Can we cure HBV infection with novel direct acting antivirals ?

Fabien Zoulim

Hepatology Department, Hospices Civils de Lyon

INSERM U1052, Cancer Research Center of Lyon

Lyon University, France

Hôpitaux de Lyon

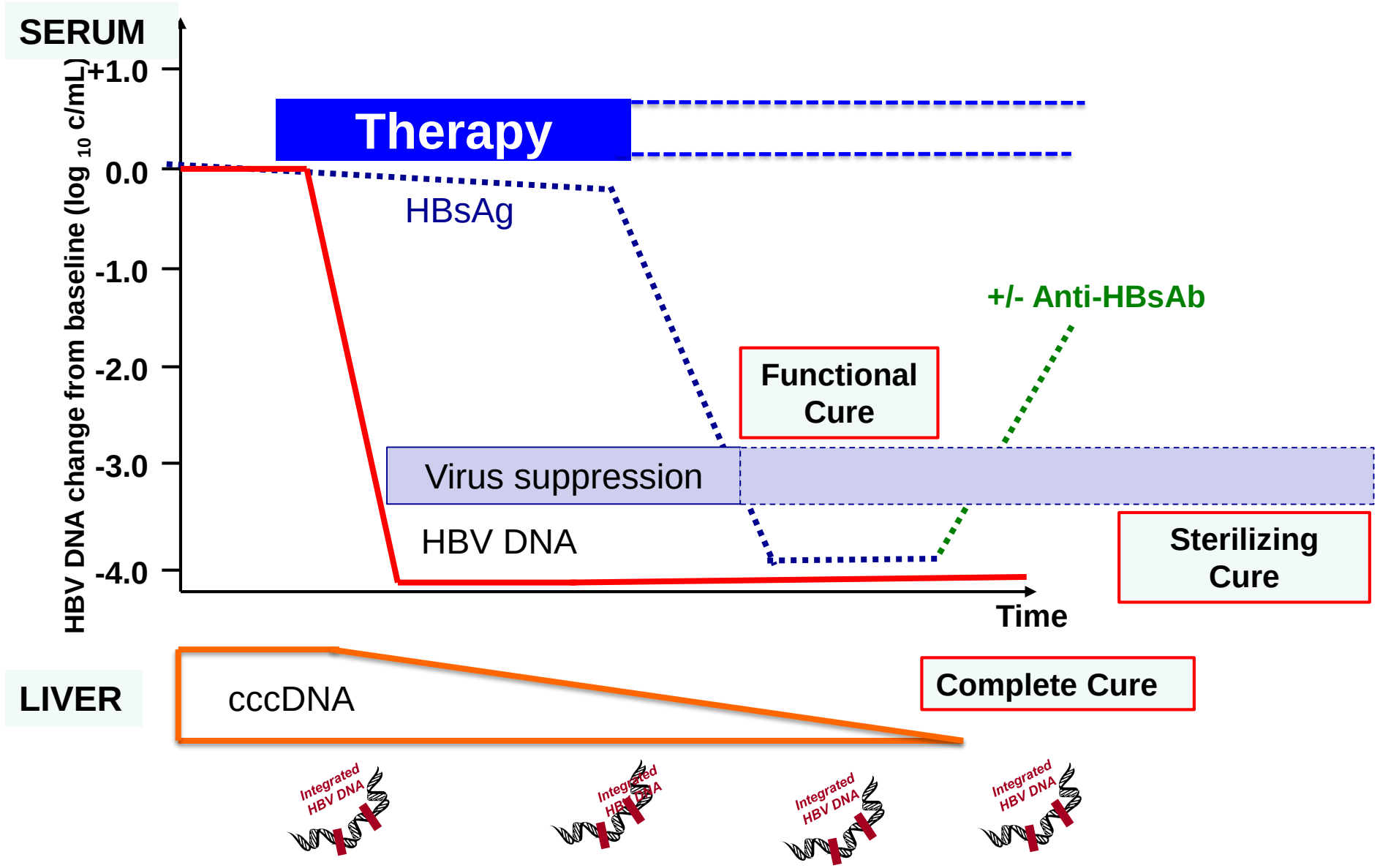
Agence autonome de l'Inserm

UNIVERSITÉ DE LYON

CRCL

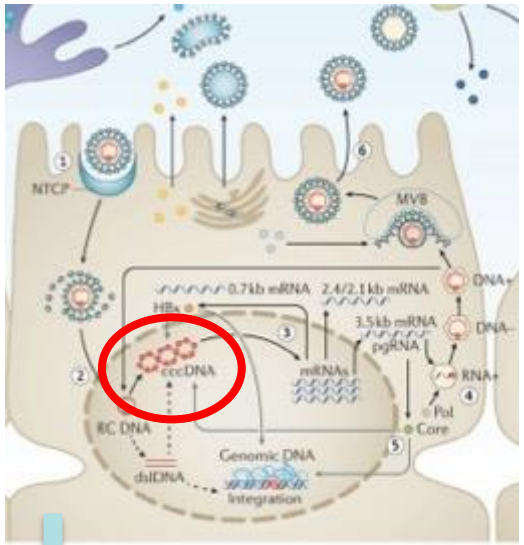
Inserm

Goals of future therapies to cure HBV infections



Lok/Cornberg et al, Hepatology / J Hepatol joint publication, 2017 & 2019; Testoni et al, Sem Liver Dis, 2017; Lok et al Hepatol Comm 2019.

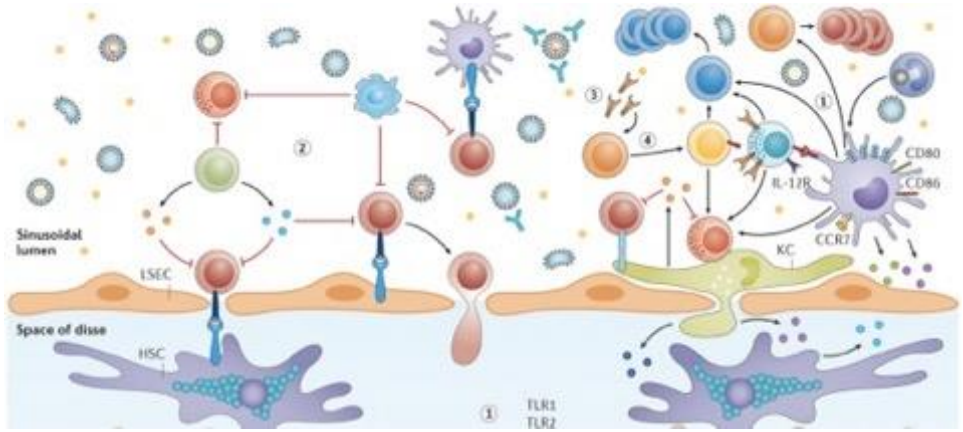
Barriers to functional cure



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

Integrated forms

HBV persistence



Defective CD8+ responses

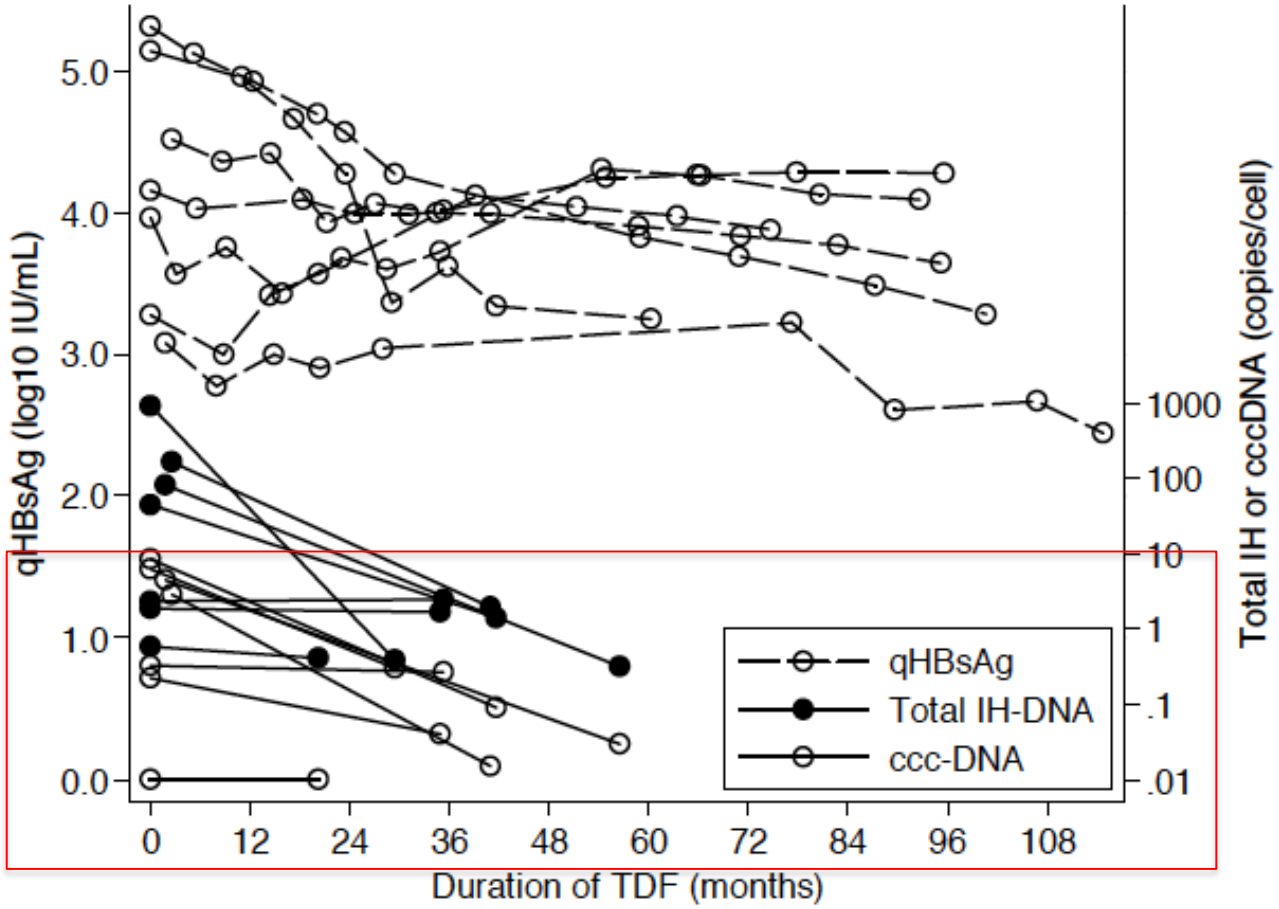
Defective B cell responses

Inefficient innate response

Defective immune responses

Revill, Testoni, Locarnini, S. & Zoulim, F. et al.Nat. Rev. Gastroenterol. Hepatol. 2016
Revill et al, Lancet Gastroenterol and Hepatol, 2019

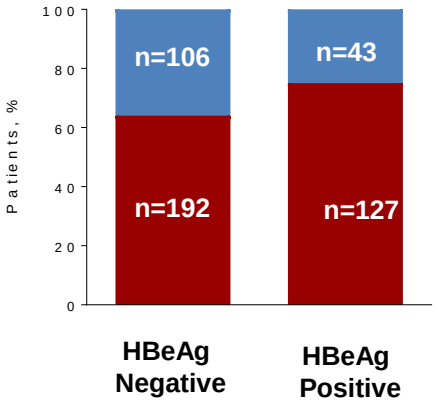
Persistence of intrahepatic viral DNA synthesis during long Tenofovir therapy (HIV-HBV cohort)

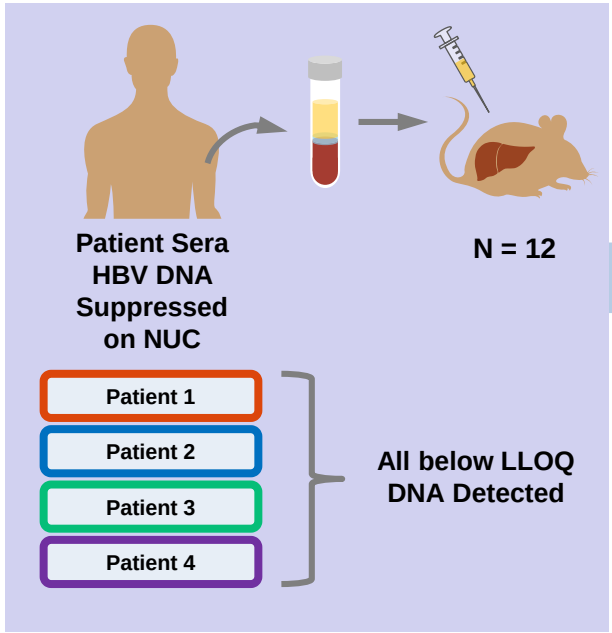


New round of infection and/or replenishment of the cccDNA pool occur despite « viral suppression »

