15th Residential Course on Clinical Pharmacology of Antiretrovirals

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Novel targets and strategies to cure HBV

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Disclosures

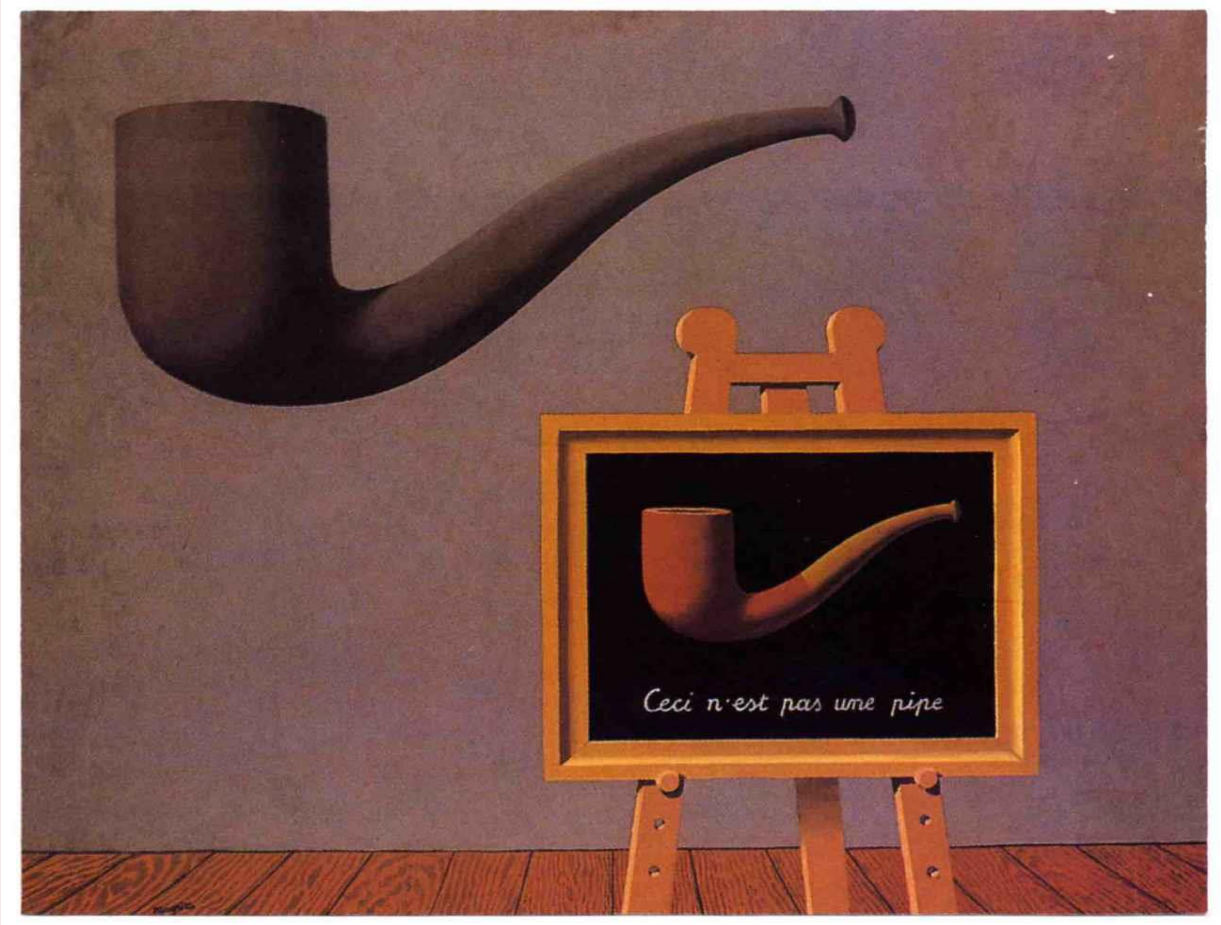
Advisory board: Abbott, AbbVie, Roche, Janssen

Speakers' bureau: AbbVie, Gilead, MSD

Research grant: AbbVie, BMS, MSD

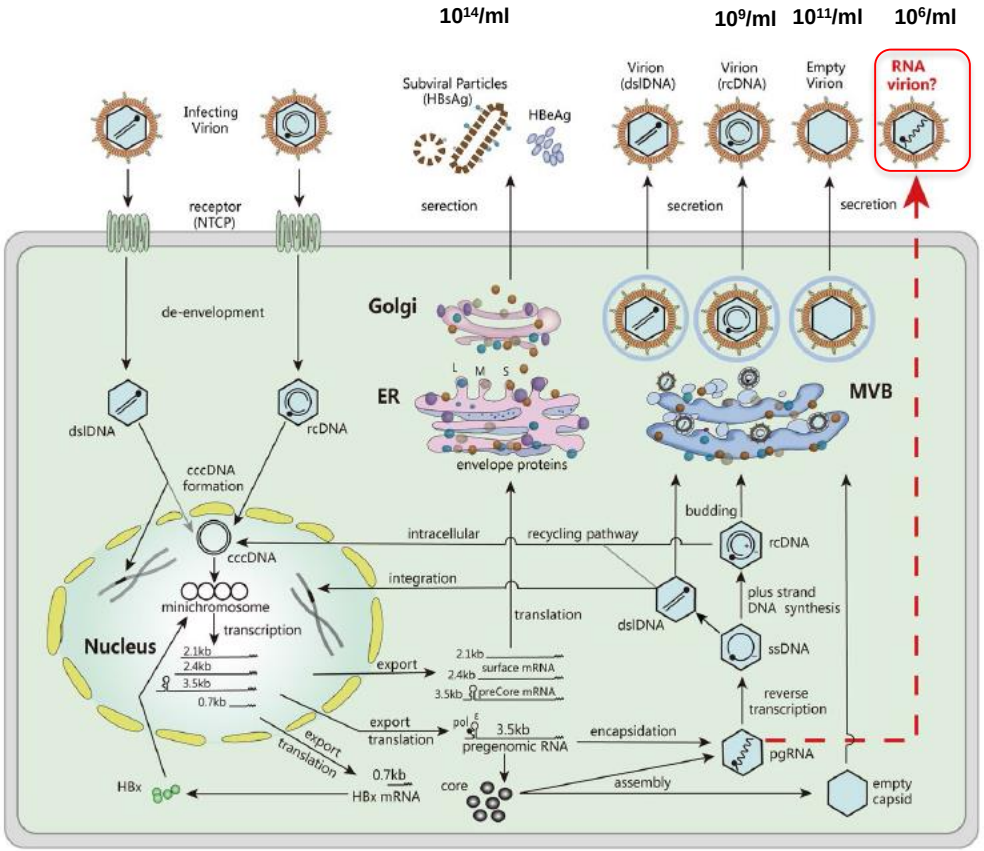
Unmet need for a personalized management of current and future antiviral treatments

- Identification of the immunological profile associated with an effective control of HBV infection
- Availability of biomarkers with high diagnostic accuracy in the identification of the carriers who achieved an effective and persistent control of HBV infection



Magritte, I due misteri 1966

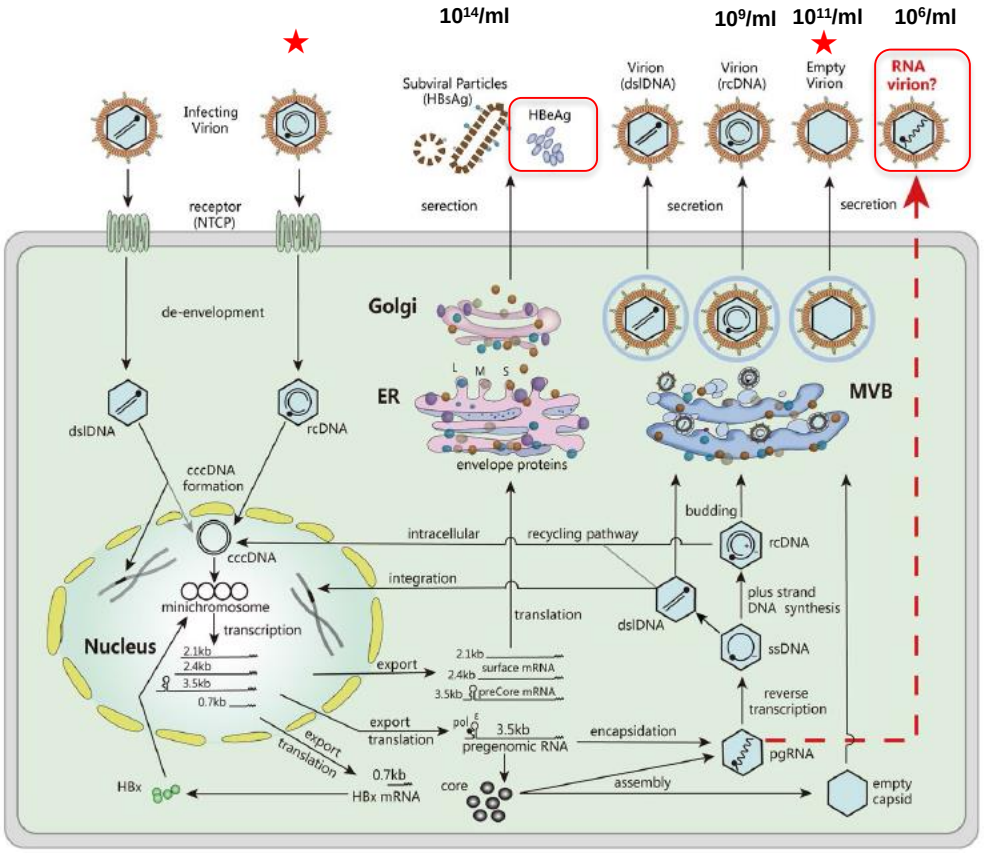
Old and new serum HBV markers



- During the HBV life cycle in addition to infective virions a **heterogenous**, not yet fully characterized, **population** of **defective particles** is produced
- **Empy virions**, **RNA containing** virions or RNA containing - naked capsid (circulating as capsid-anti-HBc complexes) are the most studied as **potential surrogate markers of cccDNA**
- However these **particles** need to be **better characterized** (empty virions contain «core» protein or an aberrant pre-core protein? Is the heterogeneity of circulating HBV-RNA influencing its detection?) as well as **their dynamics** during the course of infection or treatment
- A comprehensive understanding of their nature is mandatory to optimize the diagnostic assays and their use in clinical practice

