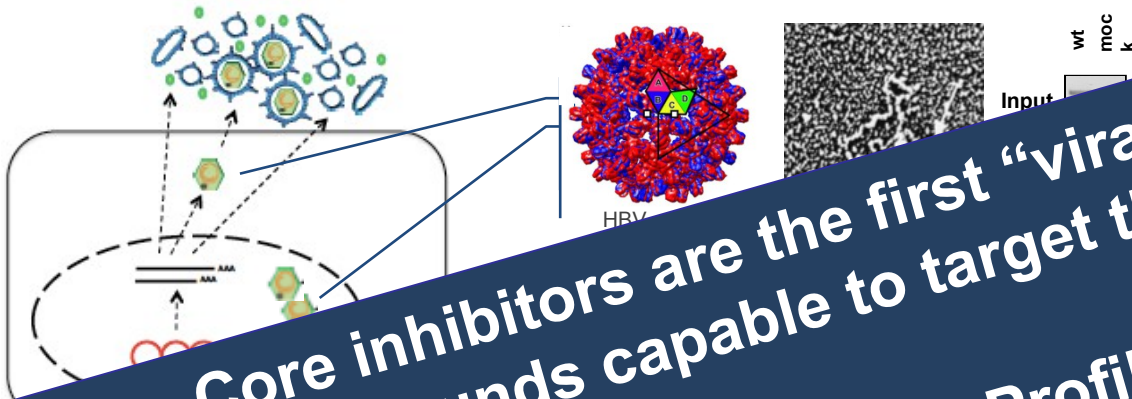
Several compounds are being developed (different spectrum of activities ??)

Core inhibitors potentially target both cytoplamic (capsid) and nuclear Hbc:

- block new cccDNA accumulation (Rc-DNA delivery and/or core particles recycling [1]) 1
- inhibit cccDNA transcription [2] 1
- inhibit Hbc recruitment on the cccDNA [3] 1
- modulate Hbc cellular target genes [4] 2

1. Belloni L. et al., AASLD 2013; 2. Durantel D et al., AASLD 2013

# Core inhibitors drugs



Core inhibitors are the first “viral specific” compounds capable to target the cccDNA

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

Gane, AASLD 2014

[NVR3-778-101 Protocol, Clinicaltrials.org # NCT02112799]

Phase 1b clinical trial [4 dosing cohorts  
[100, 200, 400 mg QD and 600 mg BD]  
Yuen, AASLD 2015

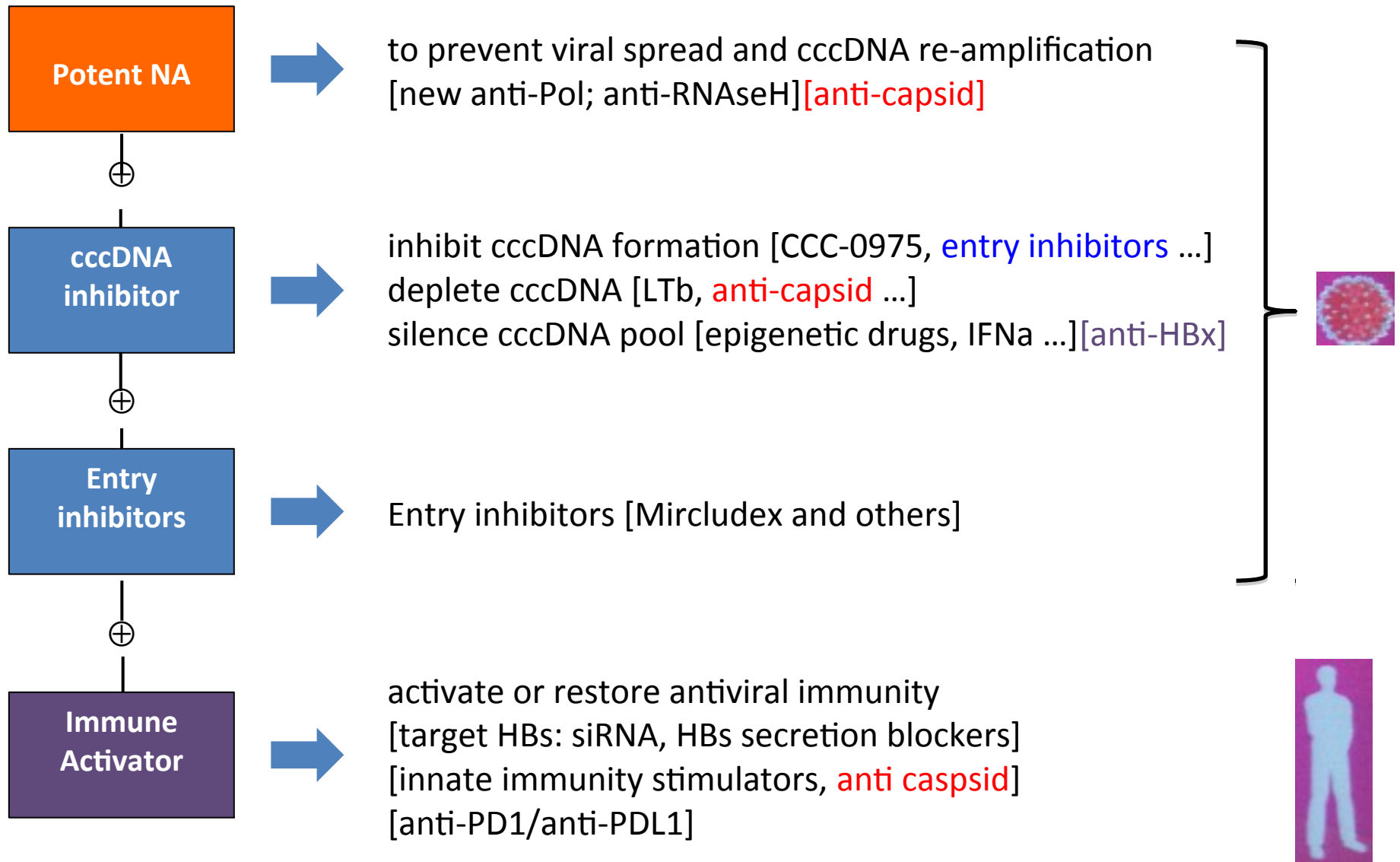
target both cytoplasmic (capsid) and/or core particles recycling [1] 1

- inhibit cccDNA transcription [2] 1
- inhibit HBc recruitment on the cccDNA [3] 1
- modulate HBc cellular target genes [4] 2