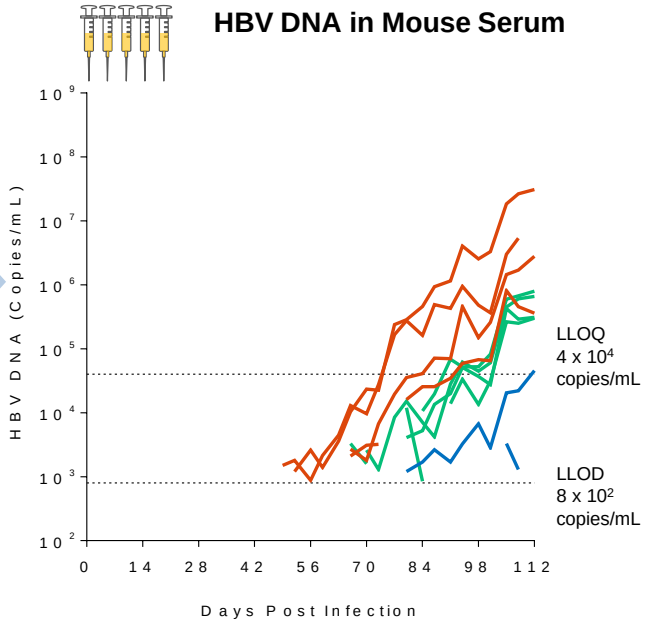
Boyd et al, J Hepatol 2016

Patient Sera with HBV DNA Below LLOQ Under TDF Is Infectious





112 days

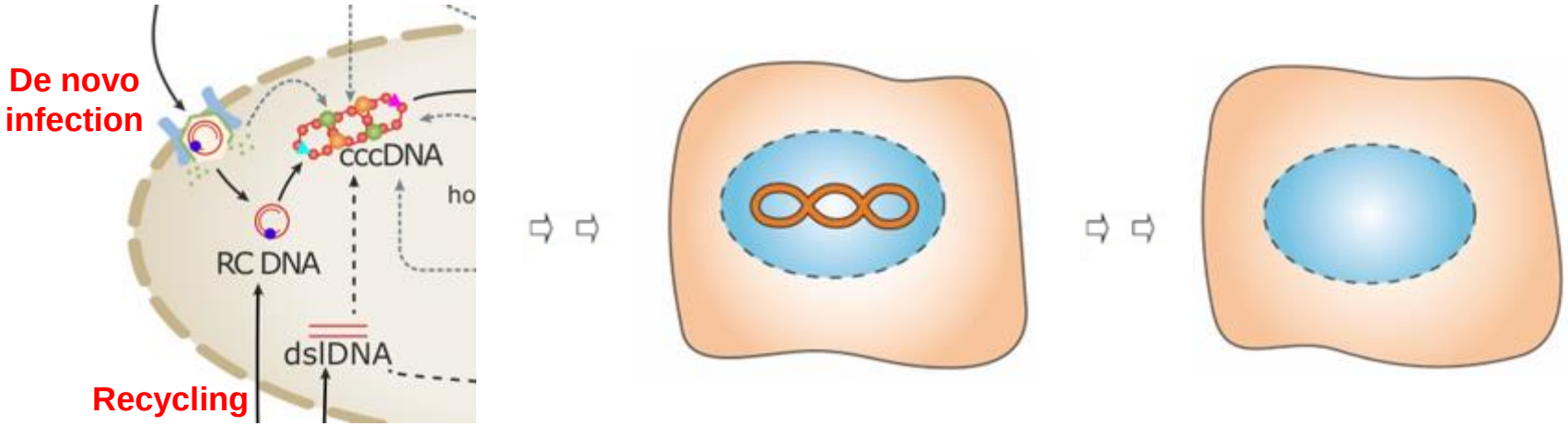


Marcellin et al.
Hepatology 2014;60:1093A.

Burdette et al, EASL ILC 2019

How is the steady-state of cccDNA pool maintained?

Non-cytolytic cccDNA elimination
Hepatocyte turnover



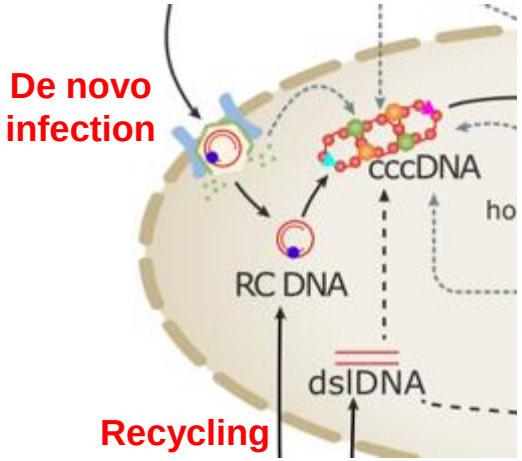
Modified from Nassal, 2015 & 2018

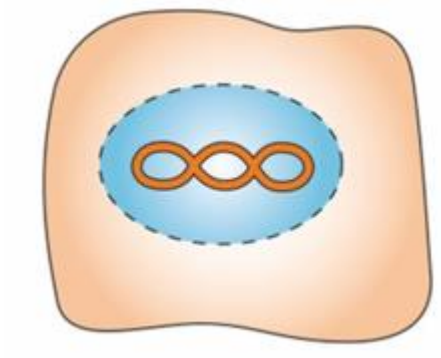
Switching the balance towards elimination ?