Adapted from *Liu S et al, Hepatology 2019;* Jansen L, J Inf. Dis 2015; Honda M et al J Infect Dise2015; Hu J &Liu K, Viruses 2017

Old and new serum HBV markers



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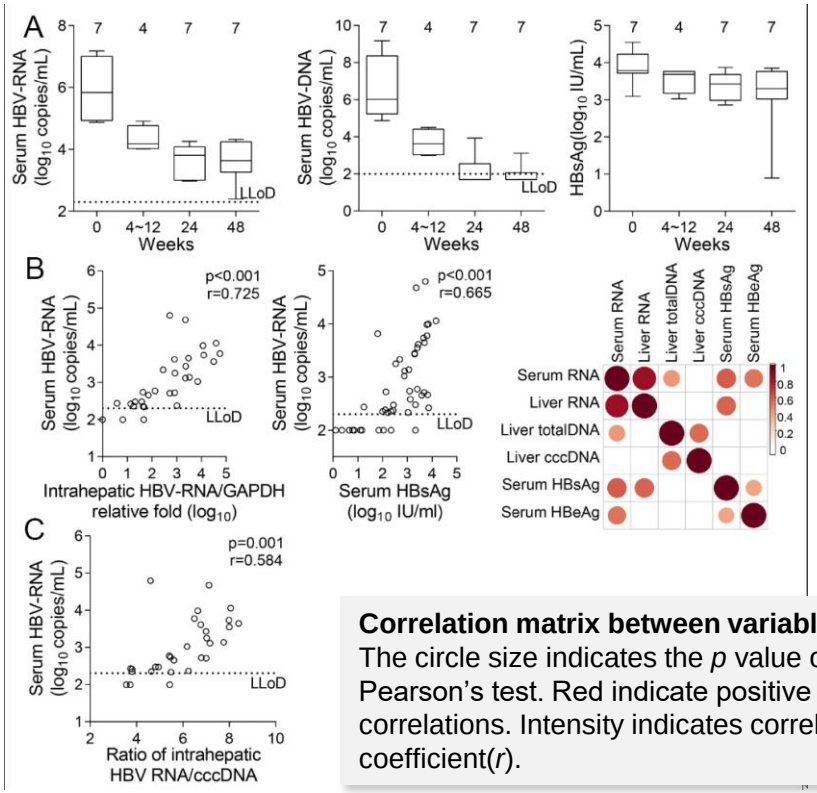
Relationship between serum HBV RNA levels and intrahepatic viral as well as histologic activity markers in 47 entecavir-treated patients



Correlation matrix between variables. The circle size indicates the *p* value of Pearson's test. Red indicate positive correlations. Intensity indicates correlation coefficient(*r*).

No significant correlation was found between between **serum HBV-RNA** and **intrahepatic cccDNA** copy number

(A) Kinetics of levels of serum HBV-RNA, HBV-DNA, and HBsAg during the 48 weeks of entecavir treatment.

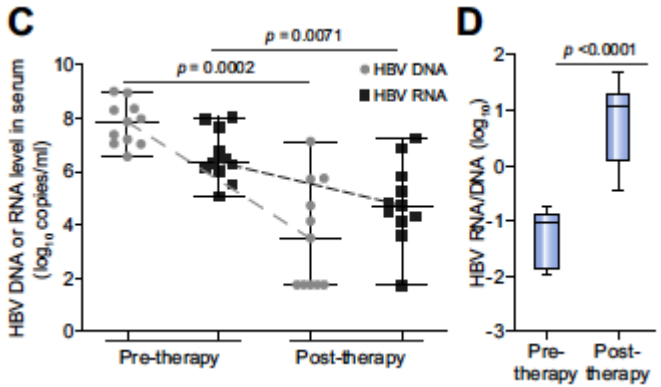
(B) Correlation of **serum HBV-RNA** and **intrahepatic HBV-RNA** or serum HBsAg.

(C) Correlation between **serum HBV-RNA levels** and **intrahepatic viral transcriptional activity** (**intrahepatic HBV-RNA/cccDNA**).

HBV-RNA serum levels appear to be a marker of cccDNA transcriptional activity

Jing Wang et al, J Hep 2017

Serum HBV-RNA is encapsidated pregenome RNA that may be associated with persistence of viral infection and viral rebound



- After the beginning of NA treatment HBV-RNA serum levels showed a slower decline as compared to than that of HBV-DNA.
- The HBV-RNA/HBV-DNA ratio increased significantly after treatment start (P< 0.0001)

Association of HBV-RNA serum levels and viral rebound after the disontinuation of NA

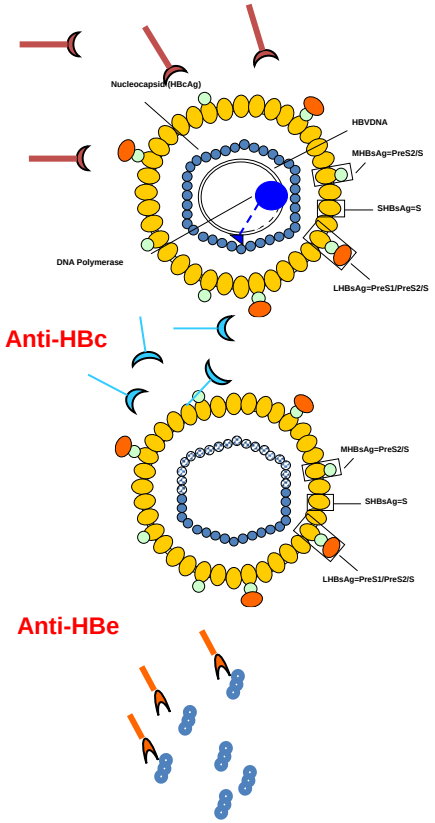
HBV RNA	Viral rebound (n)	No viral rebound (n)	Total (n)	*p value
Positive	21	0	21	
Below the LoQ	3	9	12	0.001
Total (n)	24	9	33	

- **All the patients with detectable HBV-RNA had a rebound of viral replication**
- 3/9 (25%) of pts with undetectable HBV-RNA relapsed

Serum HBV-RNA was confirmed to be pgRNA present in virus like particles and its kinetics during NA therapy could be useful to guide treatment duration

Wang J et al, J Hep 2016

HBcrAg assay



Infectious Virions

10^9/ml

Empy Virions

10^{11}/ml

HBeAg

Automated Chemiluminescence technology
(CLEIA - Lumipulse *G* HBcrAg)

- Disruption of each particle, disruption of Ag/Ab complexes
- Denaturation of the 3 Ags (HBcAg +p22cr + HBeAg), that are than linearized
- 3 monoclonal antibodies are used to capture denaturated HBcAg and HBeAg and 3 as detectors

Detection limit : 2 LogU/mL
(0.1 kU/mL) of HBcrAg

Dynamic range : 3.0 to 7.0 LogU/mL
(1.0 – 10 000 kU/mL)

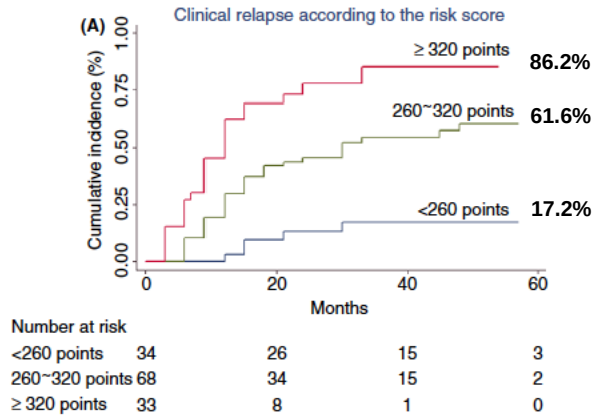
Cutoff: 1.0x 10^3 U/ml

- ✓ In **HBeAg positive patients** HBcrAg serum levels reflect the total amount of cccDNA more than the pgRNA/cccDNA, because HBcrAg is the measure of the translation of both pre-core and pgRNA cccDNA transcripts
- ✓ In **HBeAg negative patiens** HBcrAg serum levels correlate better with the HBV replicative activity (pgRNA and pgRNA/cccDNA) and serum HBV-DNA , because HBcrAg is the measure of pgRNA translation

Kimura T et al J Clin Micr 2002; Brunetto MR, London 2019

Predictors for relapse after cessation of NA in CHB pts

- 135 CHB patients, 129 (95.6%) HBeAg negative, who discontinued NA after a median time of 36.7 months and in virological remission from 25 (IQR, 9.4-30) m.
- At cessation serum HBsAg and HBcrAg levels were 2.77 (IQR 2.15-3.09) and 3 (IQR 2.3-3.9) log U/ml
- Cumulative incidence of clinical relapse (HBV-DNA > 2000 IU/ml +ALT x2 ULN) at 3 and 5 y: 52.7 and 56.1%