1. Belloni L. et al., AASLD 2013; 2. Durantel D et al., AASLD 2013

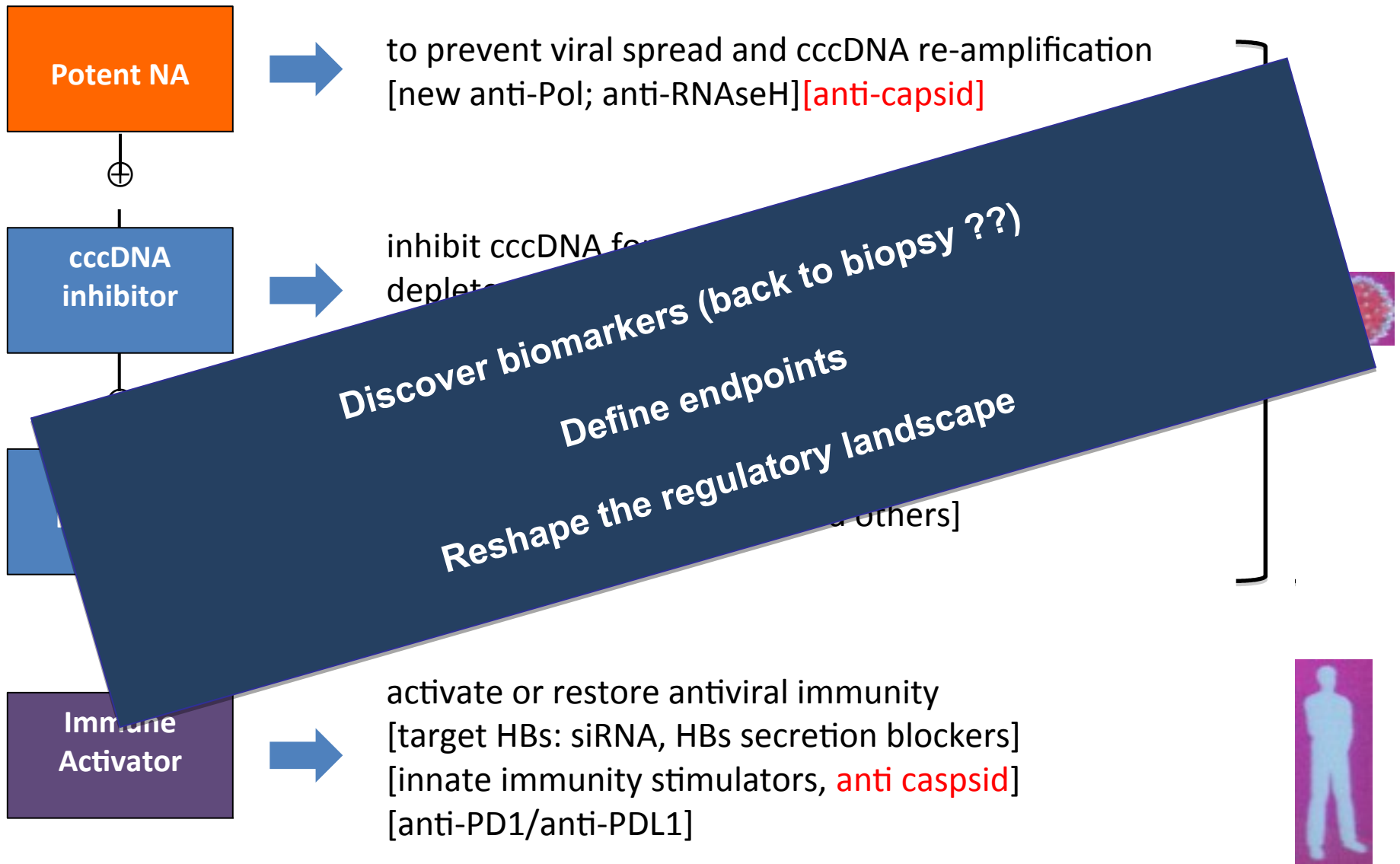
# What Might HBV Cure Will Look Like?

*let's keep an open mind*



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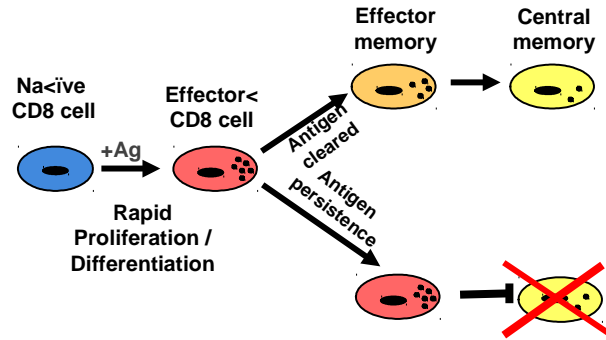
*let's keep an open mind*





# TOWARDS HBV CURE:

## RESTORING T CELL FUNCTION / DIFFERENTIATION



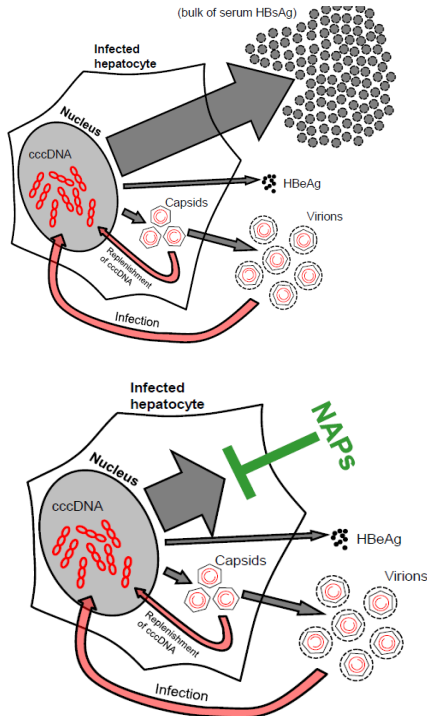
Efficient T cell function/differentiation

Acute Self-limited Infection

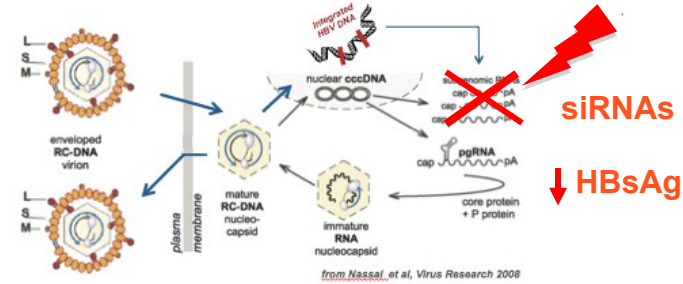
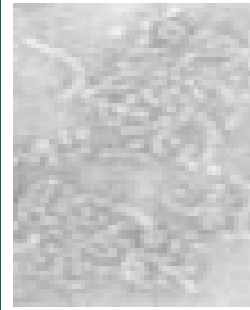
Chronic HBV infection

Inefficient T cell function/differentiation

### Nucleic Acid Polymers (NAPs)



### High viral / antigen load (HBsAg, HBeAg)



Reduction of HBsAg (and HBeAg) *should* translate in a revival of adaptive immune response and *functional cure*

Tekmira ONCORE BIOPHARMA, INC. Arbutus BIOPHARMA Aspiring to Cure Chronic Hepatitis B

Arrowhead Research CORPORATION



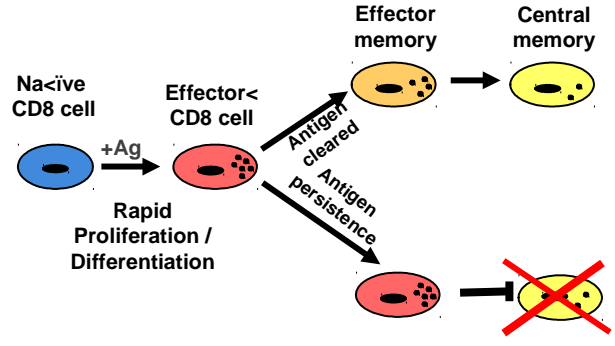
Dynamic polyconjugates





# TOWARDS HBV CURE:

## RESTORING T CELL FUNCTION / DIFFERENTIATION



Efficient T cell function/differentiation

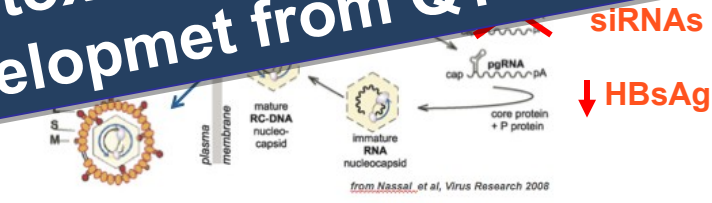
Acute Self-limited Infection

Chronic HBV infection

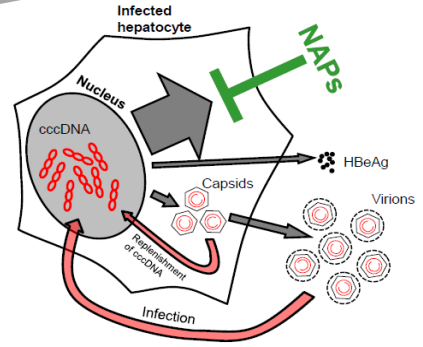
Inefficient T cell function/differentiation