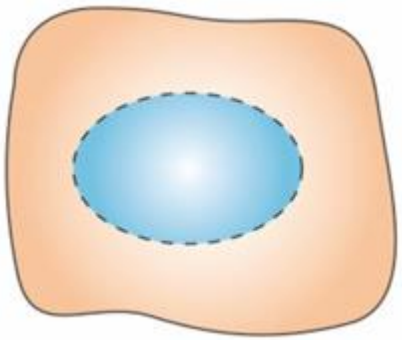
Decreased replenishment

Infected cell death
Non-infected cell division

Direct cccDNA elimination



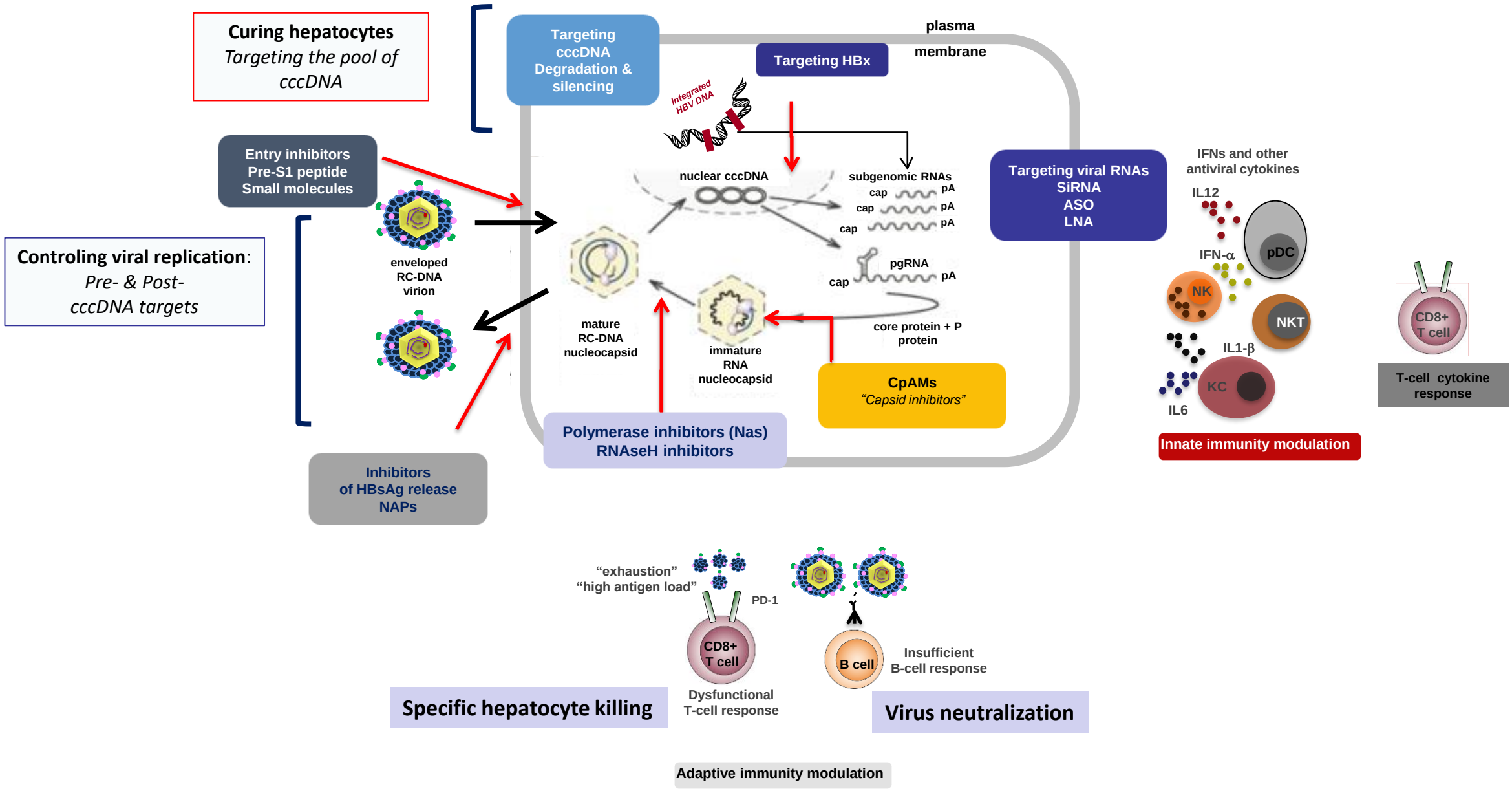




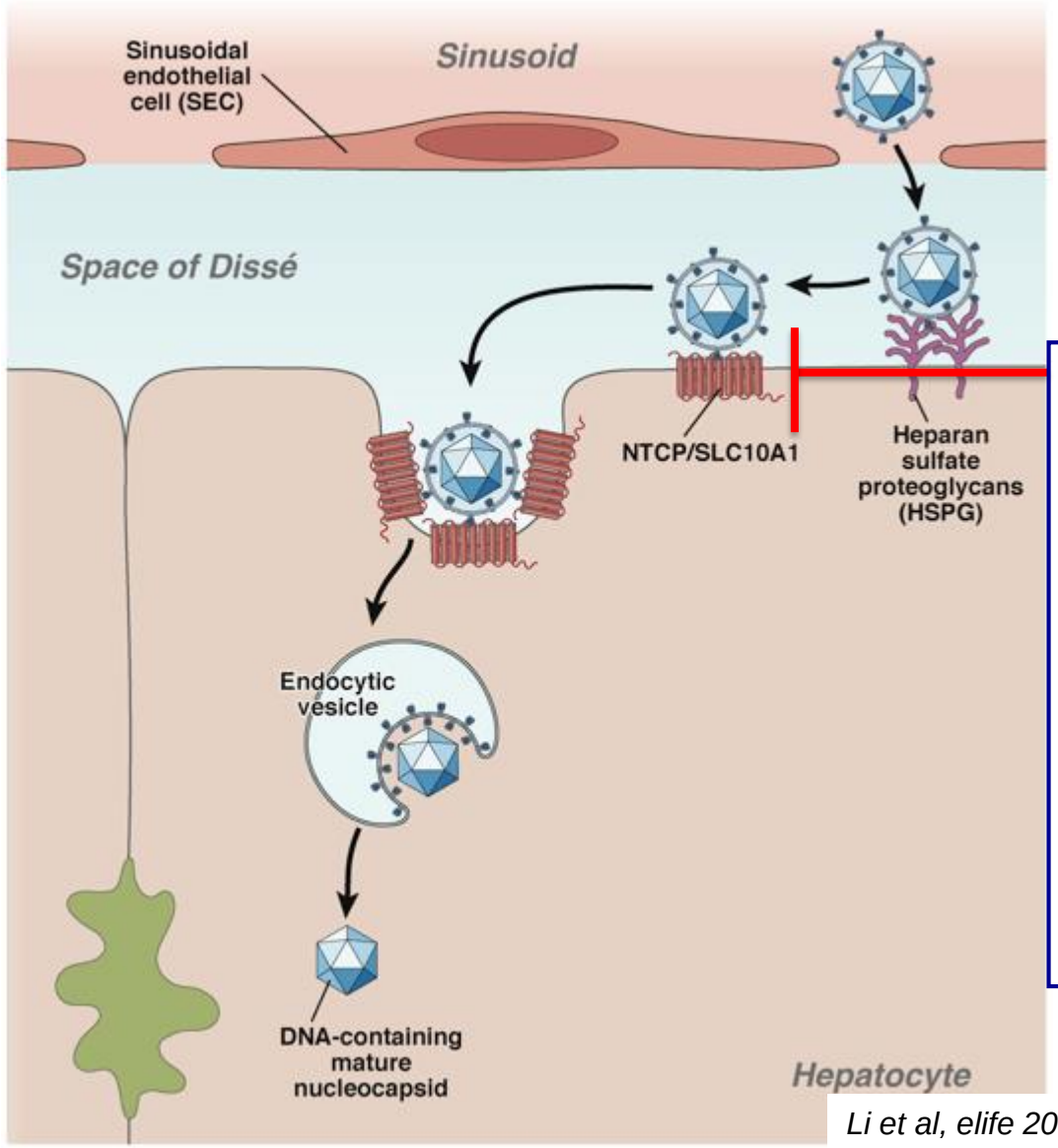
loss

intrahepatic cccDNA pool

synthesis


Curing hepatocytes
Targeting the pool of cccDNA
Targeting cccDNA Degradation & silencing
Targeting HBx
plasma membrane
Integrated HBV DNA
Entry inhibitors
Pre-S1 peptide
Small molecules
nuclear cccDNA
subgenomic RNAs
cap
pA
pgRNA
core protein + P protein
Targeting viral RNAs
SiRNA
ASO
LNA
IFNs and other antiviral cytokines
IL12
IFN-α
pDC
NK
NKT
IL1-β
KC
IL6
CD8+ T cell
T-cell cytokine response
Innate immunity modulation
Controling viral replication:
Pre- & Post-
cccDNA targets
enveloped RC-DNA virion
mature RC-DNA nucleocapsid
immature RNA nucleocapsid
CpAMs
"Capsid inhibitors"
Polymerase inhibitors (Nas)
RNAseH inhibitors
Inhibitors of HBsAg release
NAPs
"exhaustion"
"high antigen load"
PD-1
CD8+ T cell
Dysfunctional T-cell response
B cell
Insufficient B-cell response
Specific hepatocyte killing
Virus neutralization
Adaptive immunity modulation

Model for HBV entry in hepatocytes and development of entry inhibitors



Entry inhibitors
Myrcludex
(pre-S1 peptide)
Blank et al, J Hepatol 2016
Bogomolov et al, J Hepatol 2016

Ezetimibe
Lucifora, Antiviral Res 2013
Proanthocyanidin
Tsukuda, Hepatology 2017
Cyclosporin analogues
Shimura, J Hepatol 2017
HBV conference, Taormina 2018

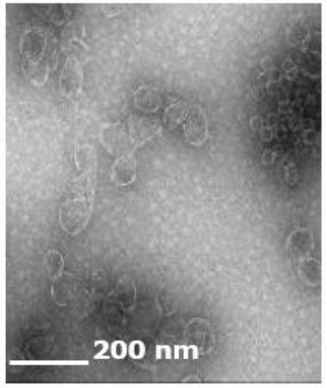
Li et al, elife 2012; Urban et al, Gastroenterology 2014

Different classes of capsid assembly modulators

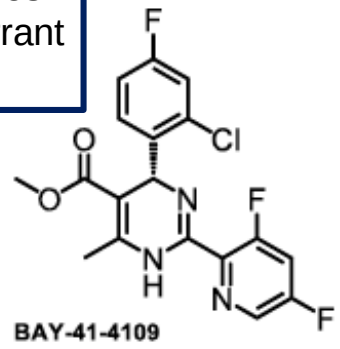
Heteroaryldipyrimidine derivatives (HAP)

Phenylpropenamide derivatives (A*T series)*

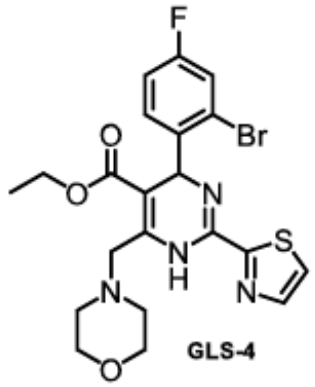
Heteroaryldihydropyrimidine (HAP) family of compounds induces formation of aggregated and aberrant capsid structures



CAM-A (Aberrant)

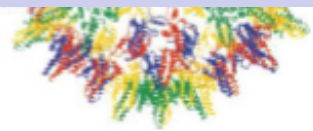


Deres et al, Science 2003



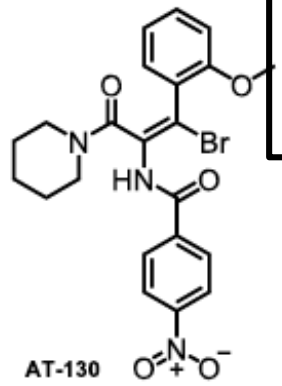
Hu et al., Ann. Rep. in Med. Chem. 2013

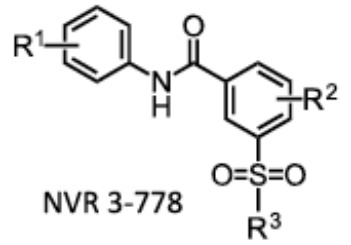
Compounds in evaluation
BAY41-4109
HAP-12
AT-130
NVR3-778
JnJ-6379
JnJ-0440
RO7049389
ABI-H0731
ABI-H0808
GLS4
GLP26
HAP_R01
SBA_R01
AB-423
AB-506
EP-027367



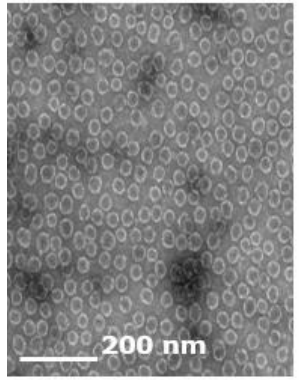
Winne et al, Mol.Cell 1999

Phenylpropenamide and Sulfamoylbenzamide chemical series accelerate formation of capsid-like particles





(*cf.* Campagna et al J.Virol. 2013)

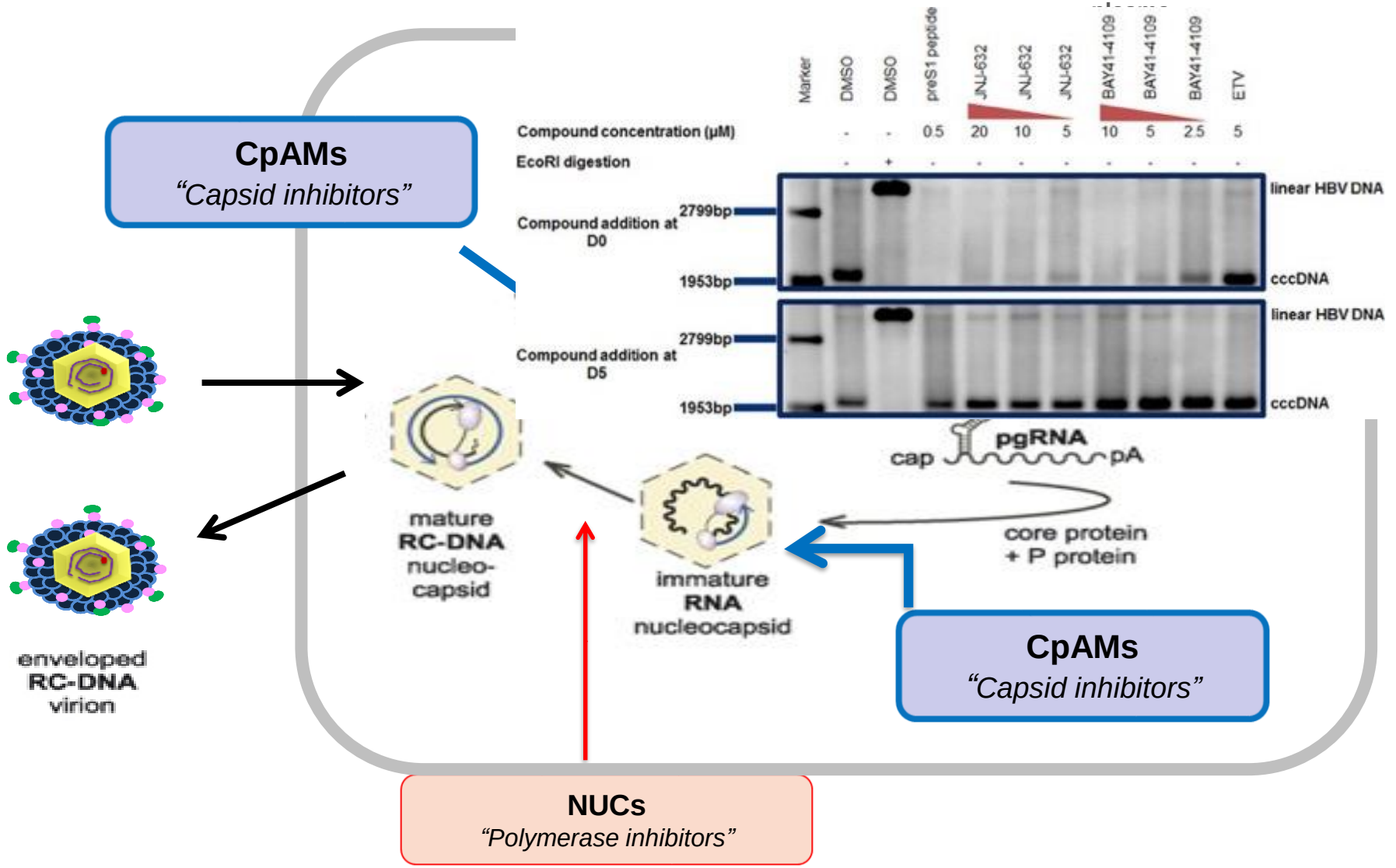


CAM-N (Normal)

scription
plication

rcDNA-containing nucleocapsid

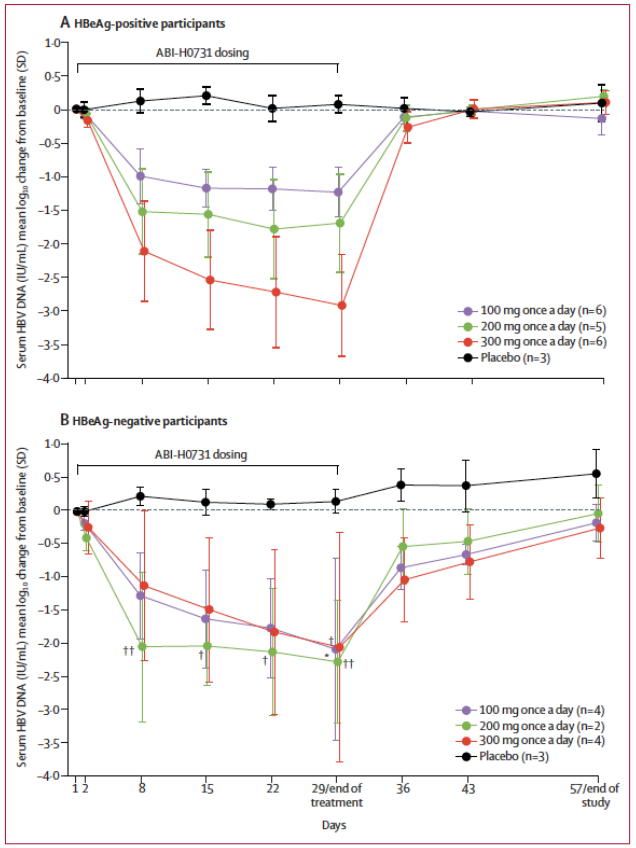
CpAMs inhibit viral genome replication and prevent cccDNA formation when administered prior to HBV inoculation



Berke et al. Antimicrob. Agents Chemother. 2017; Lam et al AAC 2017; Mani et al AAC 2018; Zhou et al Sci Report 2017