The CR risk rose by EOT increasing levels of HBsAg and HBcrAg; unajusted HR for CR were 2.01 for HBsAg and 1.48 x log U/ml for HBcrAg (P<0.0001), respectively

SCALE-B score, a model to predict relapse by 5 EOT variables: **35*HBsAg (logIU/ml) + 20*HBcrAg(logU/ml) + 2*age + ALT (U/l) + 40 TDF**

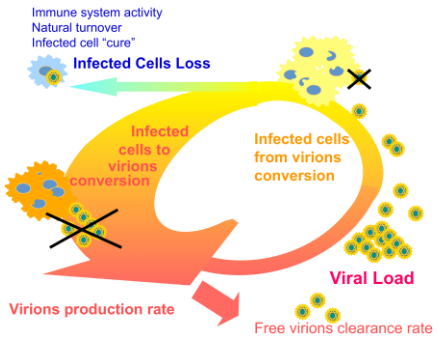
Prediction of relapse after NA cessation

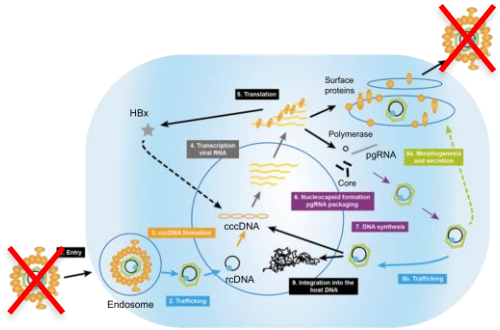
	Sensitivity	Specificity	PPV	NPV
<260, n=34	92.42%	42.03%	60.4%	85.29%
≥320, n=33	37.88%	88.41%	75.76%	59.80%

HBcrAg serum levels could contribute in combination with other markers (HBsAg, HBV-RNA…) to identify candidates to safe treatment discontinuation

Hsu Y-C et al AP&T 2019

Response to antiviral treatment: how it is changing the way to look at it





Virological responses

- *during NA* **undetectable HBV DNA** by a sensitive PCR (LoD 10 IU/ml)
- *after NA*, sustained off-therapy virological response, **HBV DNA <2,000 IU/ml** for at least 12 months
- *during PegIFNa* **HBV DNA <2,000 IU/ml** at 6 months and at the end of therapy.
- *after PegIFNa* **HBV DNA <2,000 IU/ml** for at least12 months

Serological responses

- **HBeAg** are HBeAg loss and HBeAg seroconversion
- **HBsAg** are HBsAg loss and HBsAg seroconversion

On or off therapy

EASL, HBV CPG 2017

Complete sterilising cure:

undetectable serum **HBsAg** and **eradication of HBV-DNA** including intrahepatic cccDNA and integrated HBV-DNA

Functional cure:

sustained, **undetectable** serum **HBsAg** and **HBV-DNA** with/without seroconversion to anti-HBs *(several levels of functional cure according to cccDNA status: complete shut down of cccDNA transcription or its elimination)*

Partial cure:

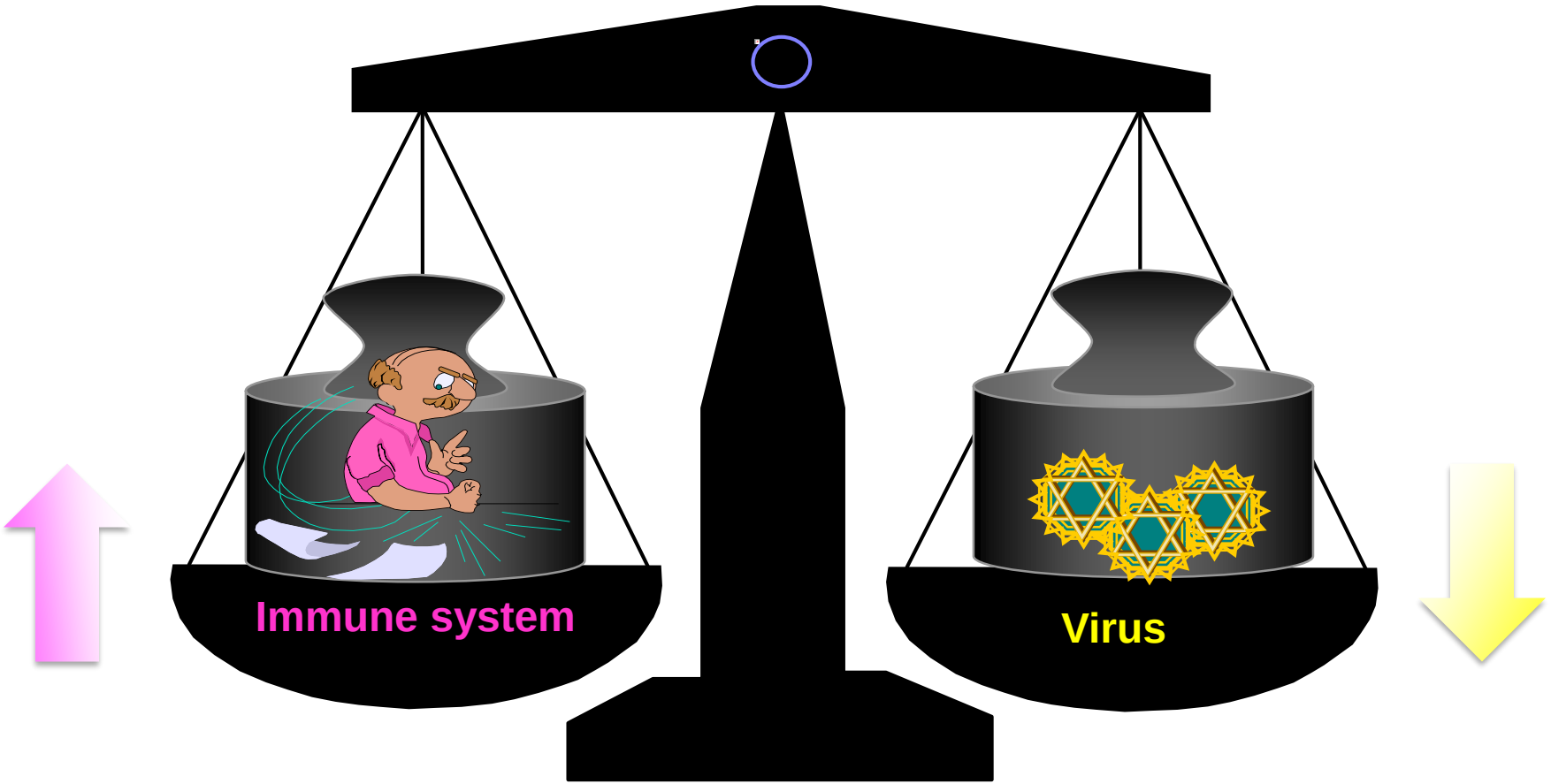
detectable serum HBsAg, but **persistently undetectable** serum **HBV-DNA**

Off therapy

Lok A et al, J Hep 2018

Magritte R, The masterpiece or the mysteries of the horizon 1955

Outcome of chronic hepatitis B infection and liver disease



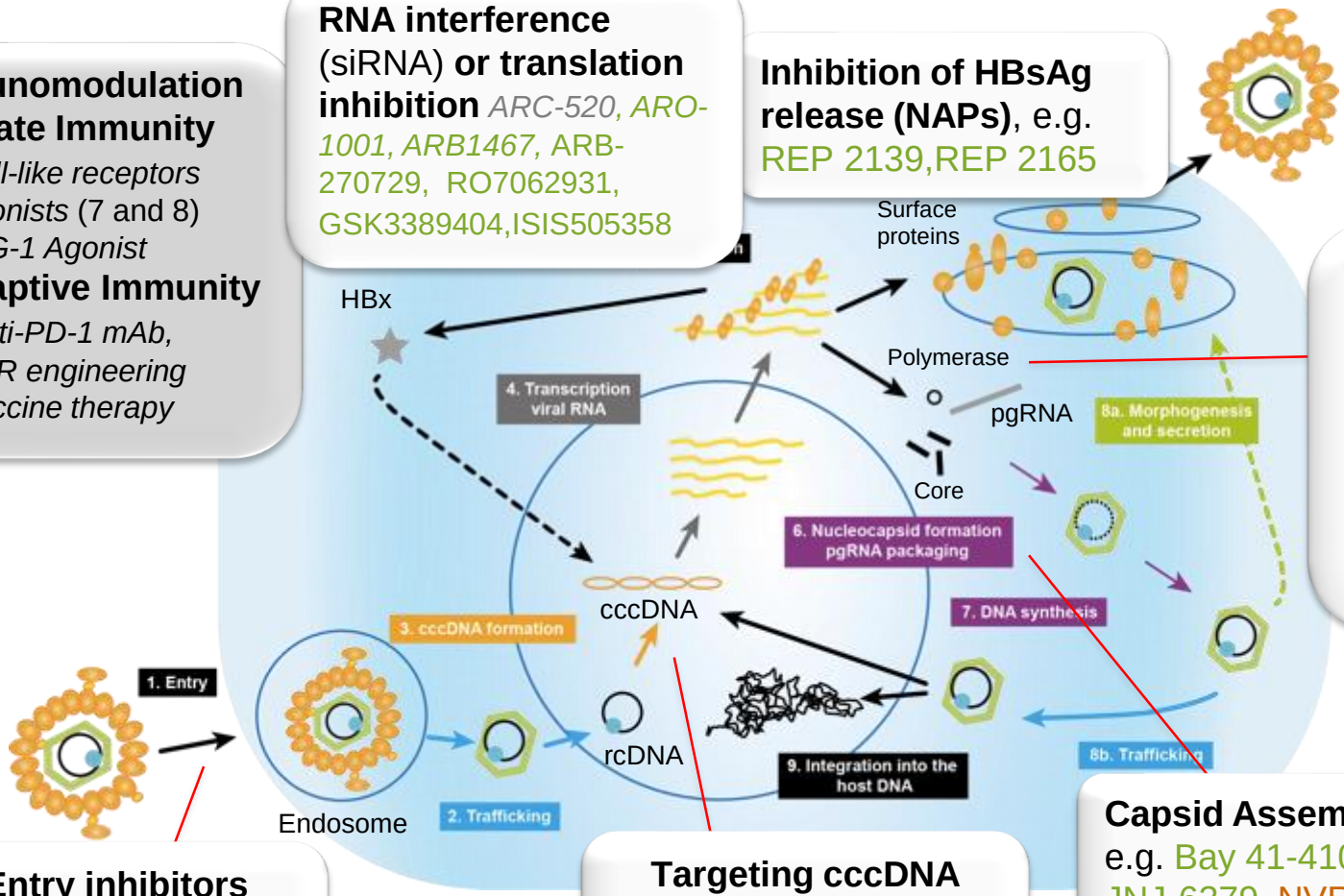
Antiviral treatment is aimed to change the “pathogenetic equilibrium” into a “non pathogenetic one”