Nucleic Acid Polymers (NAPs)

**NAPs Replicor: phase 2 at AASLD 2015**  
**ARC-520: phase 2; efficacy vs toxicity signals**  
**Tekmira/Oncor/Arbutus: clinical development from Q1 2015**



Reduction of HBsAg (and HBeAg) *should* translate in a revival of adaptive immune response and *functional cure*

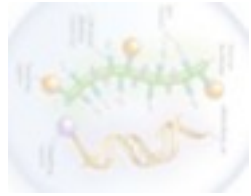


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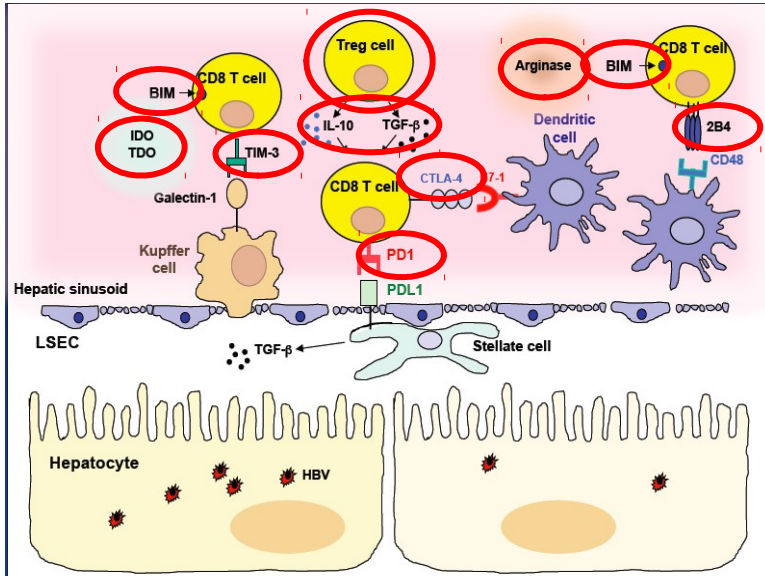
Arrowhead Research CORPORATION



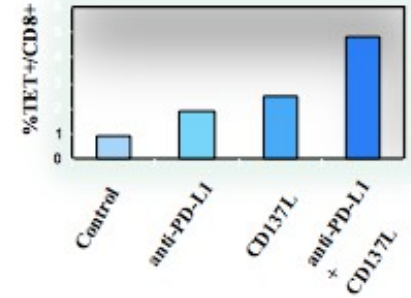
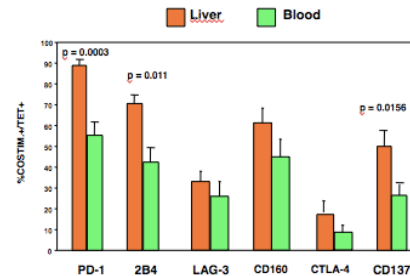
Dynamic polyconjugates



# Blocking inhibitory receptors on T cells

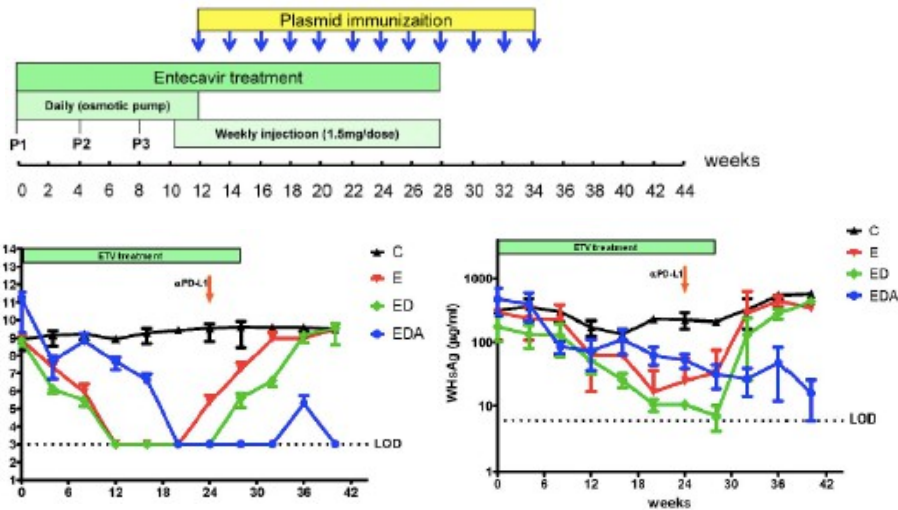


## RESTORATION OF THE T CELL FUNCTION BY COMBINED MANIPULATION OF PD-1/PD-L1 AND CD137/CD137L PATHWAYS



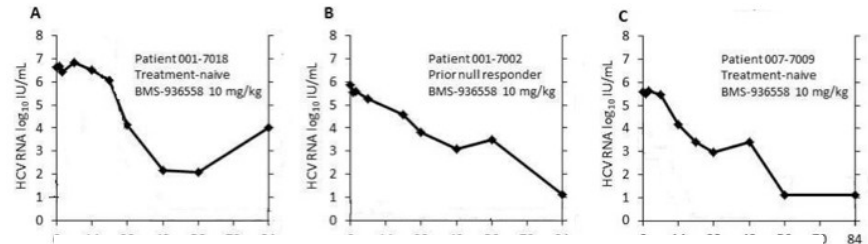
Fisicaro P et al Gastroenterology 2012

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



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

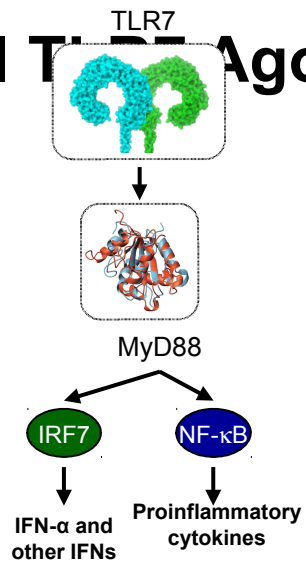
## PD-1 PATHWAY BLOCKADE Proof of concept of α-PD-1 in Chronic HCV



- 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

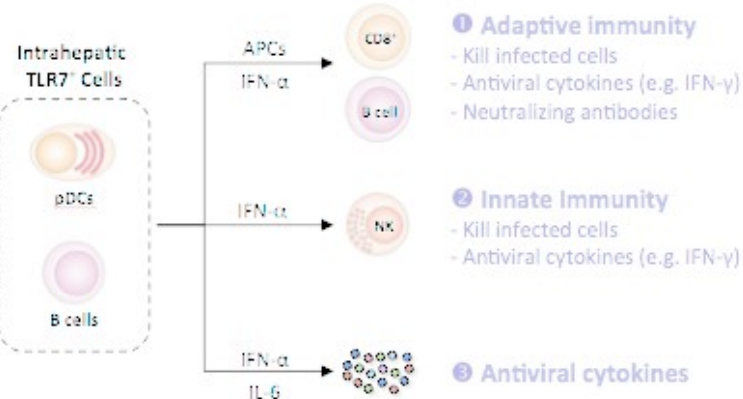
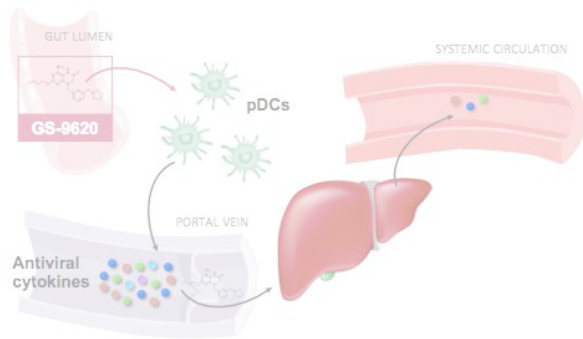
Gardiner et al. 2013. PLoS ONE 8(5): e63818.