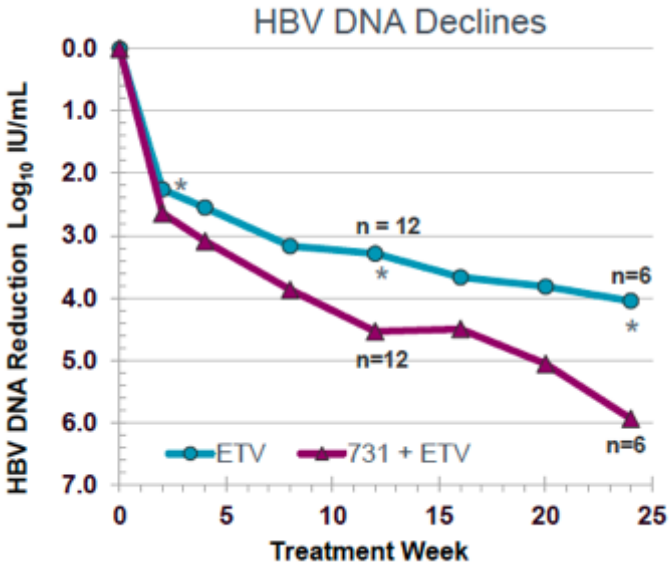
A randomised, lacebo-controlled phase 1 trial of the CAM H0731

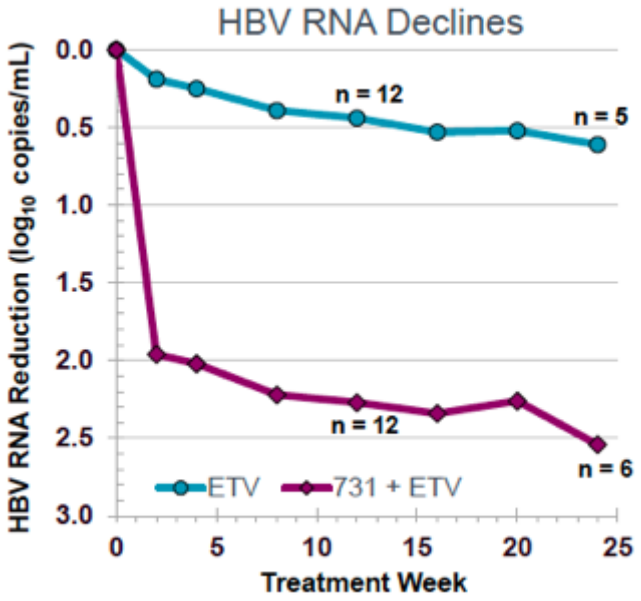
M-F Yuen et al, Lancet Gastroenterol and Hepatol, 2019



Interim Safety and Efficacy Results of the ABI-H0731 Phase 2a Program Exploring the Combination of ABI-H0731 with Nuc Therapy in Treatment-Naive and Treatment-Suppressed Chronic Hepatitis B Patients

Xiaoli MA[1], Jacob Lalezari[2], Tuan Nguyen[3], Ho Bae[4], Eugene R. Schiff[5], Scott Fung[6], Man-Fung Yuen[7], Tarek Hassanein[8], Hie-Won Hann[9], Magdy Elkhashab[10], Douglas Dieterich[11], Mark Sulkowski[12], Paul Kwo[13], Ronald Nahass[14], Kosh Agarwal[15], Alnoor Ramji[16], James Park[17], Natarajan Ravendhran[18], Sing Chan[19], Frank Weilert[20], Steven-Huy Han[21], Walid Ayoub[22], Edward Gane[23], Ira Jacobson[24], Michael Bennett[25], Qi Huang[26], Ran Yan[26], Vivian Huey[26], Eric Ruby[26], Sandy Liaw[26], Richard Colonno[26] and Uri Lopatin[26]





Target engagement confirmed

Does it decrease the pool of cccDNA and/or HBsAg expression ?

Targeting viral transcripts to suppress viral protein expression

Target gene silencing

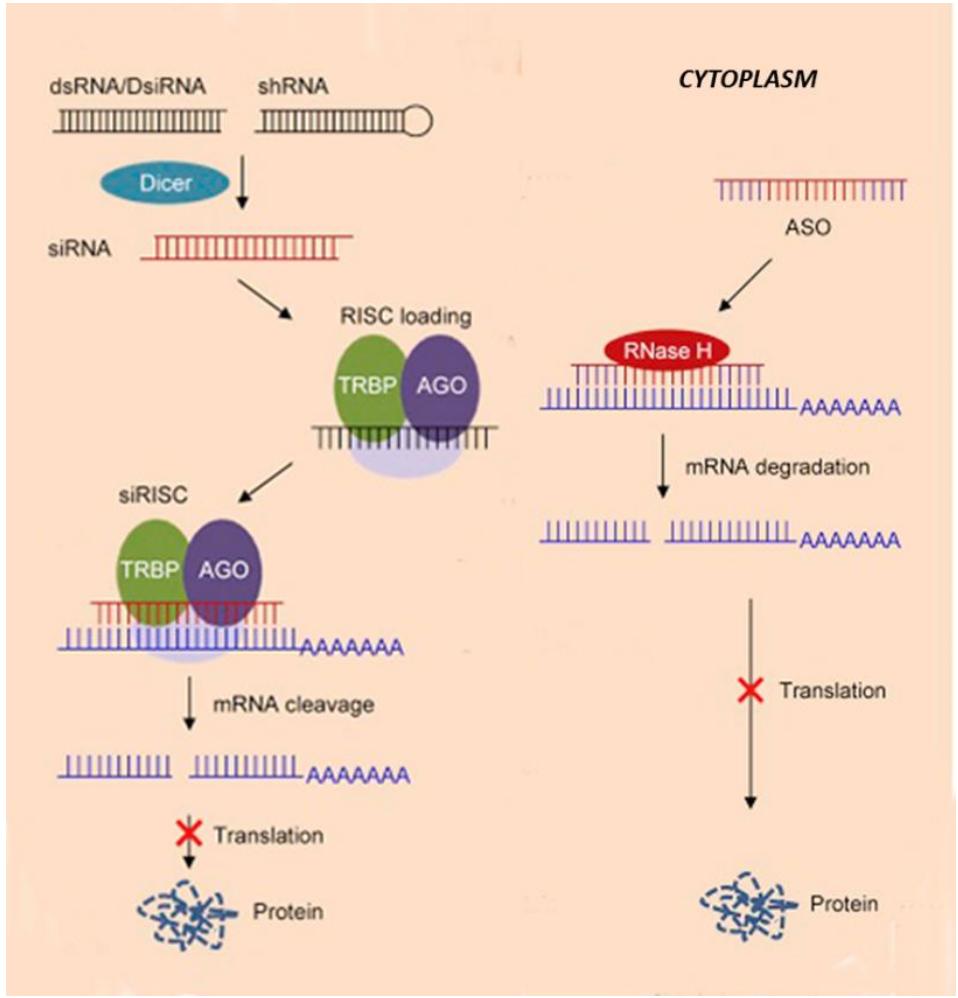
↓

Cleaving mRNA or repressing mRNA translation

↓

Antisense oligonucleotides (ASO)
Locked nucleic acids
Small interfering RNAs (SiRNA)

Wooddell et al, Science Transl Med, 2017

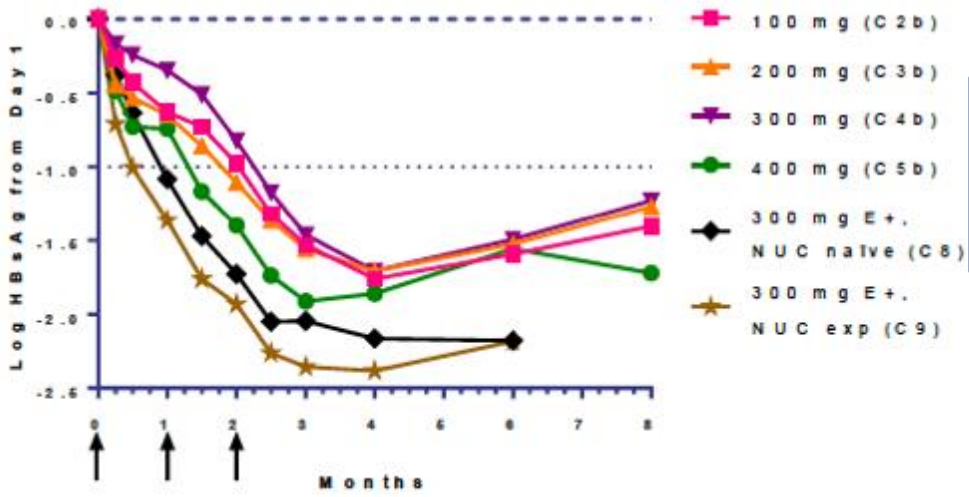


Huang, Mol Ther Nucleic Acids 2017

JNJ-3989 (SiRNA) in NUC Suppressed Patients

All patients receiving 3 monthly doses have achieved > 1 log reduction in HBsAg

Mean HBsAg reductions from baseline



Target engagement confirmed

Does it decrease the pool of cccDNA ?

- JNJ-3989 rapidly reduces HBsAg to thresholds possibly associated with improved chances of HBsAg seroclearance in many patients, even after only 3 doses
  - 88% of patients achieved HBsAg <100 IU/mL
  - 100% of patients achieved ≥ 1.0 Log10 IU/mL HBsAg reduction

MF Yuen, et al, EASL ILC 2019

GSK3389404 (antisense oligonucleotide) in NUC Suppressed Patients

Phase 2a, multicenter, randomized, double-blind, placebo-controlled study in HBeAg+/-, n=66

Mean change from baseline in HBsAg (log_{10} IU/mL) over time by treatment group

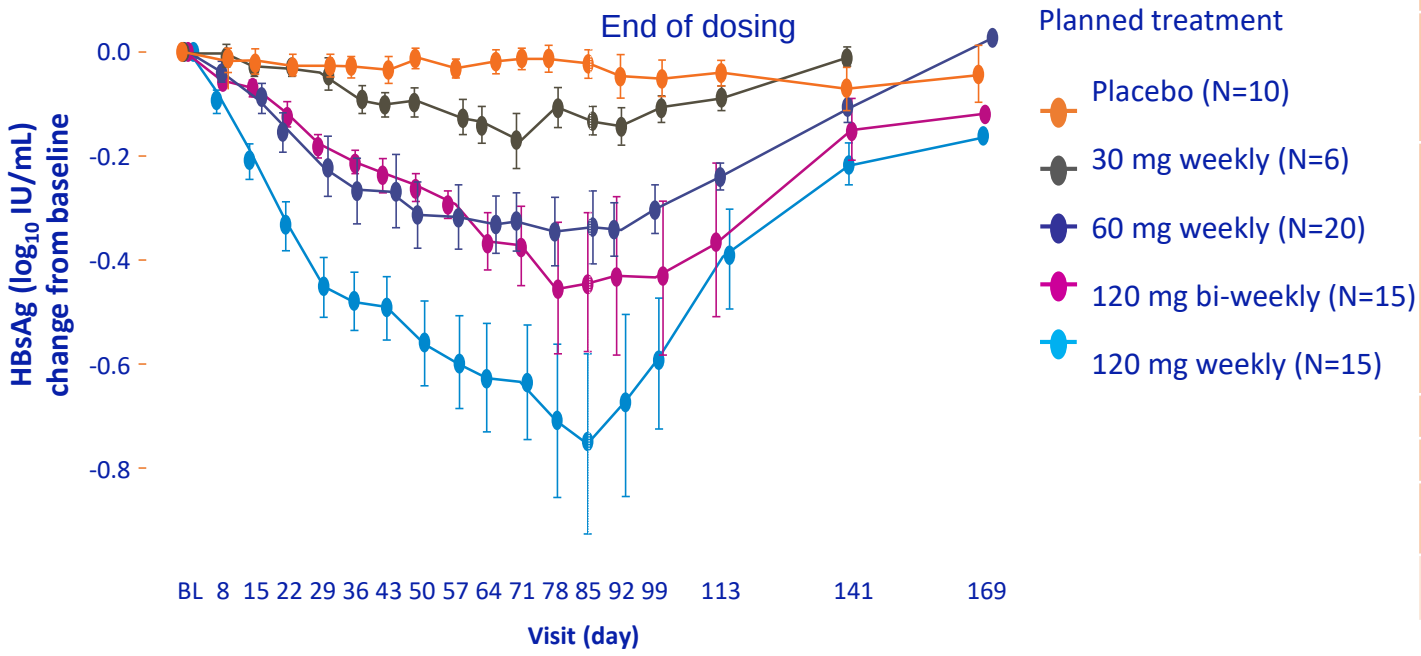


Table 1. Summary of AEs

		GSK3389404				
	Placebo (N=10)	30 mg weekly (N=6)	60 mg weekly (N=20)	120 mg weekly (N=15)	120 mg bi-weekly (N=15)	Total GSK3389404 (N=56)
Any AEs, n (%)	8 (80)	3 (50)	15 (75)	11 (73)	8 (53)	37 (66)
Mild (Grade 1)	2 (20)	2 (33)	7 (35)	4 (27)	4 (27)	17 (30)
Moderate (Grade 2)	4 (40)	0	8 (40)	6 (40)	2 (13)	16 (29)
Severe (Grade 3)	0	1 (17)	0	1 (7)	2 (13)	4 (7)
Potentially life-threatening (Grade 4)	2 (20)[a]	0	0	0	0	0
Treatment-related AEs, n (%)	4 (40)	3 (50)	10 (50)	8 (53)	7(47)	28 (50)
Serious AEs, n (%)	0	0	0	0	1 (7)	1 (2)
AEs leading to study withdrawal or treatment discontinuation, n (%)	0	0	0	0	1 (7)[b]	1 (2)

[a]Both Grade 4 lab abnormality of creatine kinase increase attributed to physical activity. [b]Grade 1 pruritus on the neck. AEs, adverse events.