Future HBV therapies: new targets, new drugs

Immunomodulation
- **Innate Immunity**
  Toll-like receptors agonists (7 and 8)
  RIG-1 Agonist
- **Adaptive Immunity**
  Anti-PD-1 mAb,
  TCR engineering
  Vaccine therapy

RNA interference (siRNA) **or translation inhibition** *ARC-520, ARO-1001, ARB1467,* ARB-270729, RO7062931, GSK3389404,ISIS505358

Inhibition of HBsAg release (NAPs), e.g. REP 2139,REP 2165



Polymerase inhibitors
- Nucleoside analogues, e.g.
- TAF, amdoxovir, MIV-210
- Non-nucleoside, e.g. LB80380

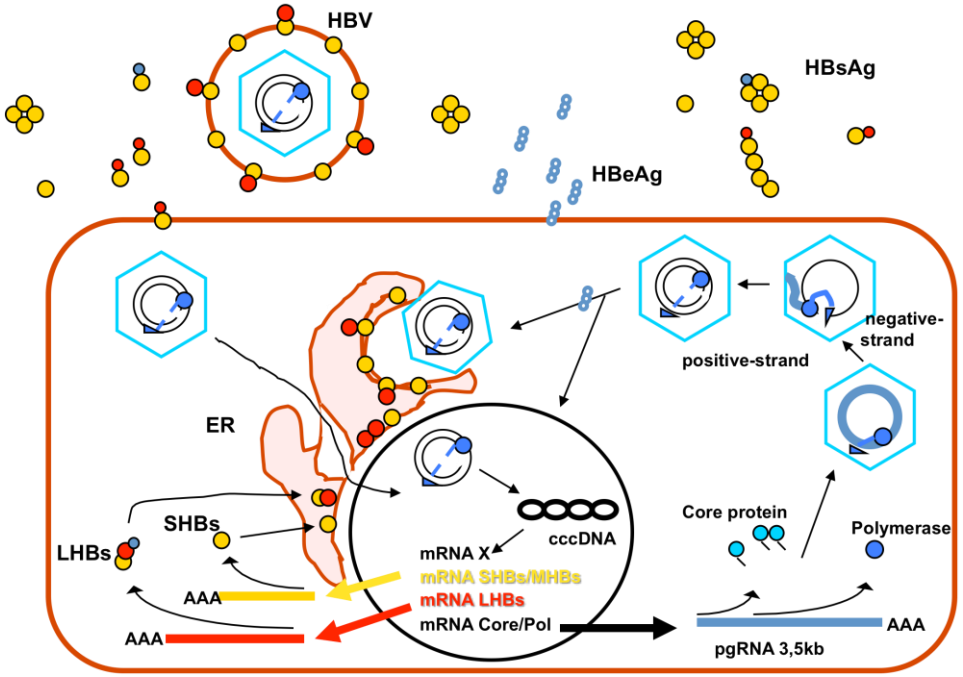
Capsid Assembly Modulators, e.g. Bay 41-4109, RO7049389, JNJ-6379, NVR1221, AB-506

Entry inhibitors (HBV/HDV)
- Lipopeptides, e.g. Myrcludex-B

Targeting cccDNA
- HAPs
- Chromatin-modifying enzymes

Development stage: **preclinical**, **clinical** ; *modified and updated from* Zoulim F, et al. Antiviral Res 2012;96(2):256–9;

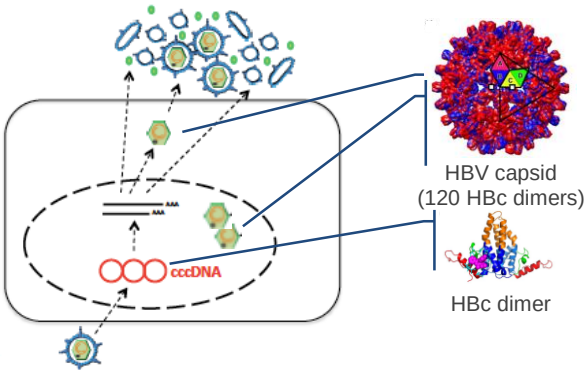
Development of anti-HBV direct acting antivirals



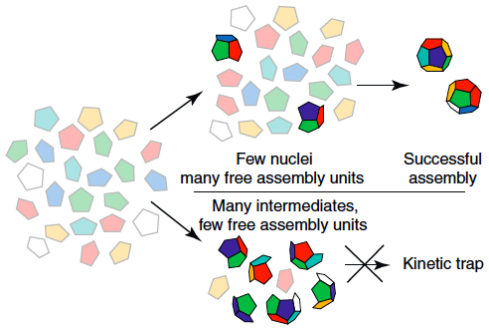
- HBV is not directly cytopathic and it persists in the infected cell without altering its homeostasis
- Major steps of viral life cycle are mediated by cellular receptors, proteins or enzymes
  (sodium taurocholate cotransporting polypeptide (NTCP); Host DNA repair mechanisms to convert RC-DNA into cccDNA; histones are bound to cccDNA forming the viral minichromosome…)

- The HBV interaction with the hepatocyte is pervasive and complex and could make it difficult to identify molecules acting exclusively on the virus machinery.
- Therefore the risk of off target effects is high

Targeting HBc protein / HBV capsid by Core inhibitors / Core Protein Assembly Modulators (CpAM)



- ❖ Capsid formation and pgRNA encapsidation pivotal for infective particles production
- ❖ HBc binds the cccDNA and modifies cccDNA nucleosome spacing



Capsid Assembly Modulators (CpAM):

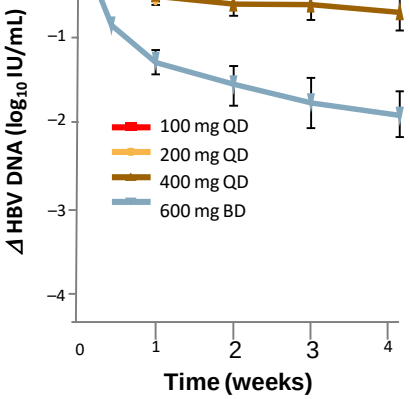
- class 1 CpAM (RO7049389) induces abberrant core protein aggregates, that are subsequently degradated
- class 2 CpAM (JNJ-6379) block pgRNA encapsidation with production of empty capsids

Gane E, AASLD 2014

Oral HBV capsid assembly modulator (CAMs)

- Antiviral effect during 28 days dosing on qHBV DNA

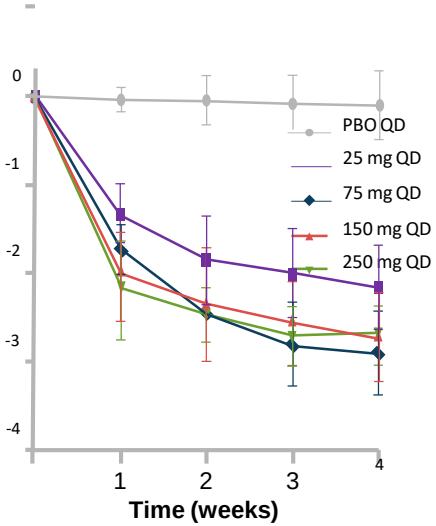
NVR3-778



Yuen M-F, et al. EASL 2016, Barcelona. LBO6

- 1200mg→2log reduction
- No effect on HBsAg
- Skin rash

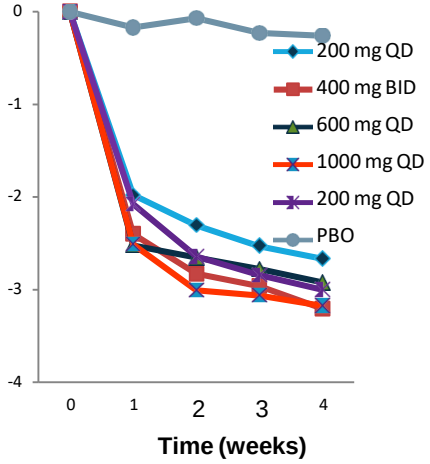
JNJ-6379



Zoulim F, et al. EASL 2018, Paris. #LBO-004

- 250mg→2.9 log reduction
- No effect on HBsAg
- Occ ALT elevation

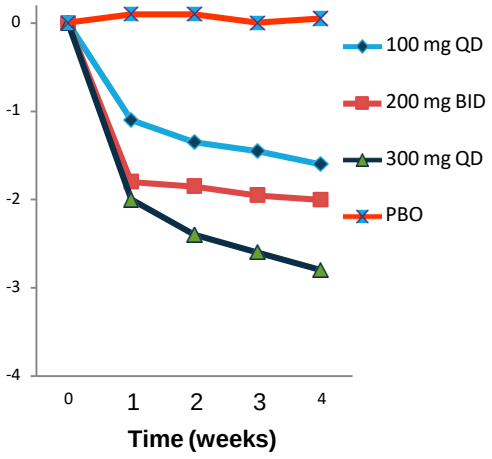
RO7049389



Gane E, et al. EASL 2018, Paris. #LBO-003 Yuen M-F.EASL 2019, Vienna., #FRI-219

- 200mg→3.2 log reduction
- No effect on HBsAg
- ALT elevation in 20%

ABI-H0731



Yuen M-F, et al. AASL D2016, San Francisco

- 400mg→3.9 log reduction
- No effect on HBsAg
- Skin rash

Oral HBV capsid assembly inhibitor (CpAMs)