# Oral TLR7 Agonist



Persistent HBV viremia suppression and increased HBsAg in chimps (Menne et al., J Hepatol 2015) and woodchucks (Landorf et al., Gastroenterology 2013)

GS-9620



## 1 Adaptive immunity

- Kill infected cells
- Antiviral cytokines (e.g. IFN- $\gamma$ )
- Neutralizing antibodies

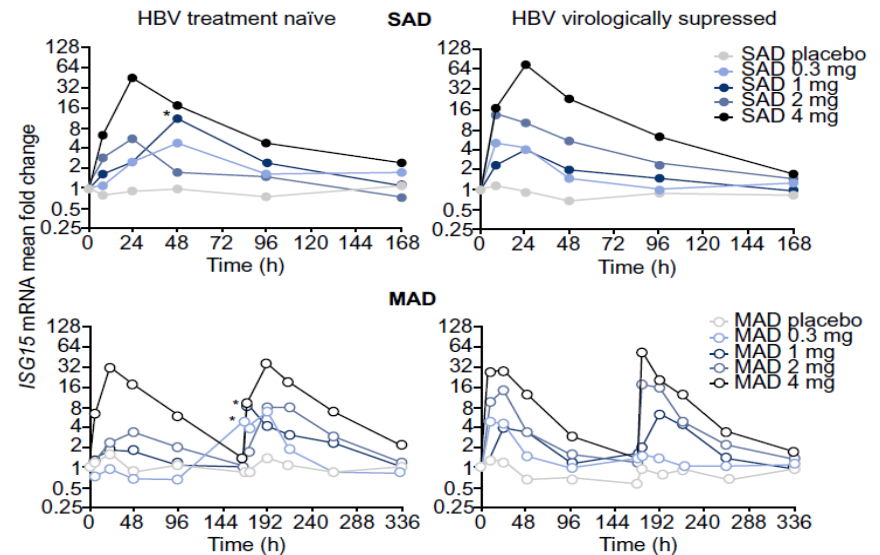
## 2 Innate Immunity

- Kill infected cells
- Antiviral cytokines (e.g. IFN- $\gamma$ )

## 3 Antiviral cytokines

## 2 double-blind phase 1b trials

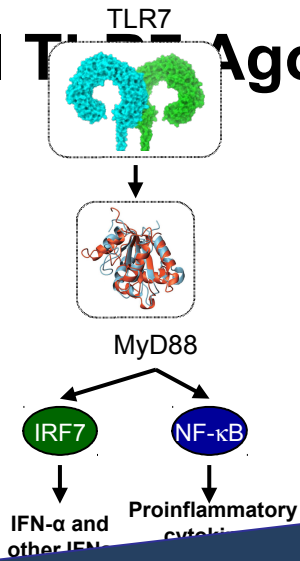
[49 treatment-naïve; 51 virologically suppressed]



**Fig. 1. ISG15 (mRNA) fold change.** The figure shows the ISG15 mRNA mean fold change over time in the SAD and MAD cohorts in each patient population. \*Induction at these time points driven by a single patient.

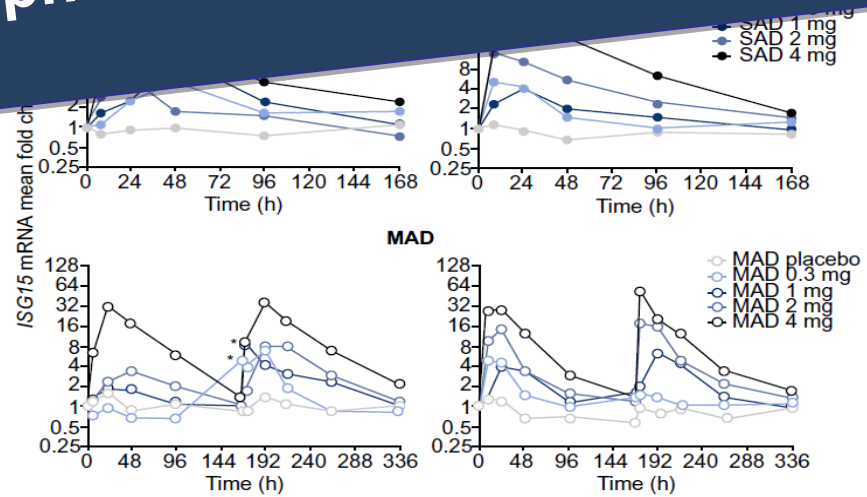
# Oral TLR7 Agonist

GS-9620

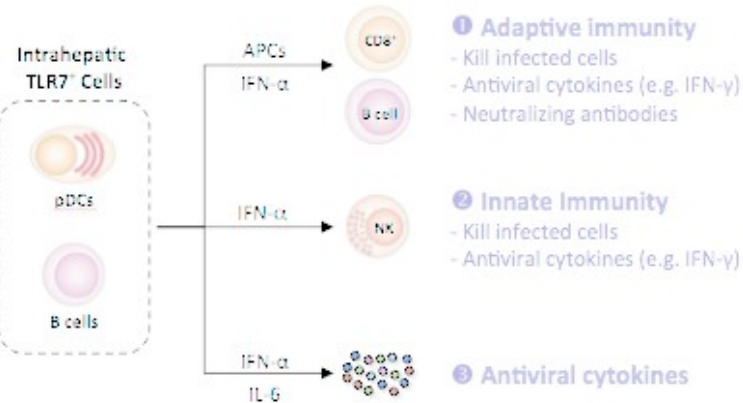


Persistent HBV viremia suppression and increased HBsAg in chimps (Menne et al., J Hepatol 2015) and woodchucks (Landorf et al Gastroenterology 2019)

Oral GS-9620 was safe, well tolerated; absence of significant systemic IFN-alpha levels or related symptoms

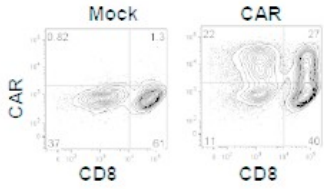


**Fig. 1. ISG15 (mRNA) fold change.** The figure shows the ISG15 mRNA mean fold change over time in the SAD and MAD cohorts in each patient population. \*Induction at these time points driven by a single patient.