Proof of principle that antisense oligonucleotides can decrease HBsAg levels

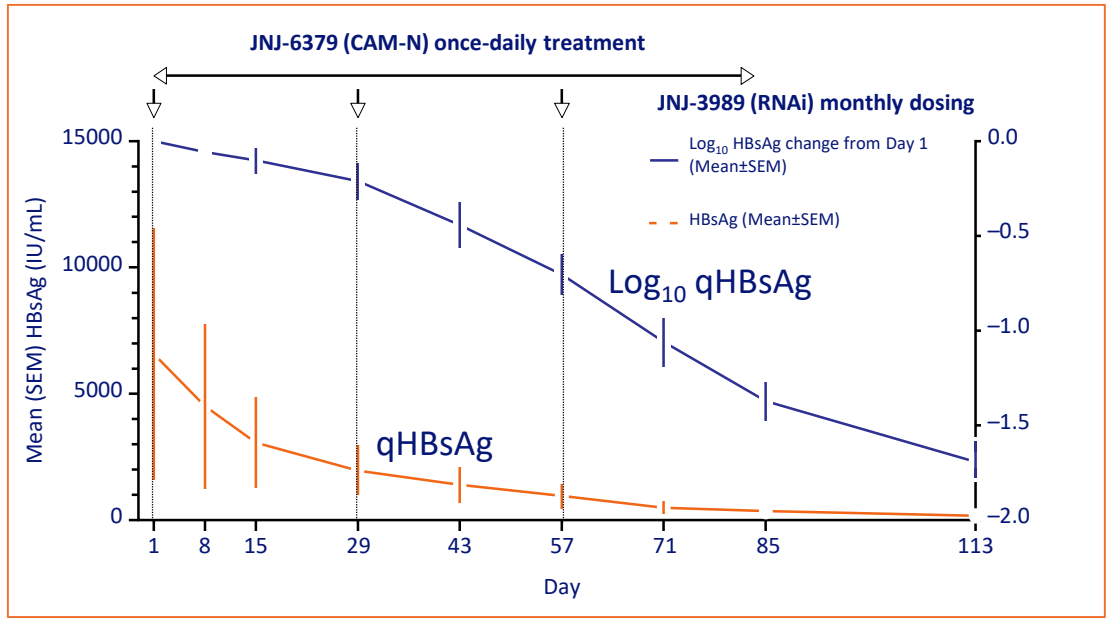
Yuen et al, AASLD, Boston 2019, Abstract 0695

Triple Therapy: NA + CAM + RNAi

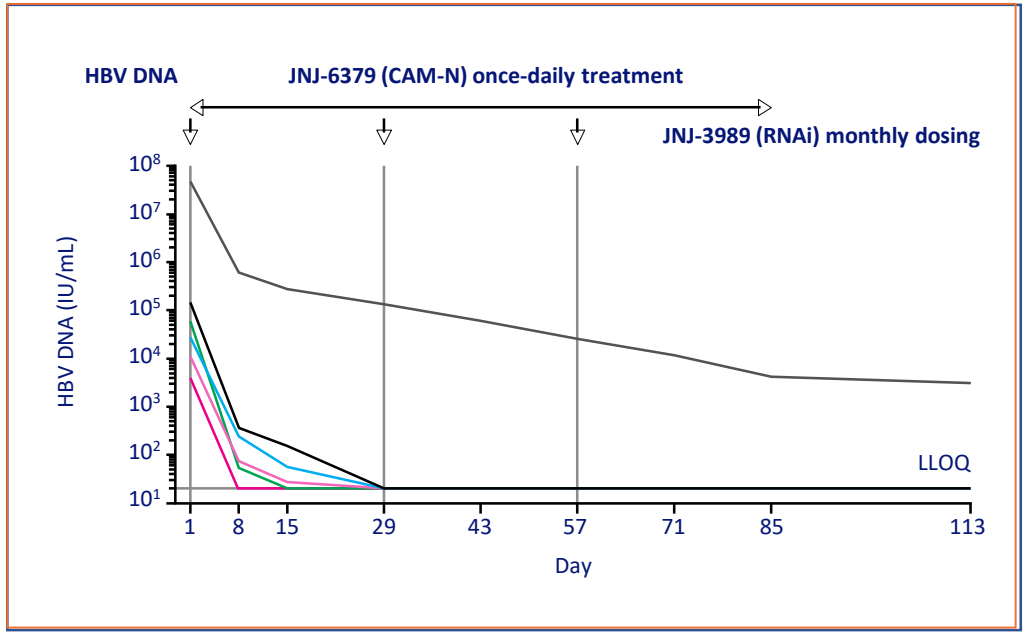
HBeAg+ n=4 / HBeAg- n=8, NA-naïve n=5 / experienced n= 7, All 12 Asian

- Three 200 mg JNJ-3989 subcutaneous doses on Days 1, 29 and 57
- Oral JNJ-6379 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-

HBsAg



HBV DNA

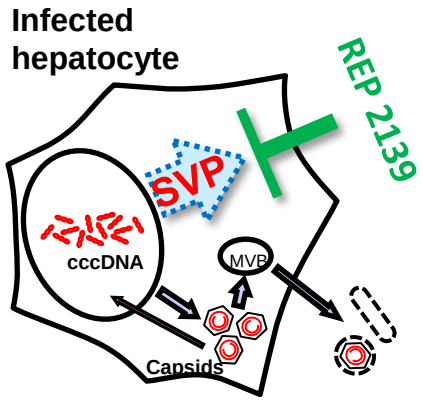


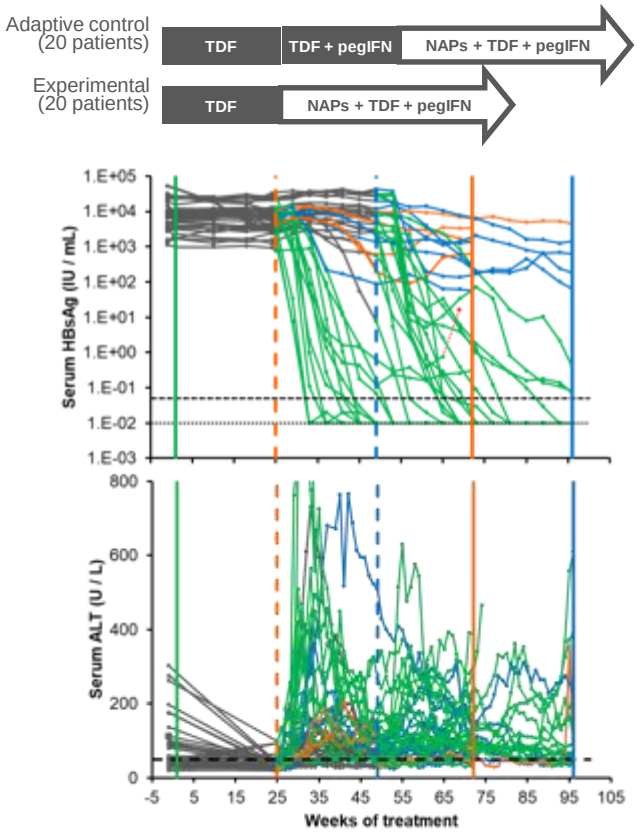
Triple therapy resulted in marked decline in HBsAg levels …? Functional cure ?

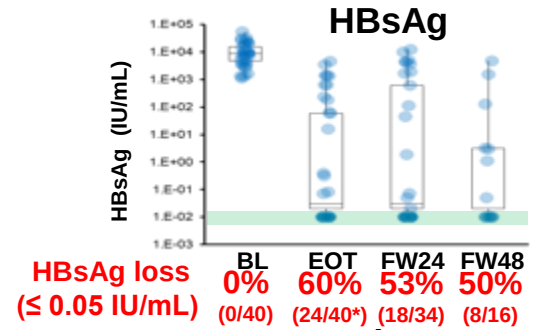
Yuen et al, AASLD, Boston 2019, Abstract LP4

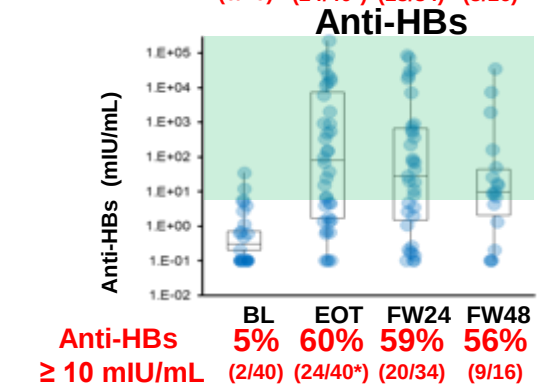
Nucleic Acid Polymers (NAPs) – Reducing HBsAg

- NAPs block assembly/release of **subviral particles**
- Aim to restore immune response → viral control









- Marked and seemingly durable HBsAg loss & gain of anti-HBs
- Interesting…need to confirm ALT flares due to immune activation → plan for Phase 2 ACTG trial to clarify

Bazinet et al, AASLD 2018, Abstract 393

Novel HBV therapies investigated in humans

Direct antivirals

Drug name	Sponsor	Mechanism of action	Class	Clinical stage	Notes	Refs
Entry inhibitors						
Myrcludex B (bulevirtide)	MYR Pharmaceuticals	Blocks NTCP	Peptide	II	2 mg Myrcludex B + IFNα treatment resulted in 40% responders with HBsAg loss observed in 26.7% of the cohort	25
CRV431	Contravir	Blocks NTCP and protein folding	Small molecule	I	Single-ascending-dose study performed up to a dose of 525 mg	172
Translation inhibitors						
JNJ3989	Janssen	mRNA degradation	siRNA	II	Most patients had HBsAg levels <100 IU mL^{-1} after 3 doses. Range of 1.3–3.8 (at nadir) log decrease in HBsAg levels	55
ARB-1467	Arbutus	mRNA degradation	siRNA	II	7 of 11 patients had >1 log decrease in HBsAg levels after 10 weeks of dosing (responders). Biweekly dosing better than monthly dosing	173
GSK3389404	GlaxoSmithKline	mRNA degradation	ASO	II	Safe and well tolerated in healthy volunteers	174
Capsid assembly inhibitors						
ABI-H0731	Assembly	Core binding	Small molecule	II	Combined with entecavir, ABI-H0731 caused a 4.54 log decrease in HBV DNA levels at 12 weeks and a 5.94 log decrease at 24 weeks	66
JNJ6379	Janssen	Core binding	Small molecule	II	Mean DNA level log decrease of 2.16–2.89 and a dose correlation for a number of patients who had a DNA level less than the LOQ at the end of the trial (28 days)	63
JNJ0440	Janssen	Core binding	Small molecule	I	Single and multiple-ascending-dose studies in healthy volunteers. Doses up to 2,000 mg QD well tolerated in the 7-day multiple-ascending-dose study	64
GLS4	HEC Pharma	Core binding	Small molecule	II	Interim (20 week) data showed DNA level log reduction of 1.48–5.58 for BID administration and 1.51–6.09 log reduction for TID administration	58
RO7049389	Roche	Core binding	Small molecule	II	Median DNA level declines of 2.7 (200 mg BID), 3.2 (400 mg BID) and 2.9 (600 mg QD) observed at the end of the trial (28 days)	59
AB-506	Arbutus	Core binding	Small molecule	I	10-day study in healthy volunteers completed	175
HBsAg secretion inhibitors						
REP 2139 and REP 2165	Replicor	HBsAg binding	Nucleic acid-based polymer	II	At the end of the trial, 60% of patients had HBsAg loss (53% had HBsAg loss at 24 weeks and 50% had HBsAg loss at 48 weeks). Anti-HBs antibodies were detectable in 56% of patients at 48 weeks	70