Longer Duration of 1st Gen CAMs → Study #211: Open label ETV+ ABI-H0731 for 52 weeks in patients who have completed Studies #201 and #202 (24 weeks ETV + ABI-H0731/Plac)

Interim results for HBeAg+ completed ≥ 32 wks ETV + ABI-H0731

- 27 patients from Study #201 (DNA suppressed on NUCs at Baseline)
  11 (41%) are HBV DNA TND, RNA <35 iu/mL and HBeAg <1 IU/mL

- 22 patients from Study #202 (Rx-naïve, DNA> 5 log iu/mL at Baseline)
  Mean HBV DNA decline 6.1 $\log_{10}$
  Mean HBV RNA decline 3.0 $\log_{10}$
  Mean HBcrAg decline 0.8 $\log_{10}$ (7 pts ≥1.0)
  Mean HBeAg decline 0.6 $\log_{10}$ (4 pts ≥1.0)
- ➡ Mean HBsAg decline 0.4 $\log_{10}$ (7 pts ≥0.5, 3 pts ≥1.0)

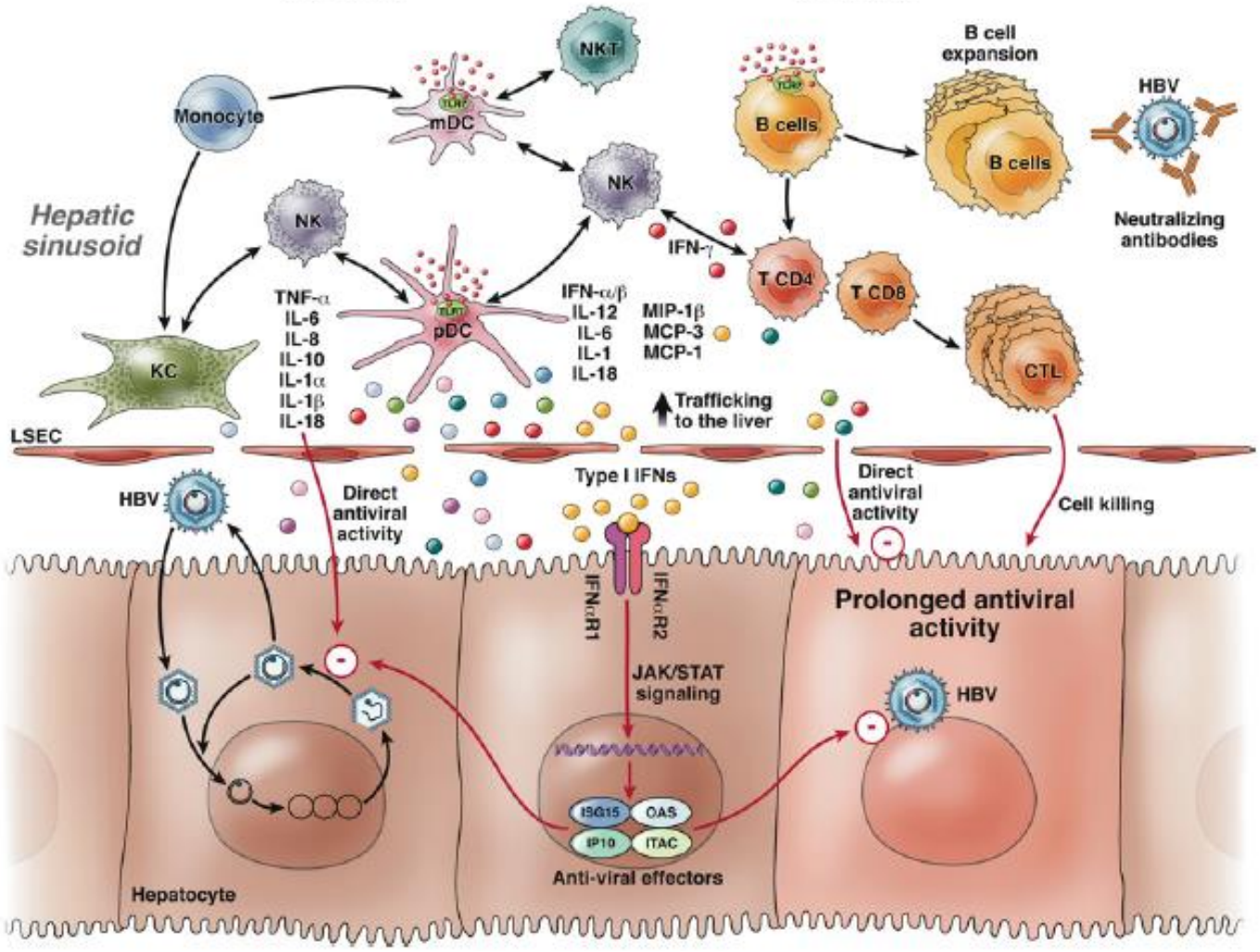
Sulkowski M , et al. AASLD 2019,. #LP1

Poor innate immune response with limited quantity of cytokines (IFN-α, IL-6, TNF-α)

HBV specific T-cell dysfunction, due to persistent exposure to high Antigen doses, T-reg suppression, Dendritic cell impairment

Innate Immunity

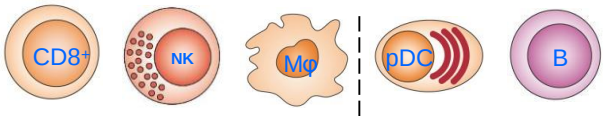
Adaptive Immunity



S.Wieland et al PNAS 2004; A.Stacey et al. J. Virology 2009,; Dunn et al. Gastroent. 2009; Zoulim F et al. Gastroent. 2014

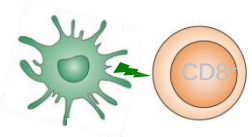
Ways to activate Antiviral Immunity against HBV

1. Stimulate Antiviral Effector Cells



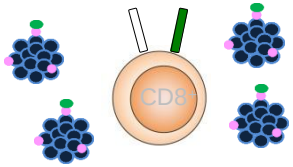
- TLR-7, TLR-8, RLRs, CLRs, NLRs
- DNA sensors

2. Generate New T cells



- Therapeutic vaccines

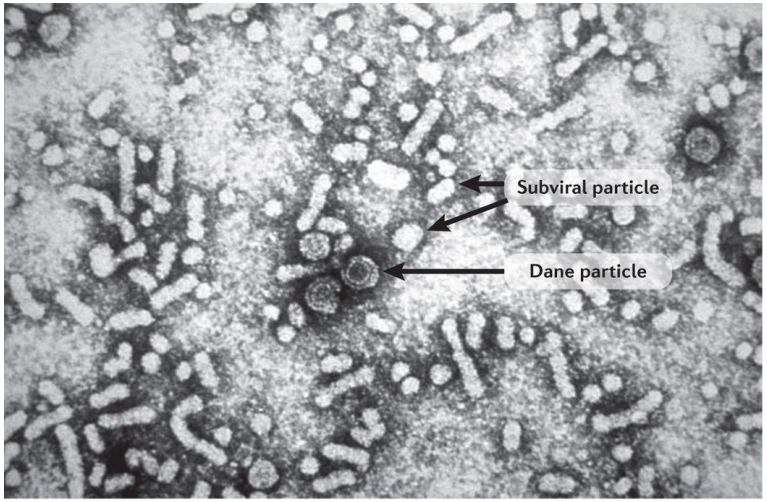
3. “Rescue” Exhausted T cells



- Reduce viral antigens
- Modulate immune receptors (PD-1)
- Relieve suppression of T cells
- Inhibit T regs