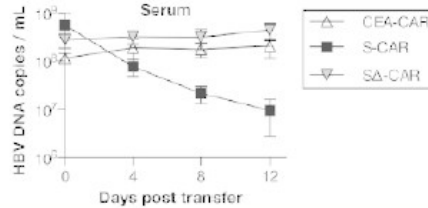


# Engineering anti-HBV immunity

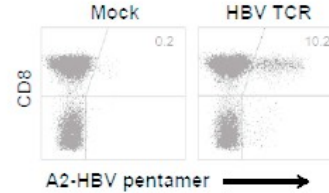
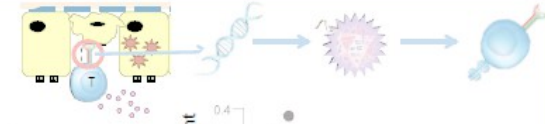
## Chimeric antigen receptor (CAR)



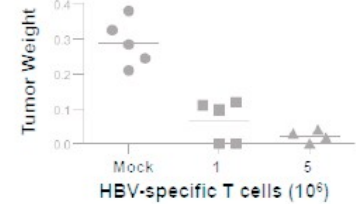
Krebs et. al. Gastroenterology. 2013 Aug;145(2):456-65.



## T cell receptor (TCR) gene therapy



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



## Case Report

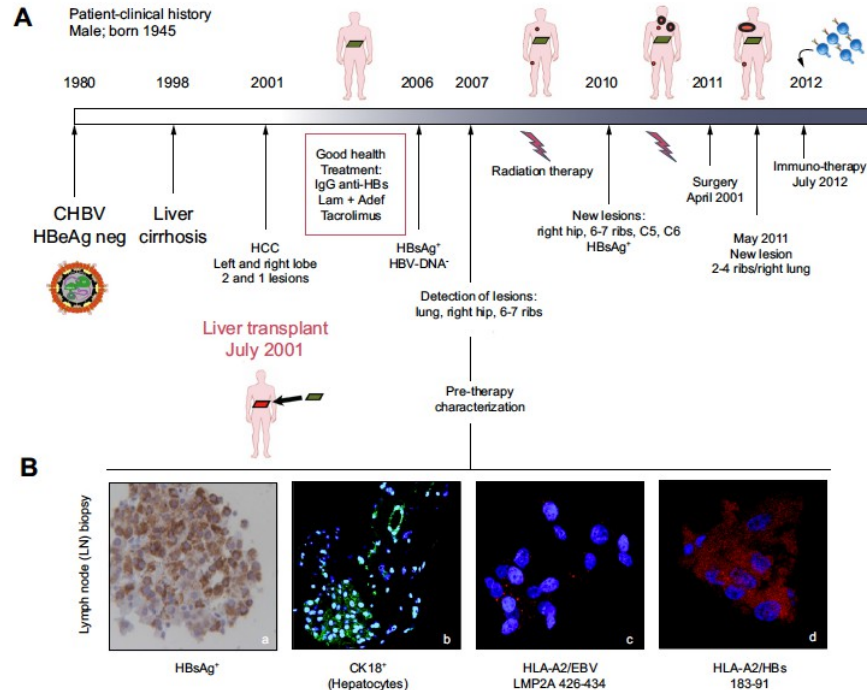
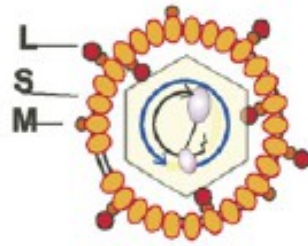


Fig. 1. Clinical history and expression of HBV antigens in HCC metastasis. (A) Schematic representation of the clinical history of the treated patient. (B) Sections (40×) of

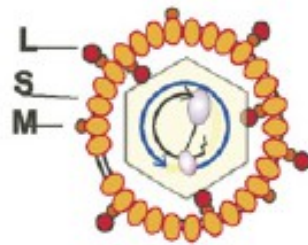
# HBV cure landscape

## Entry inhibitors

- Lipopeptides, e.g. Myrcludex-B
- Cyclosporin derivatives



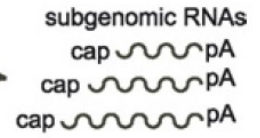
envelope  
RC-DNA  
virion



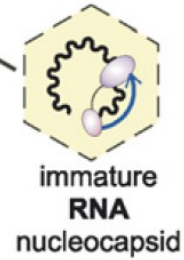
## Targeting cccDNA



## RNA interference, Arrowhead, Tekmira, Alynlam, GSK



core protein + P protein



mature  
RC-DNA  
nucleo-  
capsid

plasma  
membrane

## Inhibitors of HBsAg release Replicor

## Inhibition of nucleocapsid assembly Novira, AssemblyPHARMA, Gilead, Janssen

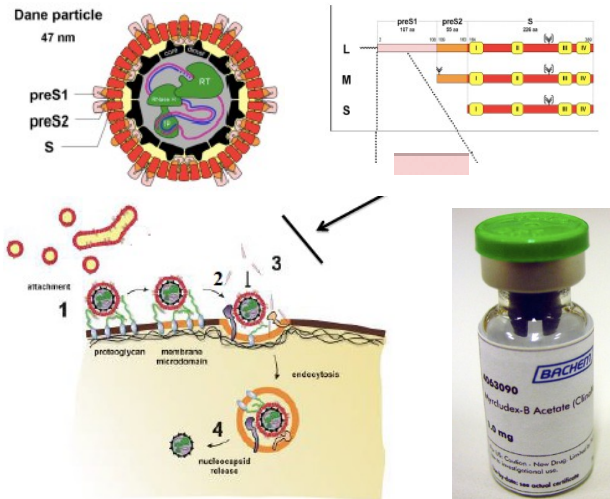
## Polymerase inhibitors

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

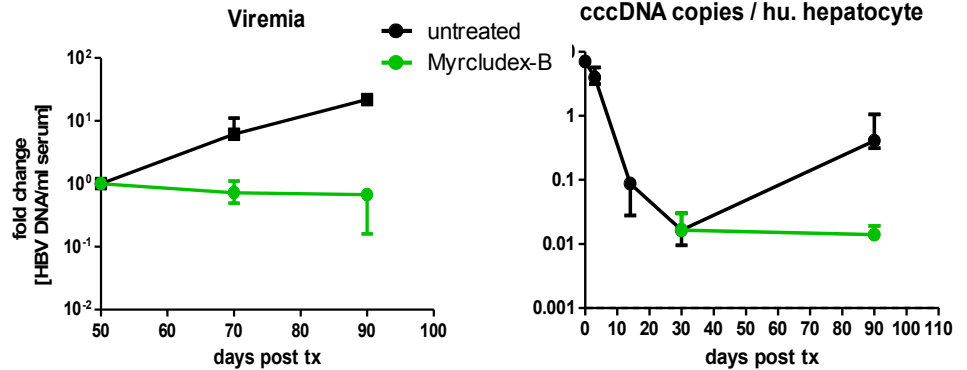
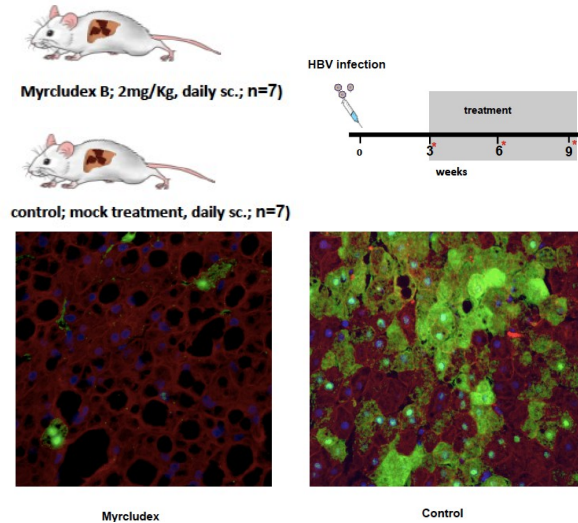
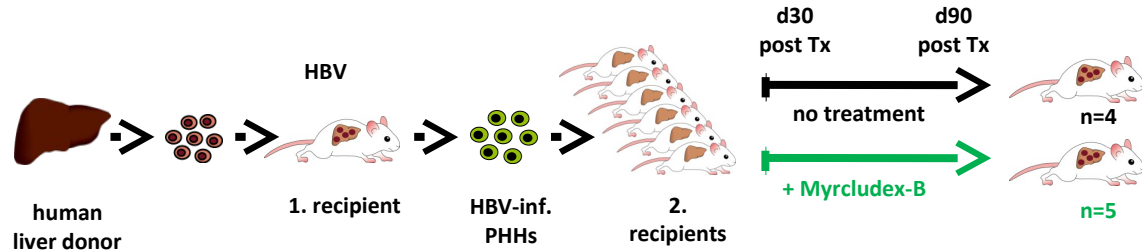
## Immune modulation