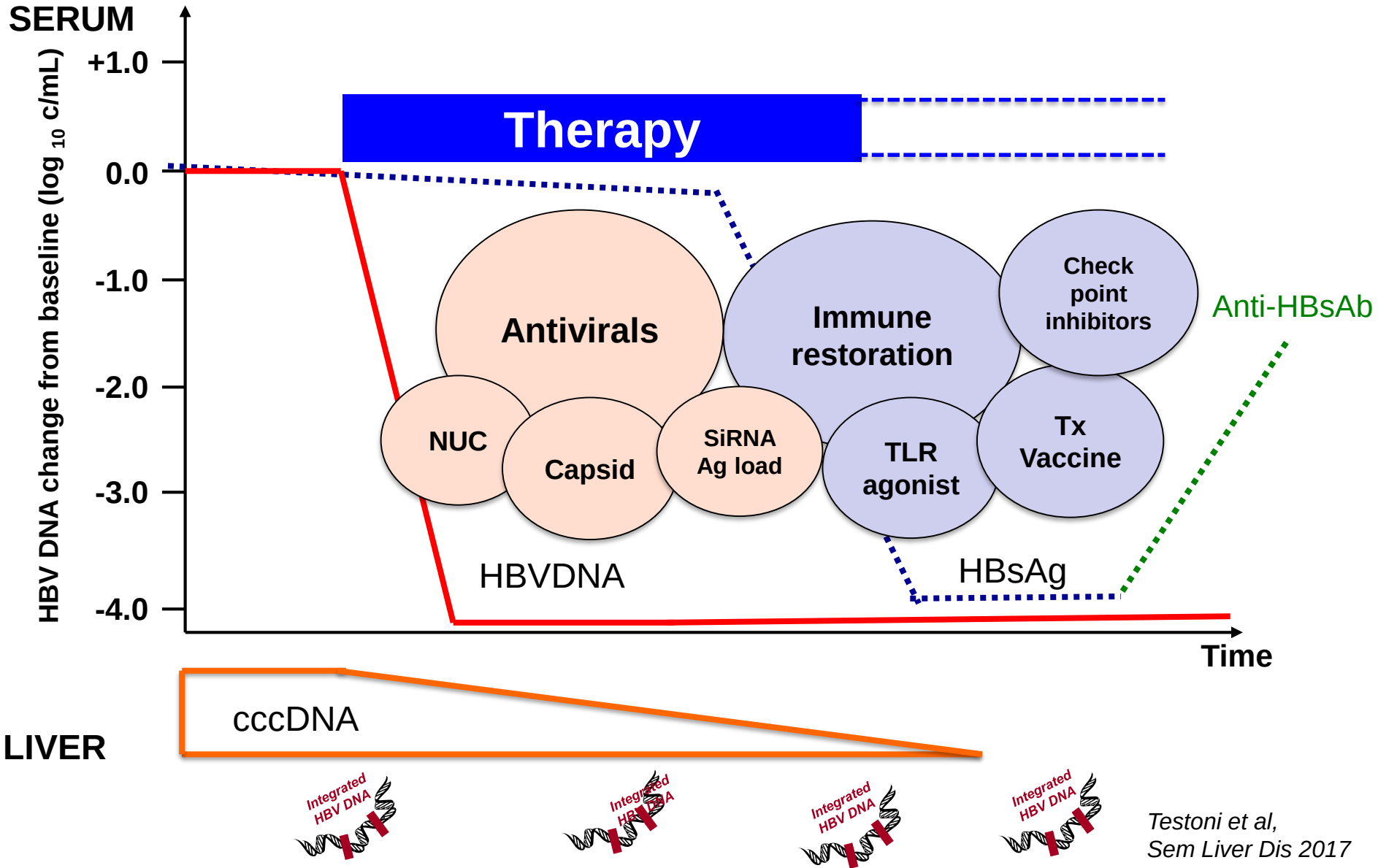
Immune modulators

Drug name	Sponsor	Mechanism of action	Class	Clinical stage	Notes	Refs
Innate immunity activators						
Inarigivir	Springbank	RIG-I agonist and polymerase inhibitor	Small molecule	II	Dose-dependent decrease in HBV DNA levels (1.54 log decrease with 200 mg). After switch to TDF, 88% of participants had DNA levels below the LOQ	108
RO7020531	Roche	TLR7 agonist	Small molecule	I	Immune activation observed in all patients. No viral data reported	176
GS-9620	Gilead	TLR7	Small molecule	II	No change in HBsAg levels. Transient dose-dependent induction of ISG15 and change in NK cell and T cell phenotype observed	111
GS-9688	Gilead	TLR8	Small molecule	I	Dose-dependent IL-12 and IL-1β production noted in healthy volunteers	177
Adaptive immunity activators						
TG-1050 (T101)	Transgene/Talsy	Vaccine	Ad5 delivery	I	HBV T cell responses induced by vaccine. Anti-Ad5 antibodies seen with higher dose. Mean 0.45 log decrease in HBsAg levels observed at day 197	178, 179
HepTcell	Altimmune	Vaccine	Peptide plus IC31 (adjuvant)	I	T cell responses strongest for vaccine plus adjuvant. Safe, but no decline in HBsAg levels was observed after three administrations of vaccine	180

Ad5, adenovirus type 5; anti-HBs, anti-hepatitis B surface protein; ASO, antisense oligonucleotide; BID, twice daily; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IFNα, interferon-α; IU, infectious units; LOQ, limit of quantification; NK, natural killer; NTCP, sodium–taurocholate cotransporting polypeptide; QD, once daily; RIG-I, retinoic acid-inducible gene I protein; siRNA, small interfering RNA; TDF, tenofovir disoproxil fumarate; TID, thrice daily; TLR, Toll-like receptor.

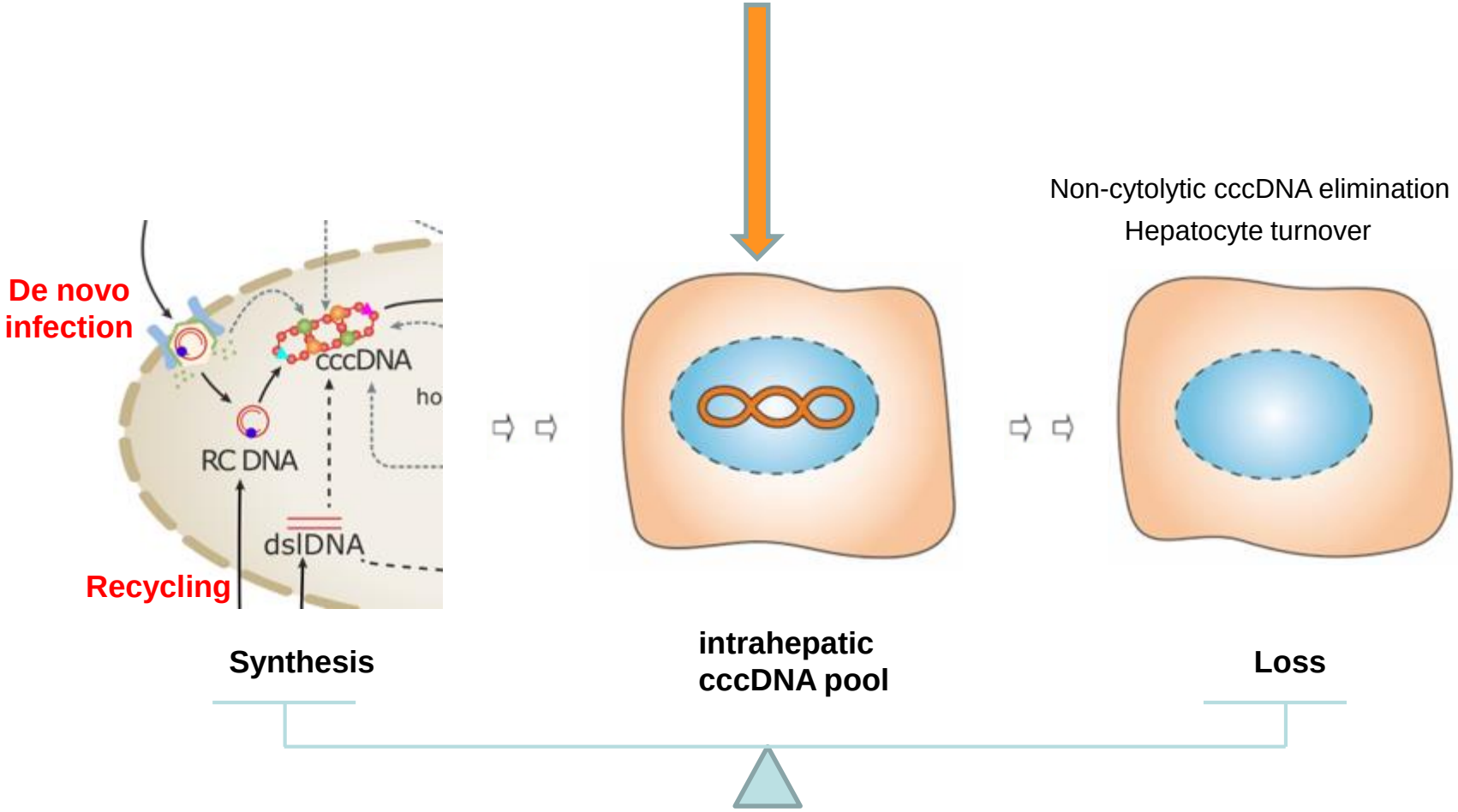
Fanning et al, Nature Reviews Drug Discovery (2019)

HBV cure - New treatment concepts – Will we need combination of DAA and immune therapy ?



Testoni et al,
Sem Liver Dis 2017

Can we intervene directly on the steady-state of cccDNA pool?

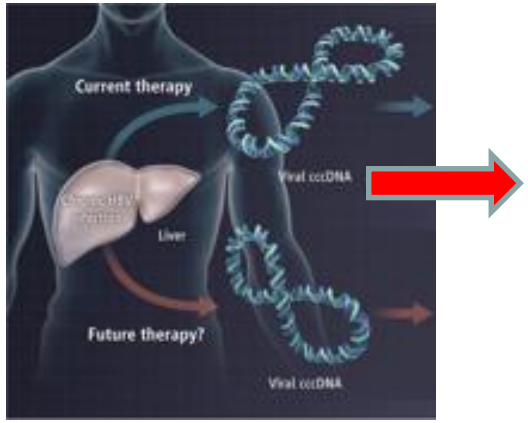


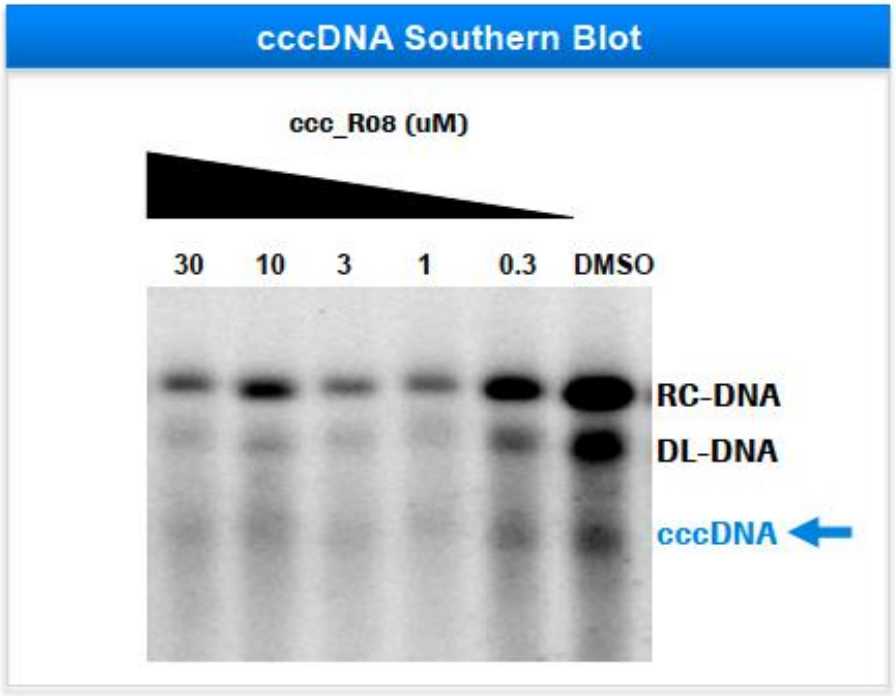
Modified from Nassal, 2015 & 2018

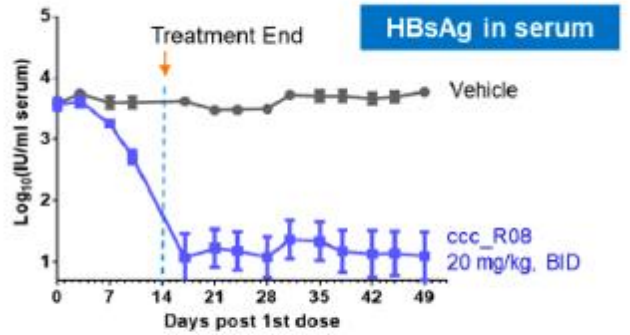
Innovations and novel perspectives for cure