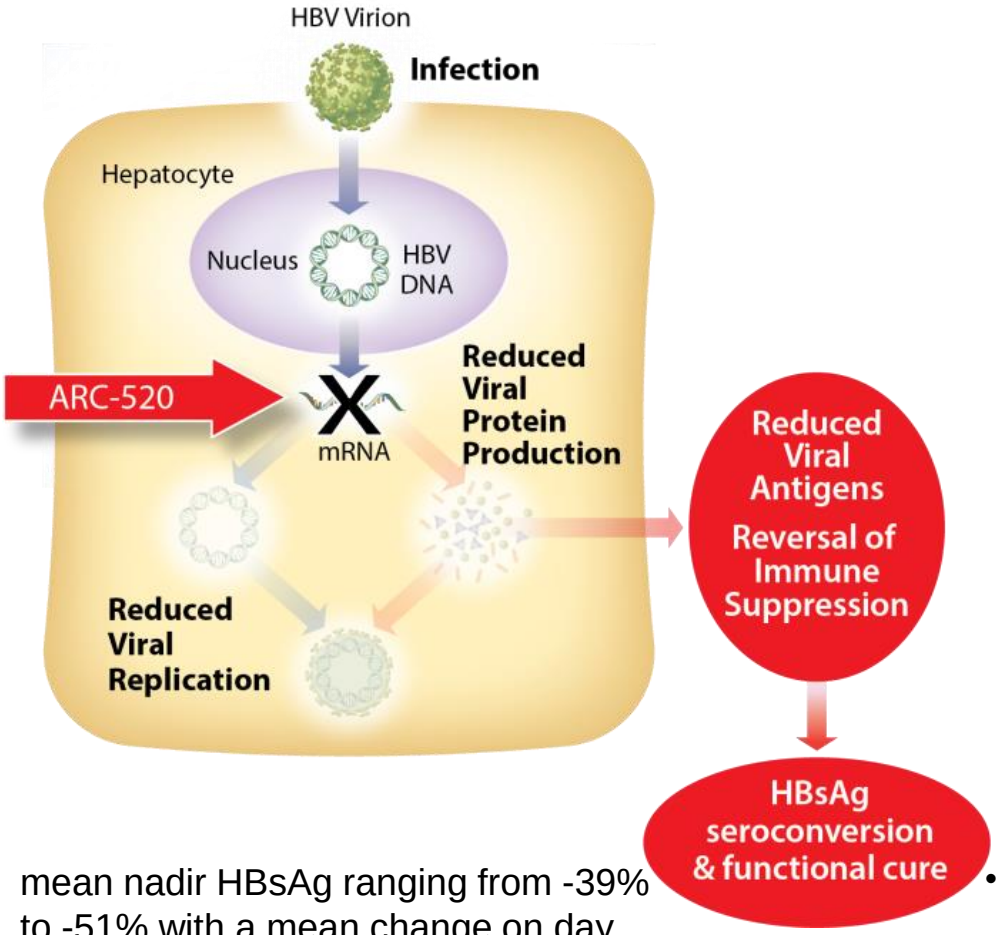
Inhibition of HBsAg production

- **Direct silencing of S gene** by direct action on the HBV-DNA sequence *(CRISPR-mediated base editing)*
- **Inhibition of HBsAg proteins translation** *(silencing RNA, antisense oligonucleotides, mRNA destabilisers)*
- **Inhibition of HBsAg particles secretion** *(NAPs)*



Nature Reviews | Disease Primers

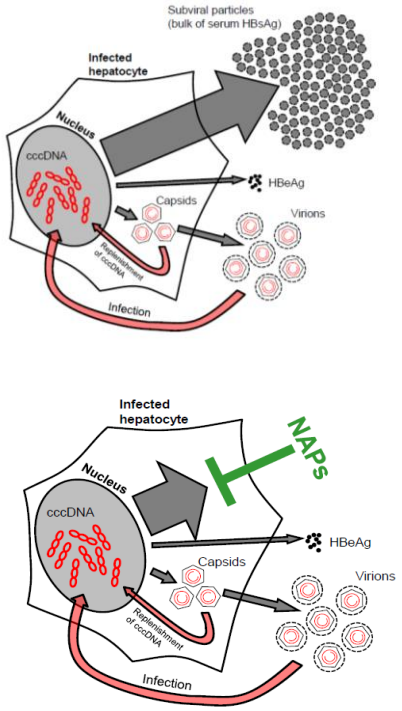
Inhibition of HBsAg production



- mean nadir HBsAg ranging from -39% to -51% with a mean change on day 85 of 31-22%
- This is the first time that a reduction in HBsAg mediated through RNA interference has been shown in chronic HBV patients.

Yuen MF et al, AASLD 2014

Nucleic Acid Polymers (NAPs)



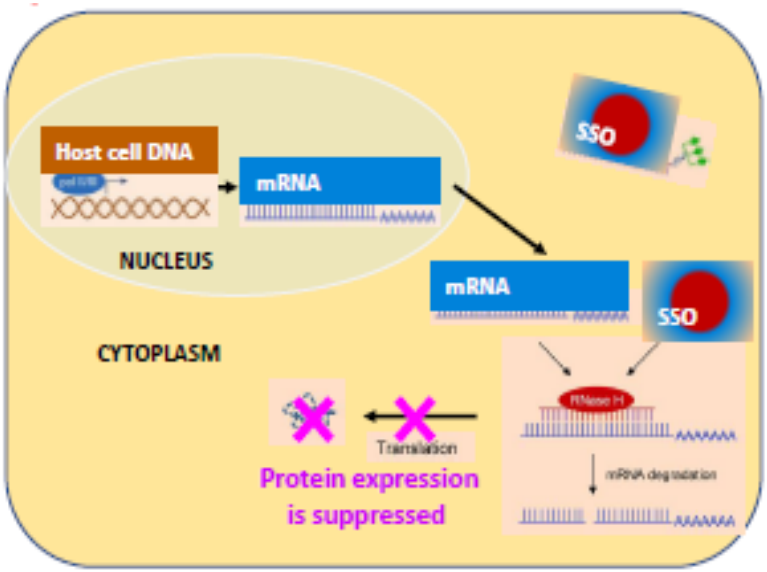
- REP 9-AC HBsAg serum clearance in 7/8 HBeAg + pts with durable HBV-DNA reduction in 2/7 responders
- REP 2139 monotherapy, followe by combination with Peg-IFN and Peg-IFN monotherapy led to undetectable HDV-RNA in 10/12 HDV pts, without rebound of HBsAg in pts with lelev < 10 IU/ml

Mahtab MA et al Hepatology 2011, Bazinet et al J Hep 2016

Other approaches to inhibit translation

Antisense oligonucleotides

- ASOs silence HBV gene expression by hybridising to HBV mRNA and activating host RNAse H mediated degradation (not RISC)
- Gal-Nac-conjugation should reduce ASO toxicities of renal dysfunction, low platelets



RO7062931: Phase 1a (Gane #704)
Safe, no toxicity

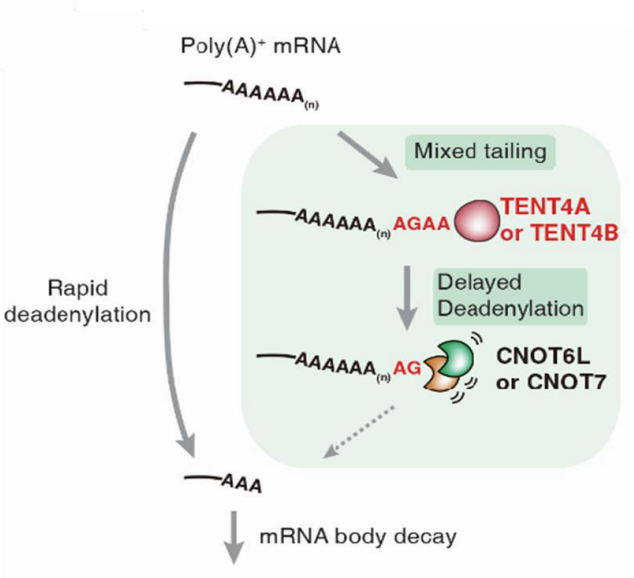
GSK3389404: Phase 1b in NUC-suppressed CHB patients (Yuen #695)
12 week dosing 120mg
HBsAg decline 0.75 log

ISIS505358: Phase 1b in treatment naïve-CHB patients (Yuen #700)
3 week dosing 300mg - HBsAg decline 1.6 log ; HBV DNA decline 1.7 log
HBsAg and HBV DNA <LLOQ in 2 pts maintained for 1-4 months post-treatment

Other approaches to inhibit translation

mRNA destabilisers

Mixed tailing by PPAD5/PPAD7 shields mRNA from rapid deadenylation and decay



Small molecules target host poly-A polymerases PAPD5/7 (TENT4A/4B) which destabilise HBV transcripts from both integrated and cccDNA

Initial compounds associated with preclinical toxicity

Gal-NAC LNA ASOs ASO [locked nucleic acid (LNA)-modified antisense oligonucleotides (ASOs)] targeting host PAPD5/7 POC study in AAV-HBV mouse model (Poster #704)

- Subcut injection Q2 weekly x3
- decrease HBsAg in all animals - mean 2.3 $\log_{10}$
- **4/8 mice had sustained HBsAg loss with anti-HBs**