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

# Myrcludex B: Targeting Entry of HBV into Hepatocytes

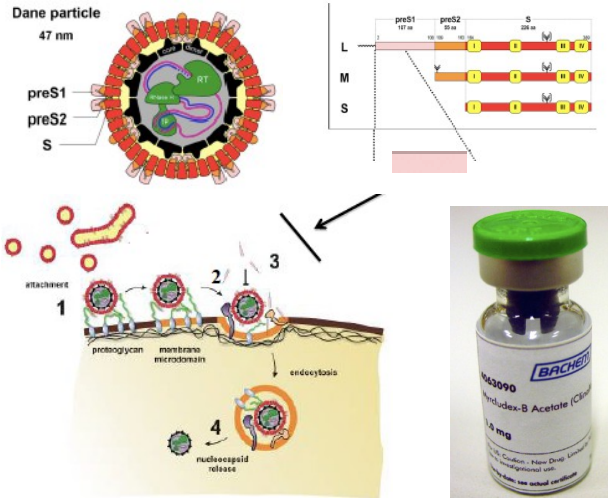


## Entry inhibitor plus cell proliferation support loss of cccDNA and HBsAg

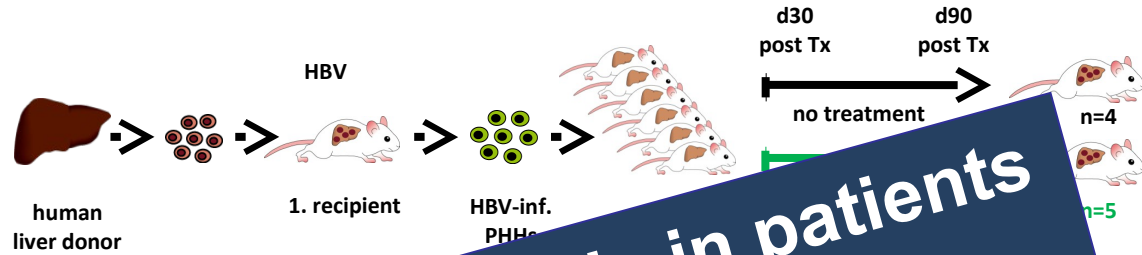


Cell proliferation combined with antiviral treatment to block re-infection (Myrcludex B) promoted cccDNA clearance in the majority of the human hepatocytes.

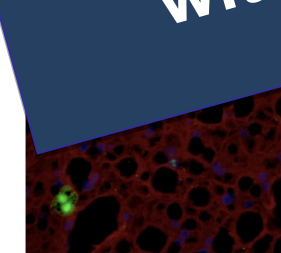
# Myrcludex B: Targeting Entry of HBV into Hepatocytes



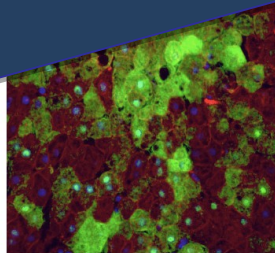
Entry inhibitor plus cell proliferation support loss of cccDNA and HBsAg



Myrcludex B

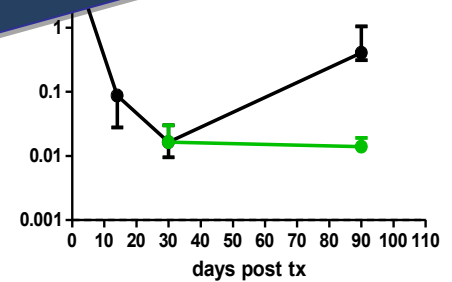
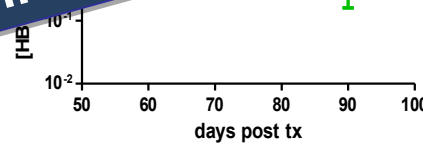


Myrcludex



Control

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

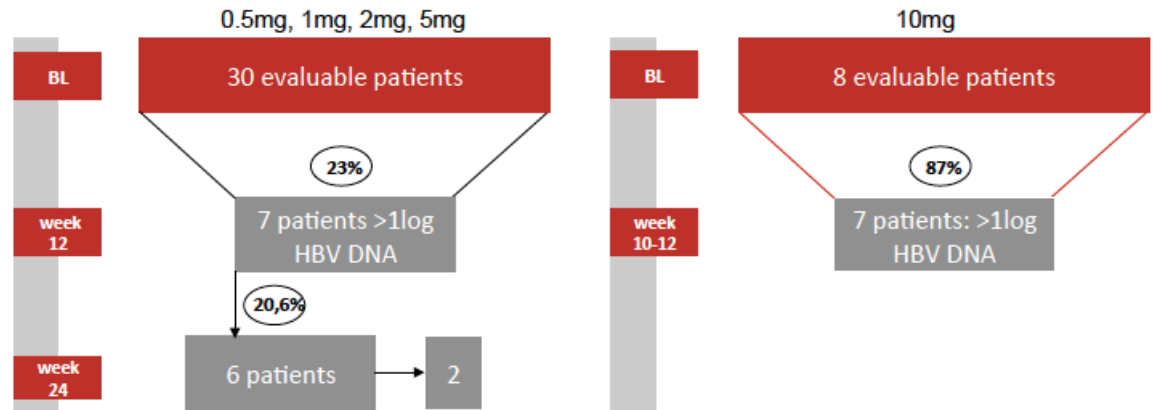
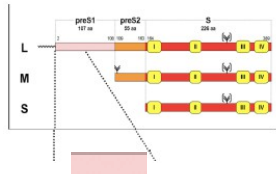


Cell proliferation combined with antiviral treatment to block re-infection (Myrcludex B) promoted cccDNA clearance in the majority of the human hepatocytes.