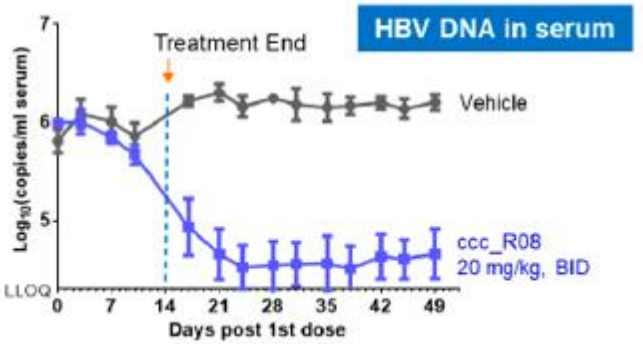
Direct cccDNA targeting strategies

Gao et al, EASL ILC 2019









Cure of HBV infection with direct acting antivirals - Conclusions

- **Curing infected hepatocytes with pre- and post- cccDNA targets**
  - Accelerate the kinetics of cccDNA decay
  - Combination therapy likely required
  - Duration of treatment will depend on the rate of hepatocyte turn-over
  - Major issue: cccDNA half-life, number of infected cells, infected hepatocyte half-life ?
- **Direct targeting of cccDNA**
  - Cytokine-mediated degradation
  - Nuclease-based gene editing
  - Small molecules
  - Still a long way to go: specificity, safety profile, delivery issues…
- **Will this be sufficient or will we need combinations with immunomodulatory approaches ?**
- **Strategies decressing viral antigen expression may represent the backbone of future combination therapies with immune modulators**

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Back-up slides

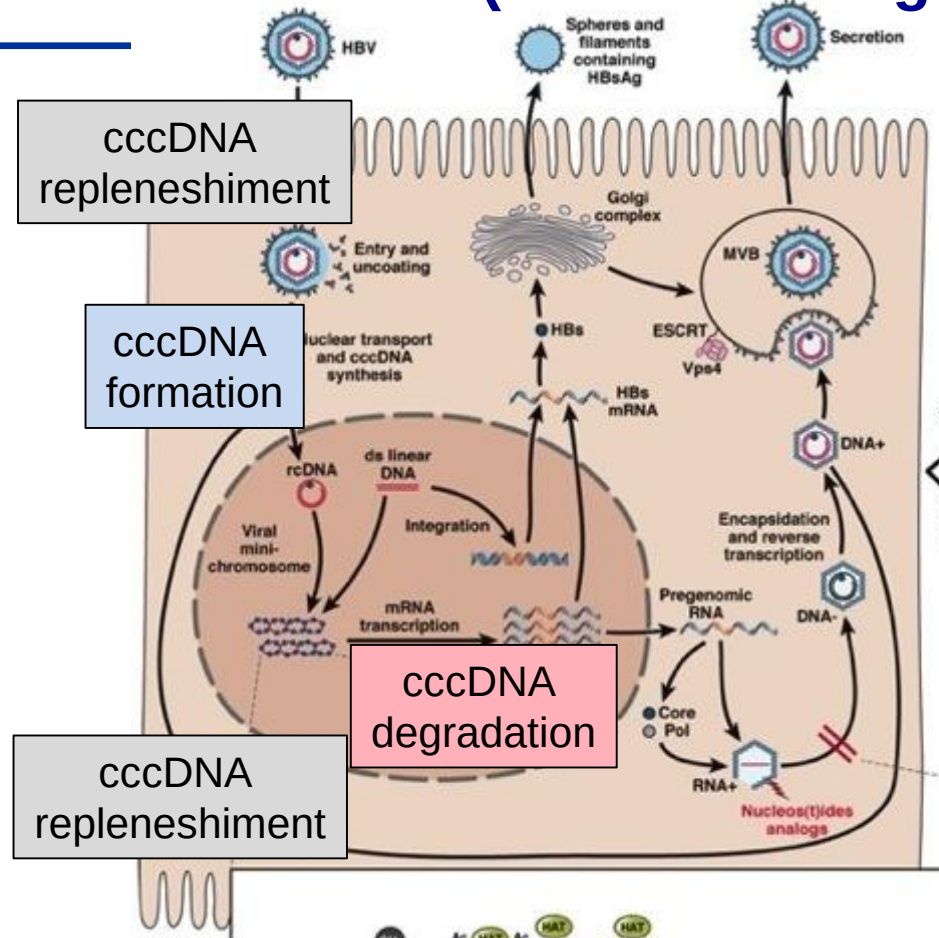
How to decrease the pool of cccDNA (without killing infected cells) ?