Mueller Hepatol 2019; 69: 1398; Mueller H, et al. AASLD 2019; #704

Challenges of Translation Inhibitors in CHB

Identification of the most effective HBV targets

- ✓ First gen siRNA (ARC-520) had little effect in HBeAg negative patients

  target sequences downstream from DR1-DR2 region to silence integrated S

  Wooddell C, et al. Sci Transl Med 2017

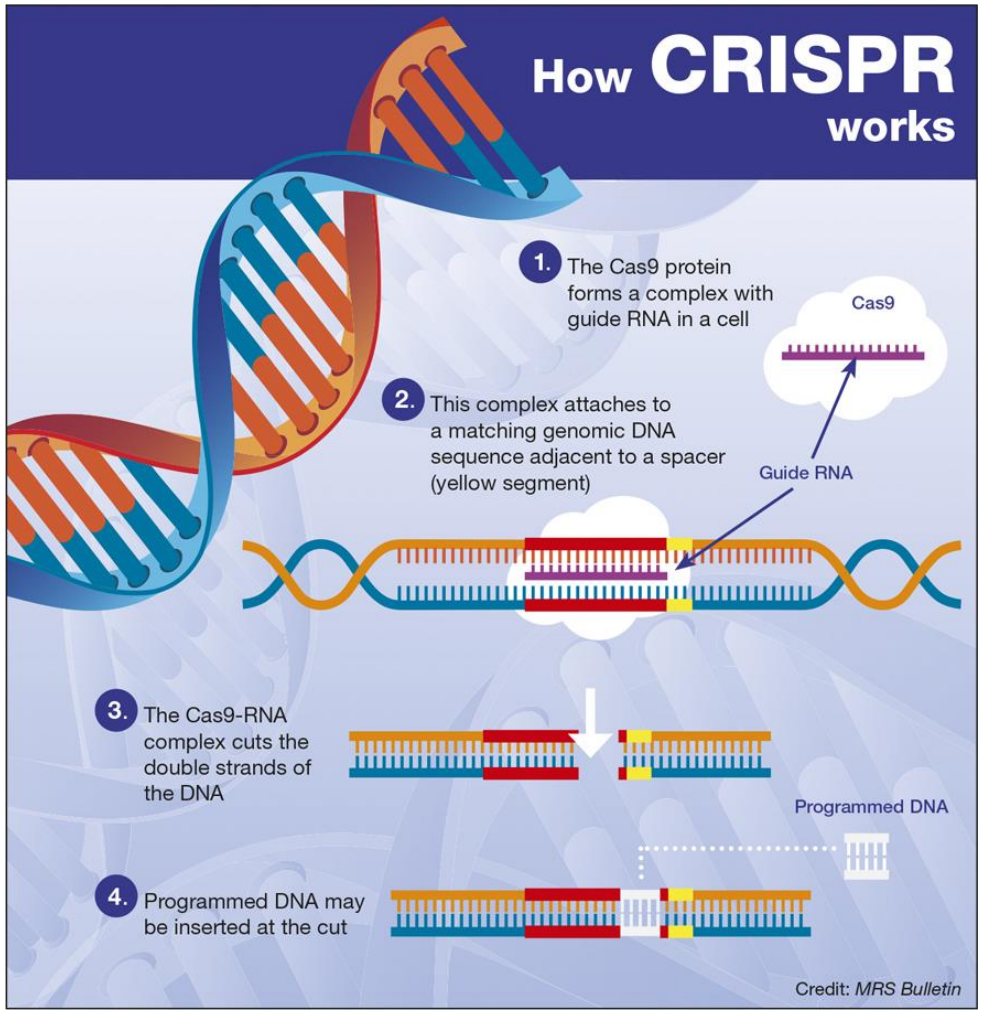
- ✓ Are multiple targets needed to prevent resistance?
- ✓ Should “X” be targeted as well as “S”?

What is best delivery system to hepatocytes

- ✓ 1st Gen NAG-MLP siRNAs (ARC-520/521); LNP siRNAs (ARB-1467/1740) required weekly intravenous dosing, infusion reactions and premeds

- ✓ Gal-NAC conjugated: subcutaneous , monthly dosing, no premeds xyz-**GalNAc binds** to the Asialoglycoprotein **receptor** that is highly expressed on hepatocytes resulting in rapid endocytosis

Efficient silencing of HBV s Gene through CRISPR-mediated base editing

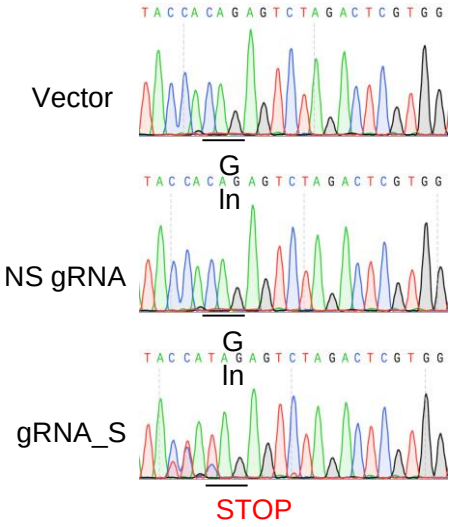


- CRISPR (clustered regularly interspaced short palindromic repeat)-Cas9 (CRISPR-associated nuclease 9) system is an effective approach to edit HBV genome and reduce HBV replication through double-strand DNA break (DSB).
- In addition, the Cas9-mediated DSB is able to cause instability of host genome since integrated viral sequences are common in patients with chronic HBV infection.
- Recent studies show that CRISPR base editors could precisely change single nucleotide without DSB and efficiently silence genes by generating stop codons (CRISPR-STOP).

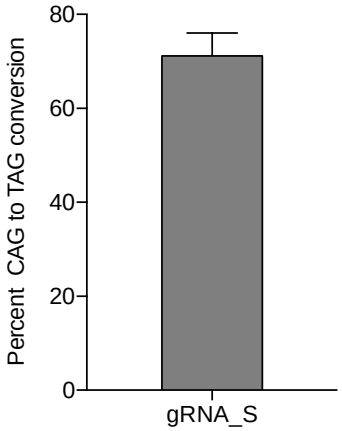
Efficient silencing of HBV s Gene through CRISPR-mediated base editing

- A single guide RNA (gRNA_S) was designed to modify the 30th codon of HBV S gene, which encodes HBV surface antigen (HBsAg), from CAG (glutamine) to stop codon TAG.
- PLC/PRF/5 cell line stably expressing a base editor AncBE4max was constructed through lentivector-mediated transduction.
- AncBE4max expressing PLC/PRF/5 cell line was transduced with either empty lentivector or lentievectors encoding non-specific single guide RNA or gRNA_S.

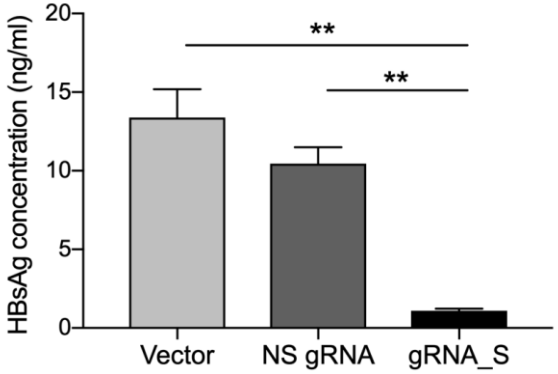
Example sequencing profiles of integrated HBV S gene



71% of cells transduced with lentivector encoding gRNA_S developed a premature stop codon



CRISPR-STOP led to a reduction of HBsAg in the supernatant by 92%



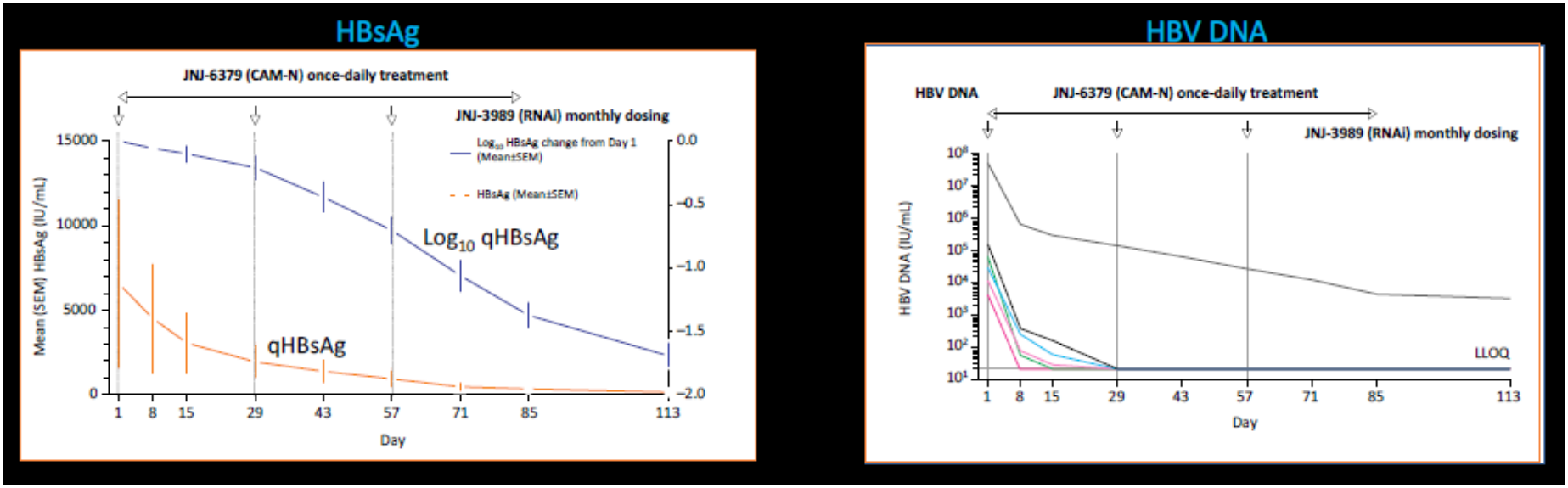
CRISPR-mediated base editing is an efficient approach to silence the HBV S gene *in vitro*, suggesting its potential in the eradication of chronic HBV infection.