# Myrcludex B: Targeting Entry of HBV into Hepatocytes

## HBV Phase 2a Results

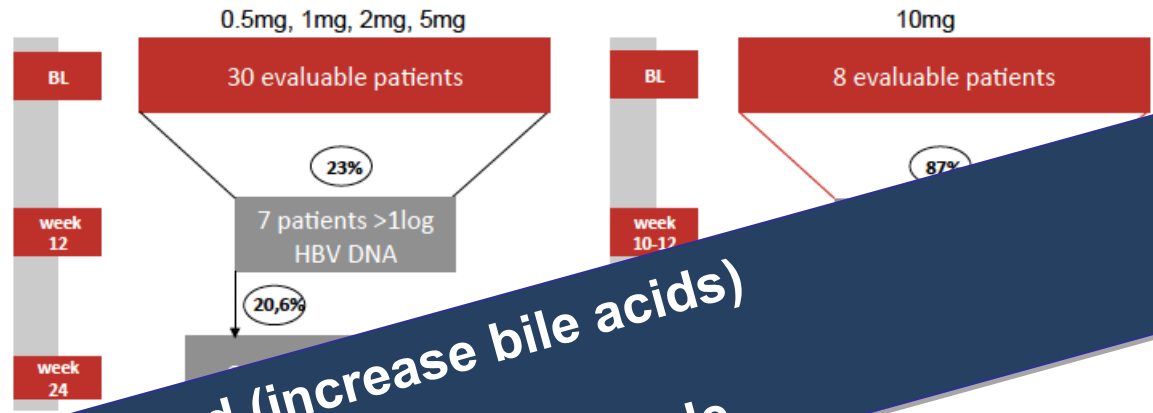
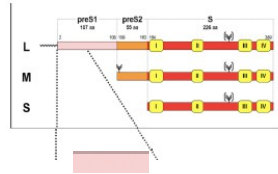


## HDV Pilot Study



# Myrcludex B: Targeting Entry of HBV into Hepatocytes

## HBV Phase 2a Results



Well tolerated (increase bile acids)  
efficacy vs treatment schedule

## HDV Pilot Study



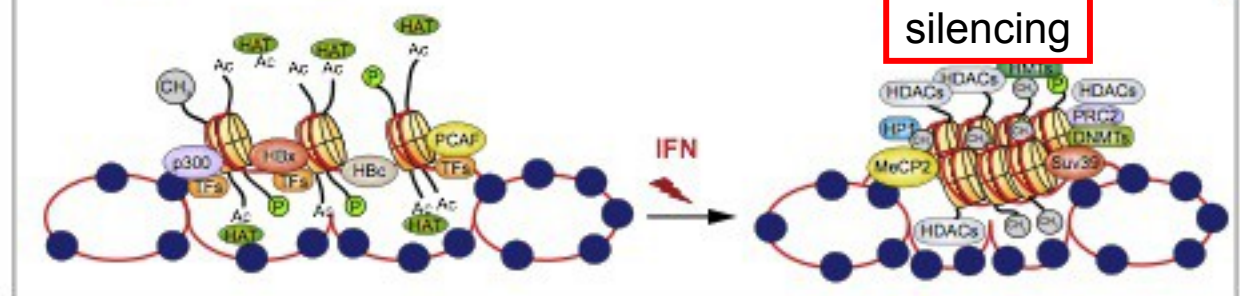
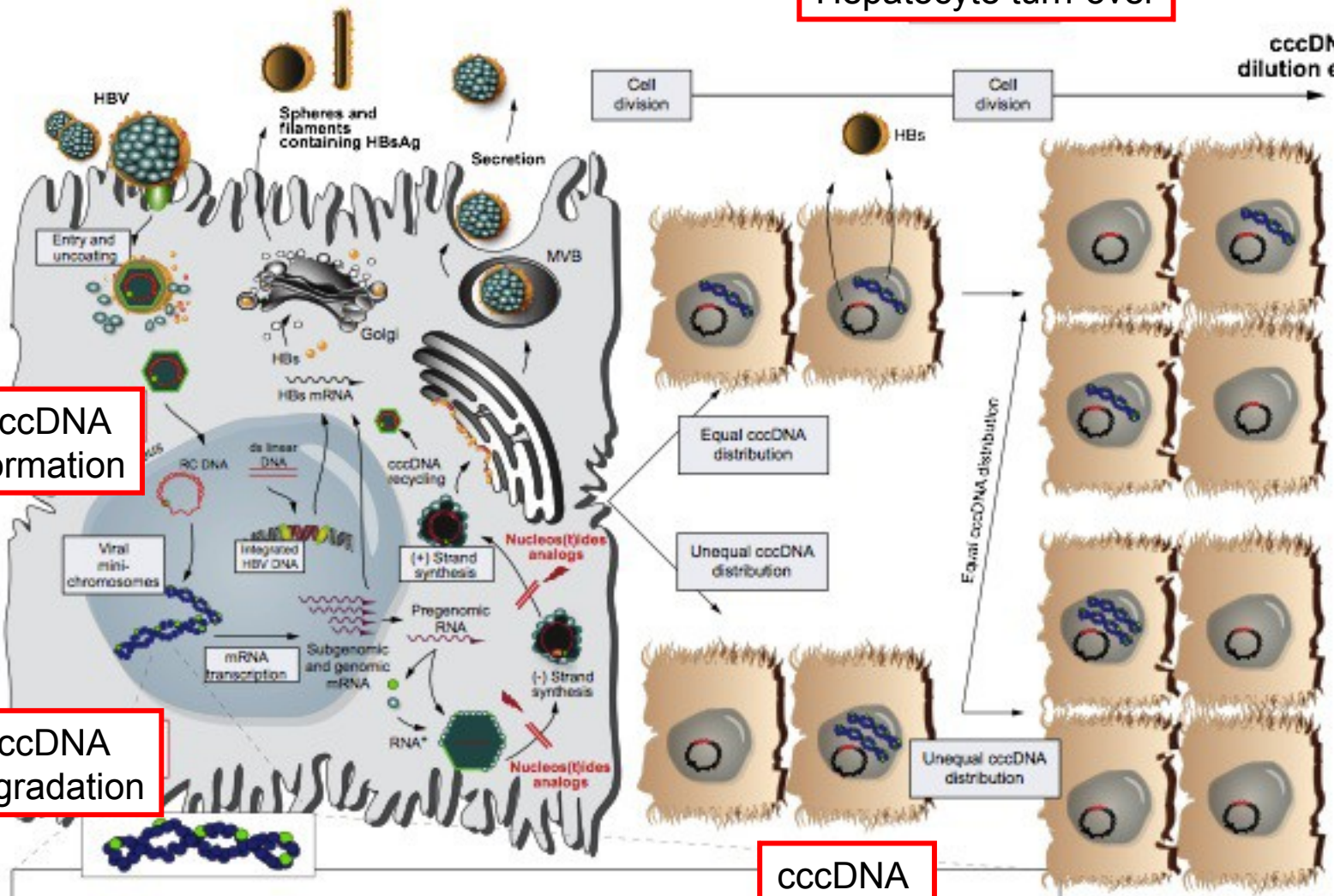
Hepatocyte turn-over