cccDNA repleneshiment

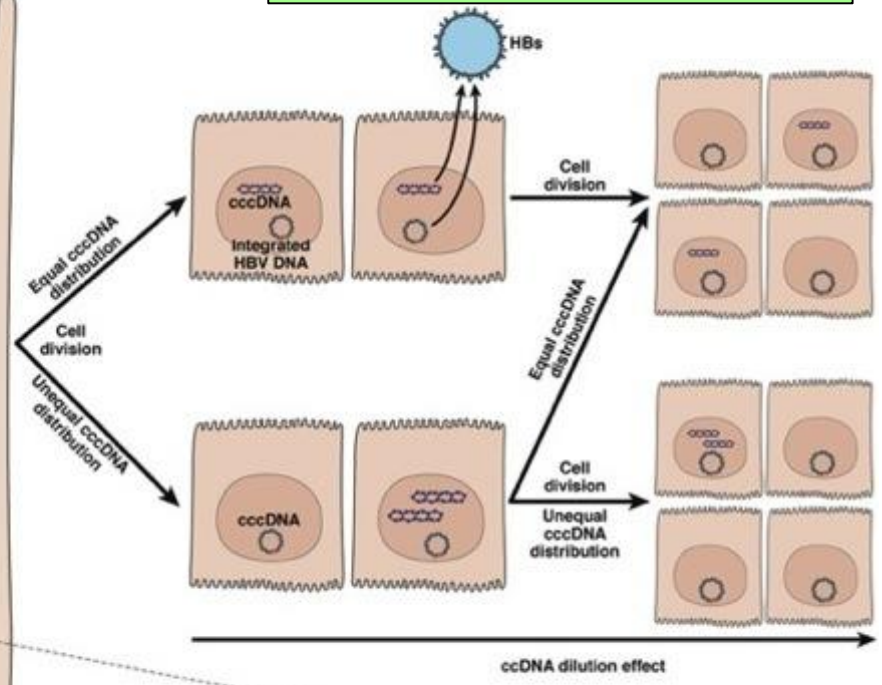
cccDNA formation

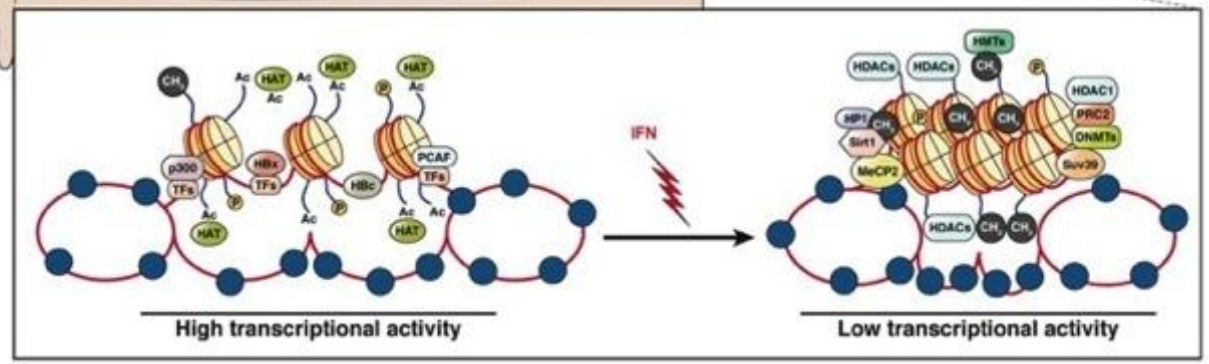
cccDNA degradation

cccDNA repleneshiment



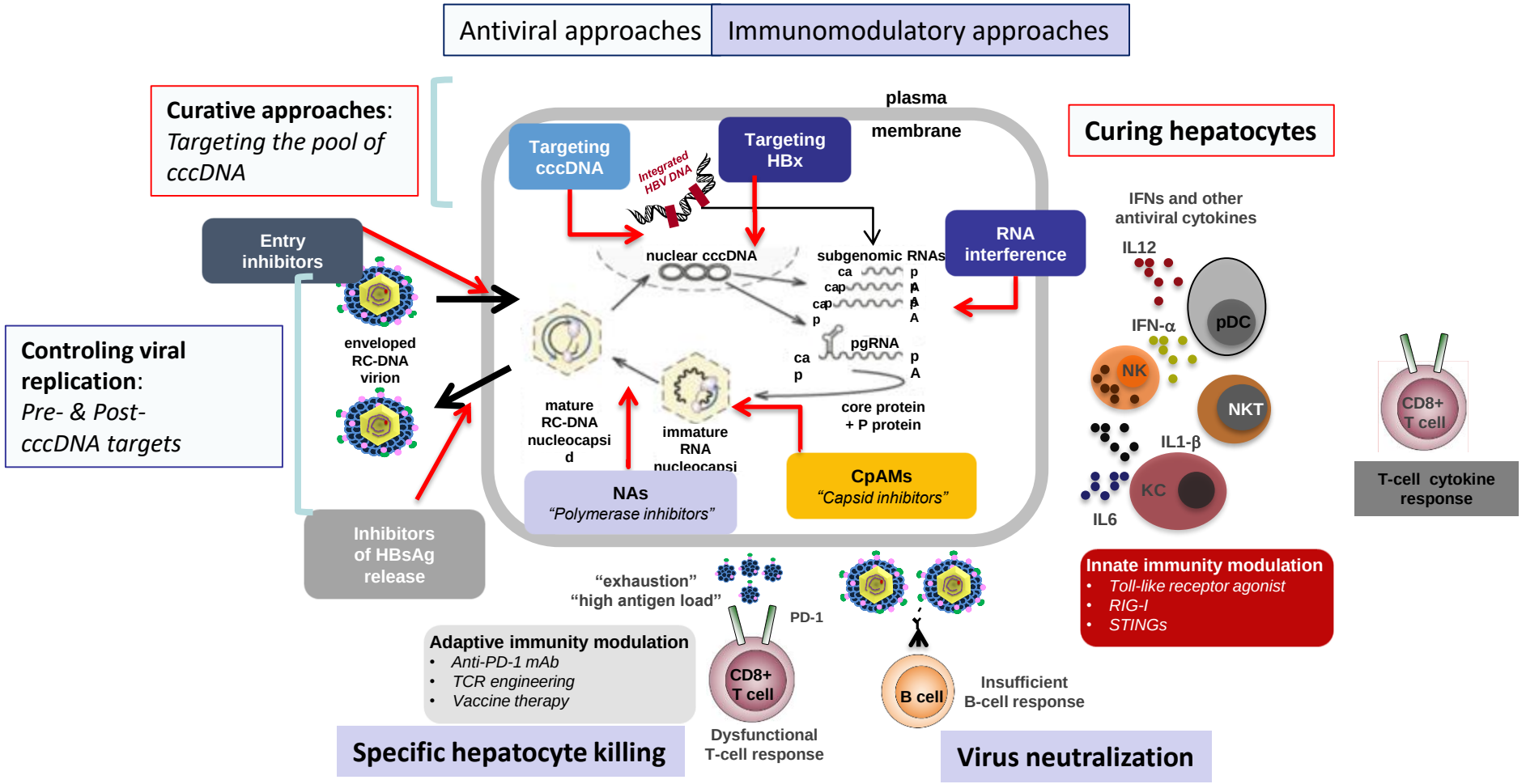
cccDNA loss through mitosis





cccDNA silencing

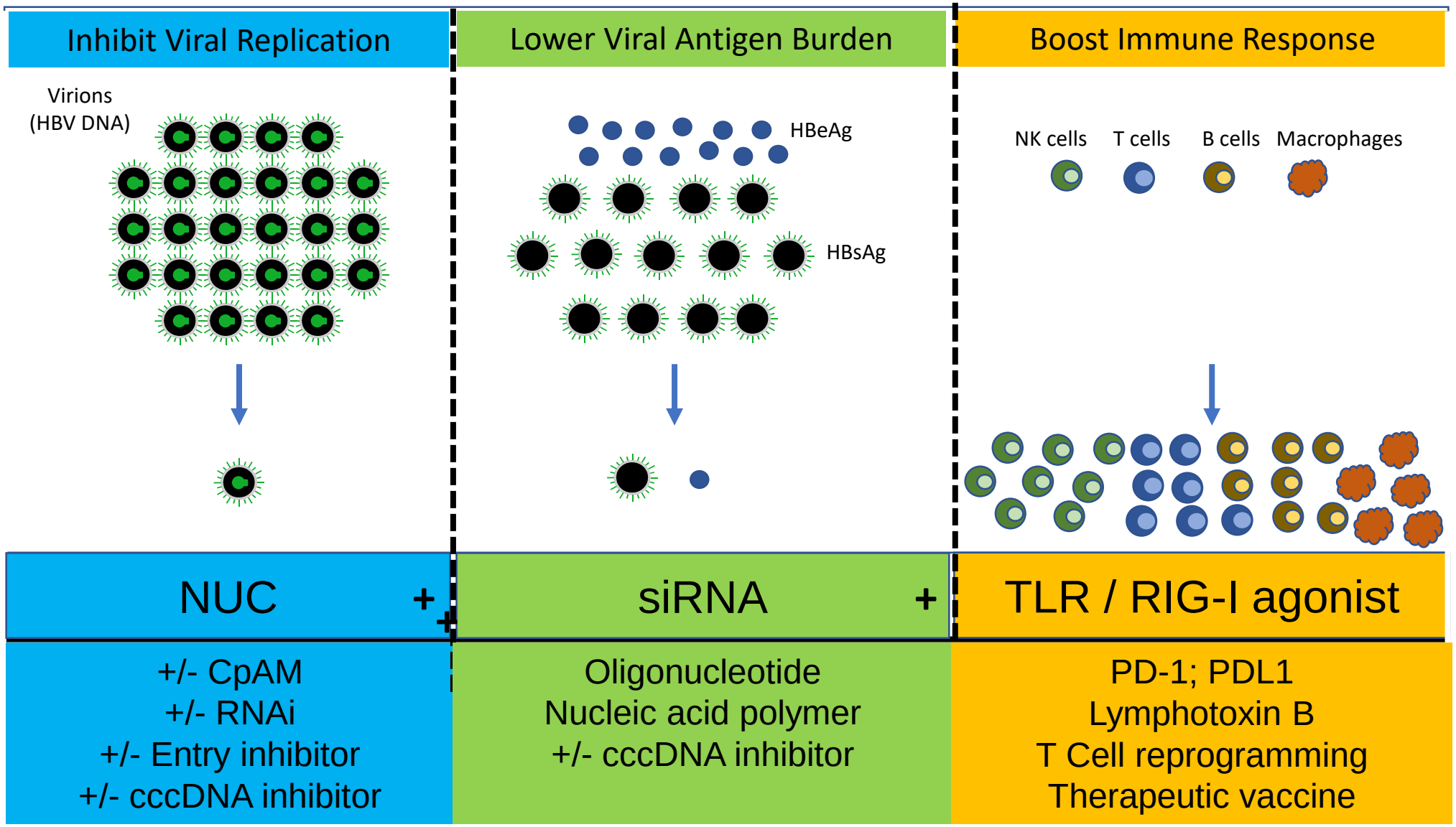
Zoulim, et al, Clin Gastroenterol Hepatol 2013
Revill et al, Lancet Gastroenterol and Hepatol 2019

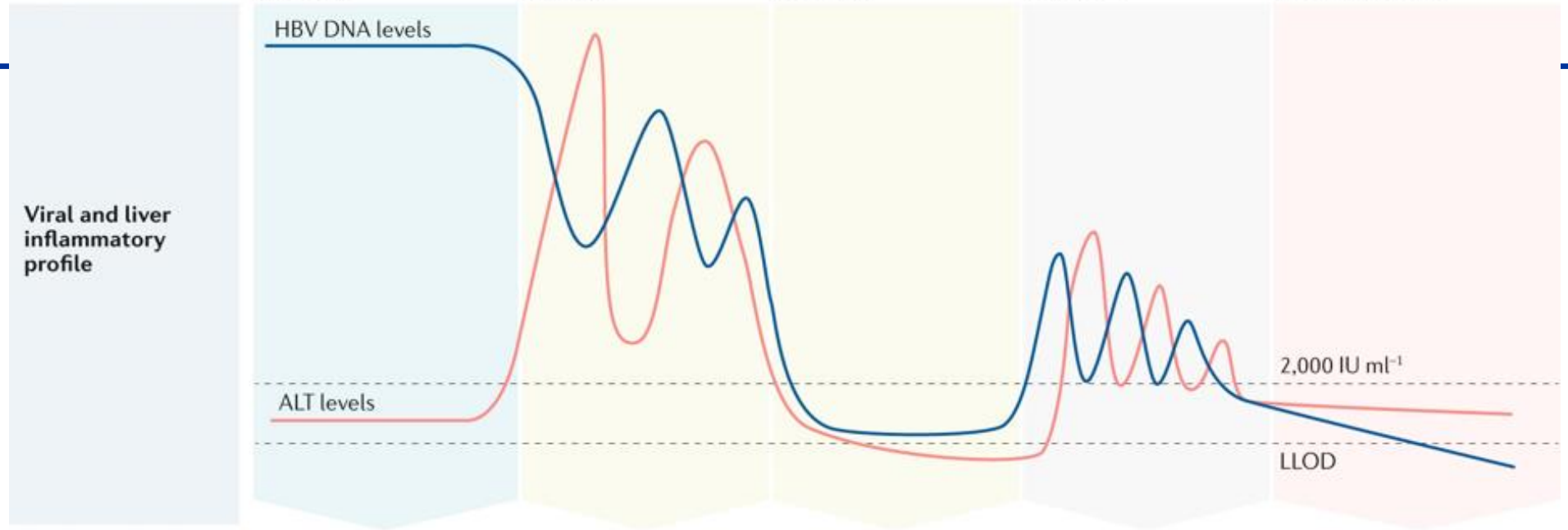


Adapted from: Levrero M, et al. Curr Opin Virol 2016;18:135–43

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: plasmocytoid dendritic cell; PD-1: programmed cell death-1; TCR: T cell receptor

Pathways to Achieving Functional Cure





	HBeAg⁺ chronic infection	HBeAg⁺ chronic hepatitis	HBeAg⁻ chronic infection	HBeAg⁻ chronic hepatitis	Functional cure
Viral and liver inflammatory profile					
Serum HBsAg	+++++	++++	+	++	–
cccDNA	+++++	+++++	++	+++	+
Fibrosis	Minimal fibrosis	Variable degree of fibrosis (progressing)	Variable degree of fibrosis (progressing)	Moderate to severe fibrosis (resolving)	Variable fibrosis (sequelae of previous phases)
Liver inflammatory infiltrate	Absent	High	Absent/moderate	Moderate	Absent
Profile of adaptive immunity	T cells • HBs –/+ • HBc –/+ • HBp –/+	T cells • HBs –/– • HBc –/+ • HBp –/+	T cells • HBs –/– • HBc –/+ • HBp –/+	T cells • HBs –/– • HBc –/+ • HBp –/+	T cells • HBs –/– • HBc –/+ • HBp –/+
	B cells • HBs –/– • HBc +	B cells • HBs –/– • HBc + +	B cells • HBs –/– • HBc + +	B cells • HBs –/– • HBc +	B cells • HBs + • HBc +

Fanning et al, Nature Reviews Drug Discovery (2019)

Core Assembly Modulator (CAM) JNJ-0440

Two cohorts of 10 treatment-naïve HBeAg +/- patients randomized to JNJ-0440 or placebo x 28 days

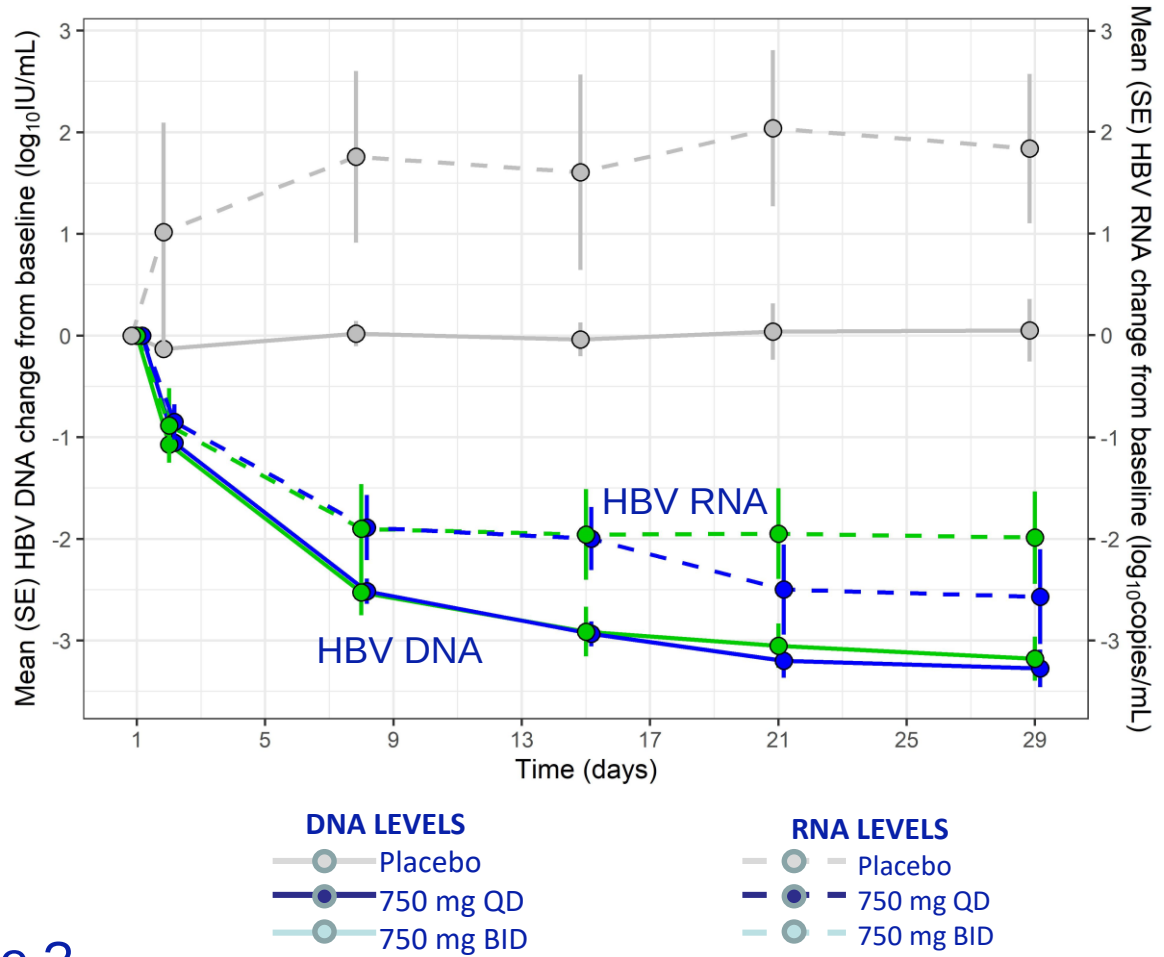
Efficacy

	750 mg QD	750 mg BID
Mean change in HBV DNA vs. BL $\log_{10}$ IU/mL	-3.2	-3.3
Mean change in HBV RNA vs. BL $\log_{10}$ copies/mL	-2.0	-2.6

- Mean change in HBeAg vs. BL $\log_{10}$ IU/mL -0.2
- No relevant changes in HBsAg levels

Safety

No treatment discontinuations/serious AEs



Potent inhibition of viral replication ? functional cure ?

Gane et al, AASLD, Boston 2019, Abstract 0089