Zhou H, Abstract #0086, AASLD 2019

Triple Therapy: RNAi + CAM + NA

HBeAg+ n=4 / HBeAg- n=8, NA-naïve n=5 / experienced n= 7, All 12 Asian

- Three 200 mg JNJ-3989 (ARO-HBV dual iRNA), subcutaneous doses on Days 1, 29 and 57
- Oral JNJ-6379 (CAM) 250 mg once daily for 12 weeks (until Day 85)
- Started or already on ETV or TDF treatment on Day 1 to beyond the end of JNJ-6379 dosing
- Response rates similar between HBeAg+ and HBeAg-



Triple therapy resulted in significant decline in HBsAg levels

Yuen et al , AASLD 2019, Abstract LP4

Combination therapy with capsid allosteric modulator and IFNα promotes innate immune response in HBV-infected hepatocytes

Objective:

To investigate the effect of capsid allosteric modulator (CAM) treatment on intracellular innate immune response in HBV-infected hepatocytes

Methods:

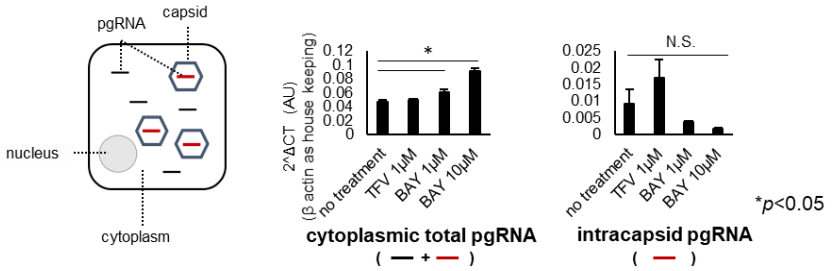
HBV-infected HepG2-hNTCP cells, primary human hepatocytes (PHHs), and human liver chimeric TK-NOG mice were treated with CAM (Bay41-4109) alone or in combination with IFNα and changes of cytoplasmic pgRNA levels, gene expression levels of RLRs-mediated innate immune signaling and antiviral effects were analyzed.

Conclusions:

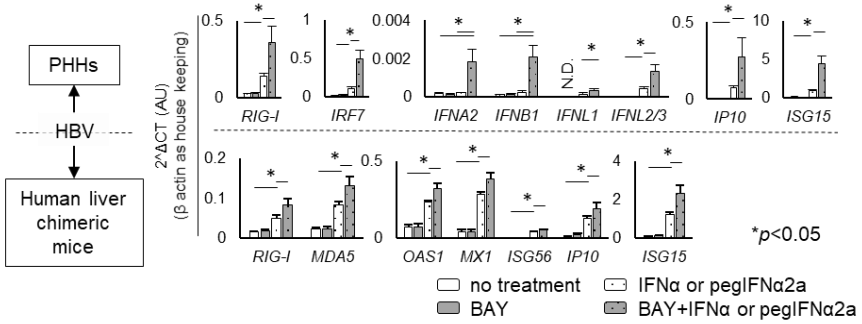
- CAM treatment led to an accumulation of cytoplasmic extracapsid pgRNA in HBV-infected heaptocytes.
- Combination therapy with CAM and IFNα could be a new anti-HBV strategy by activating host innate immune response.

Fukutomi K, et al., Abstract 90

Evaluation of cytoplasmic total pgRNA and intracapsid pgRNA levels suggested accumulation of "extracapsid" pgRNA in cytoplasm of HBV-infected hepatocytes by CAM treatment.

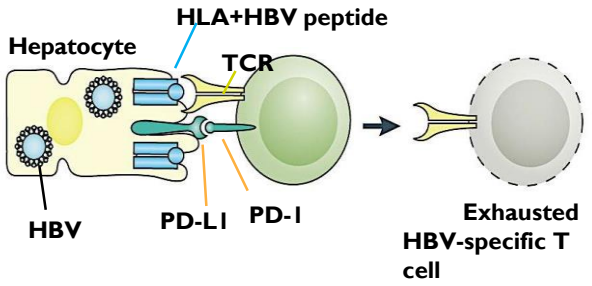


CAM treatment enhanced the IFNα-induced upregulation of innate immune gene expressions in HBV-infected PHHs and liver of human liver chimeric mice.



PD1 blockade

- CHB characterized by immune exhaustion
- PDL1 blockade should restore effective intra-hepatic HBV-specific T-cell responses



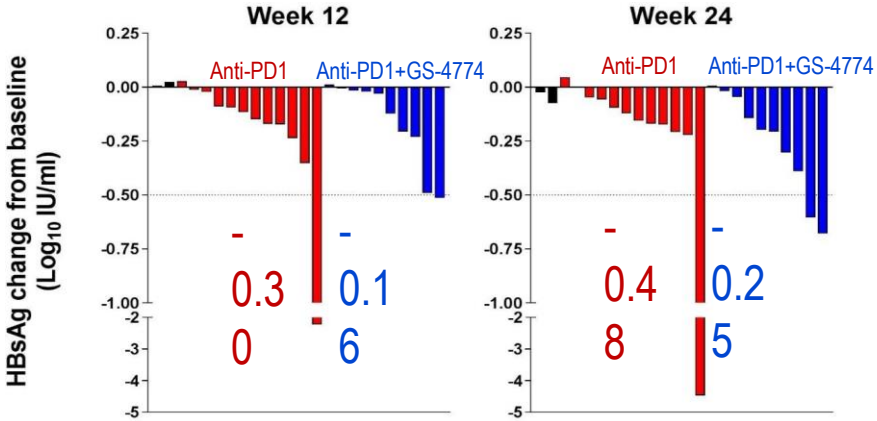
Single dose IV nivolumab 0.3mg/kg in CHB

- 20/22 had reduction in HBsAg
- One functional cure
- Overall effect was small

Dose will be limited by IR-AEs which can be prolonged and life-threatening

ACTG study exploring repeated doses

Need new approaches to PD1/PDL1 blockade



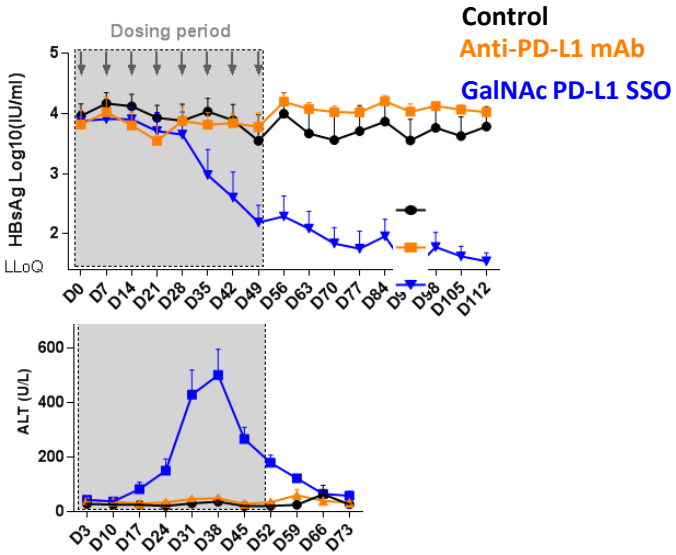
Gane E, et al. J Hepatol 2019; 71: 900–7

PD1/L1 blockade: new approaches

Inhibition of PD-L1 synthesis by LNA

GalNAc-conjugated LNA ASO [locked nucleic acid (LNA)-modified antisense oligonucleotides (ASOs)] directed against PD-L1

Mice received 5 weekly subcut doses 5 mg/kg



- 50% reduction in PD-L1 maintained for 8 weeks
- 40-fold increase in liver HBV specific IFN-γ cells
- 2.4 log reduction in HBsAg which was sustained

Inactivation of PD-L1 by small molecule inhibitors

- Several small molecules can bind to and dimerise PD-L1 and inactivate the receptor
- Short lived PD effect improving safety if IR-AEs develop

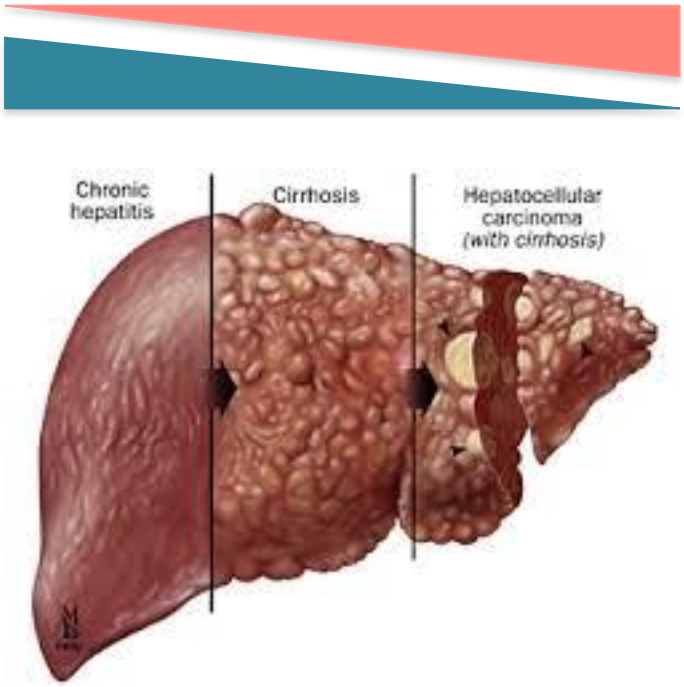
Luangsay S et al., AASLD 2019 #691; Ganesan A, et al. Nature (Scientific Reports). 2019; 9:12392

Control or Cure of HBV infection for a personalized treatment

Type of drugs' combination according to:

- ✓ Virus and infecting viral population
- ✓ Phase of HBV infection
- ✓ Stage of liver disease
- ✓ Treatment status (naive or on treatment) and response to treatment
- ✓ Host features (age, gender, co-morbidities)

HBV cure vs
HBV infection control



Liver disease control

Antifibrotic, antiangiogenetic drugs

- Standardized methods/assays are required to warrant the use in clinicsl practice of the new HBV markers

- A better understanding of the physiopathological meaning of viral markers kinetics during antiviral therapy is mandatory to define new treatment strategies

- The combined use of old and new antiviral and immune modulatory drugs will allow the tailoring of antiviral treatment at single HBV carrier level

Magritte R, La voix des airs 1931

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INNOVATIVE MODEL OF PERSONALIZED CARE

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