cccDNA formation

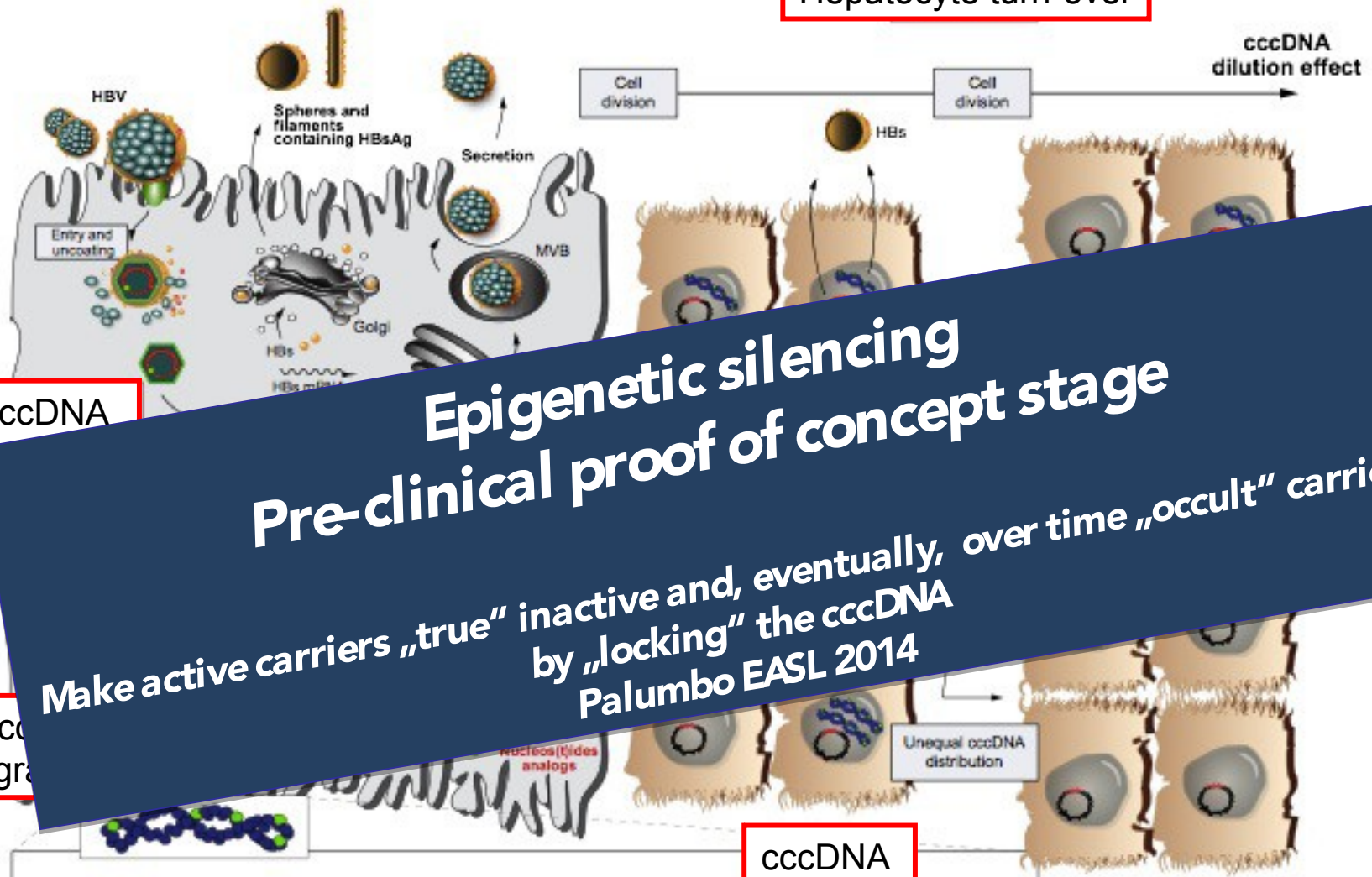
cccDNA degradation

cccDNA silencing

cccDNA dilution effect



# Hepatocyte turn-over

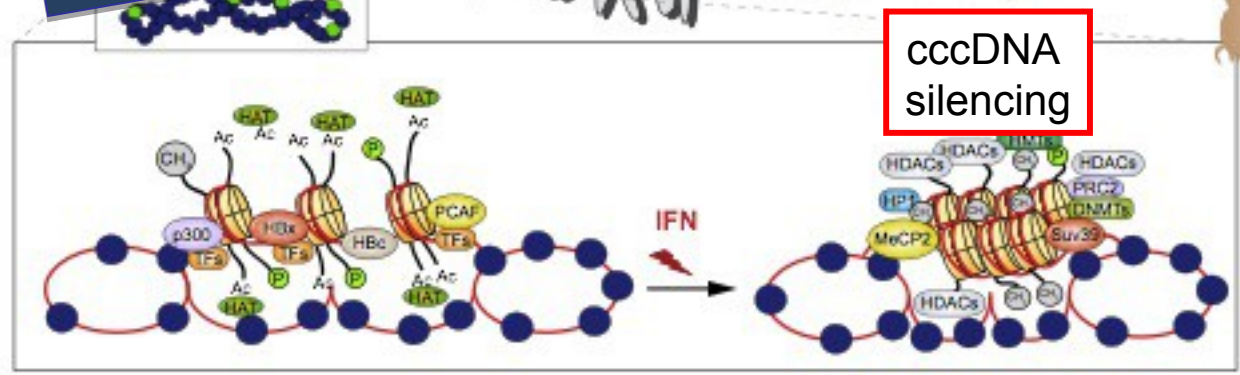


cccDNA

## Epigenetic silencing Pre-clinical proof of concept stage

Make active carriers „true“ inactive and, eventually, over time „occult“ carriers  
by „locking“ the cccDNA  
Palumbo EASL 2014

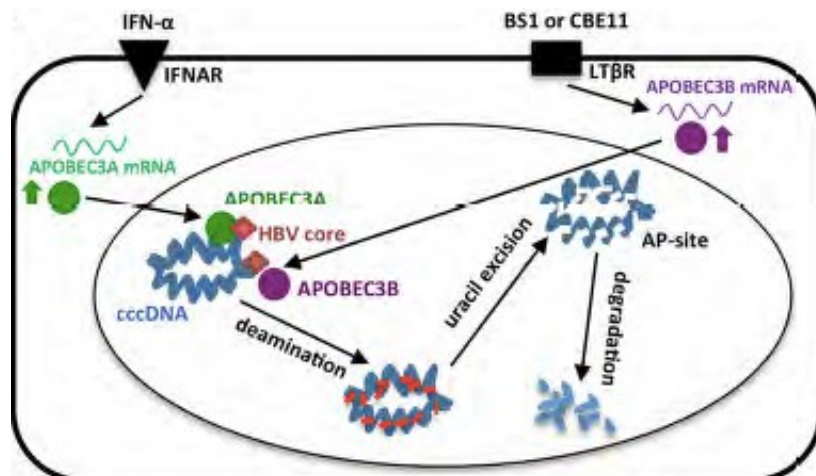
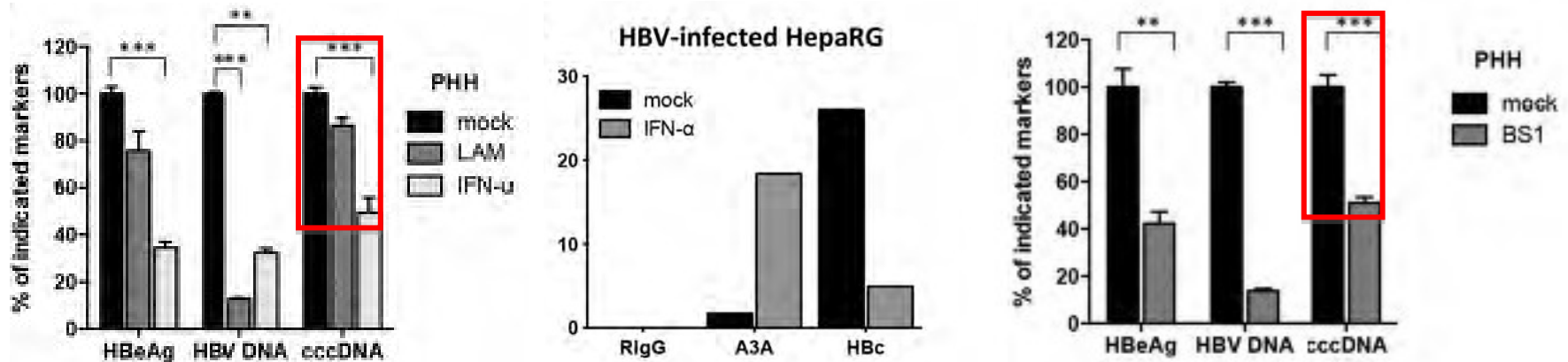
cccDNA  
degr



cccDNA  
silencing

cccDNA  
Integrated HBV DNA

# Specific and Nonhepatotoxic Degradation of Nuclear Hepatitis B Virus cccDNA



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