HBV cure: how and when?

The city of Light

Le Palais des Congress

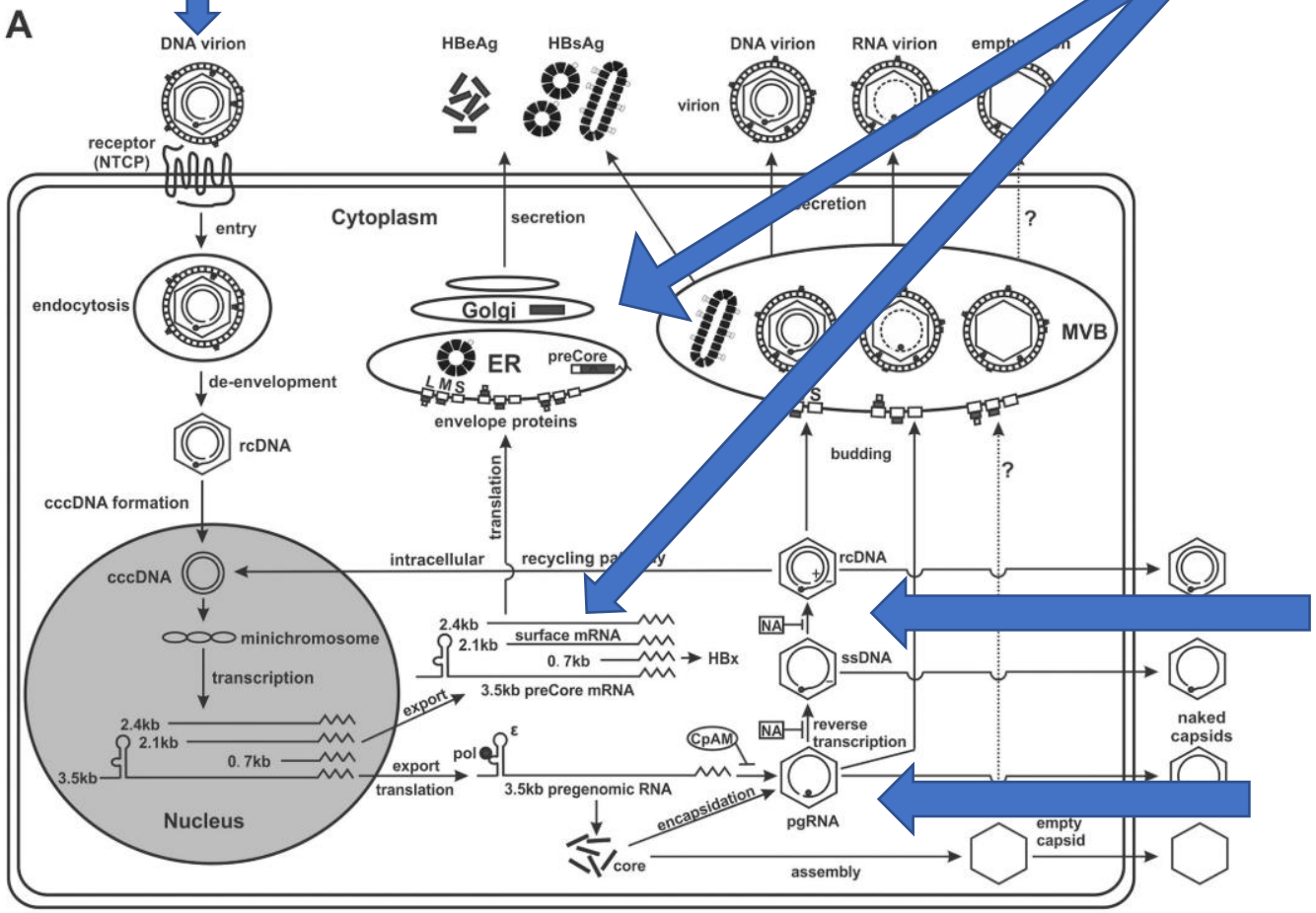
Introduction

- Presumptuous
  - I do not know how, and I do not know when
  - Data is emerging
  - Proof of principles established
- The needle is moving?

HBV replication cycle

Entry

RNAi and HBsAg assembly



Nucleoside Analogues

CAMS

Promise of new druggable targets

Several steps targets for new treatments

Considerable pipeline multiple strategies

Next generation compounds in trial

Reviewed at this meeting

Progress:

Improved on-treatment reductions in HBV DNA*

Reduction in serum HBV RNA

Decline in HBsAg with RNAi /HBsAg strategies

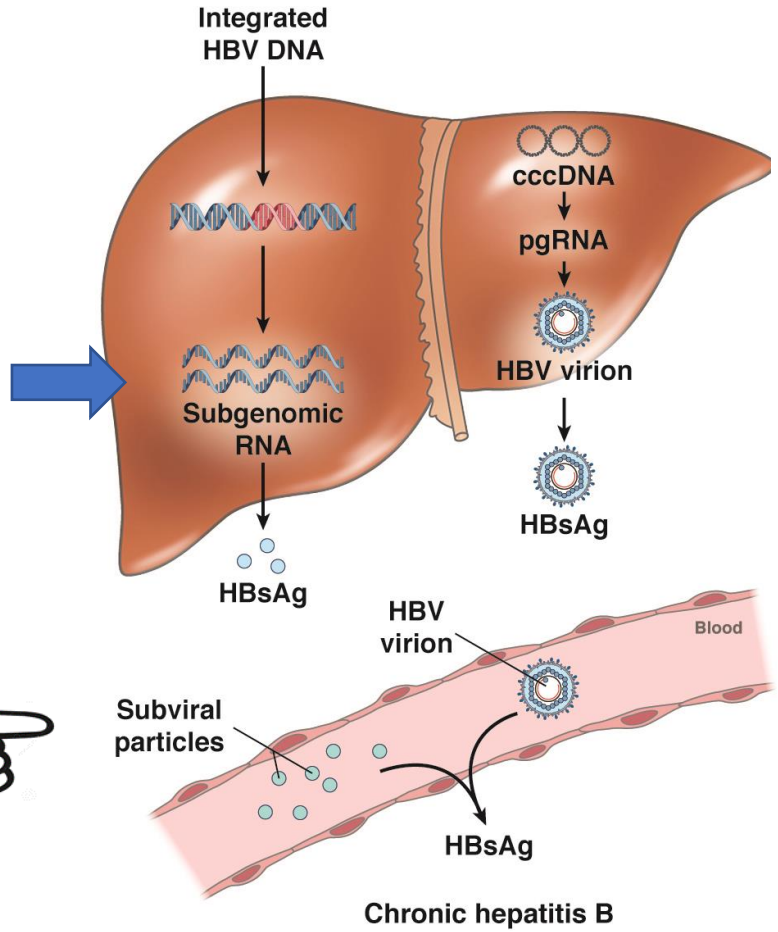
Shen PLOS Pathogens doi.org/10.1371/journal.ppat.1008945 October 20, 2020

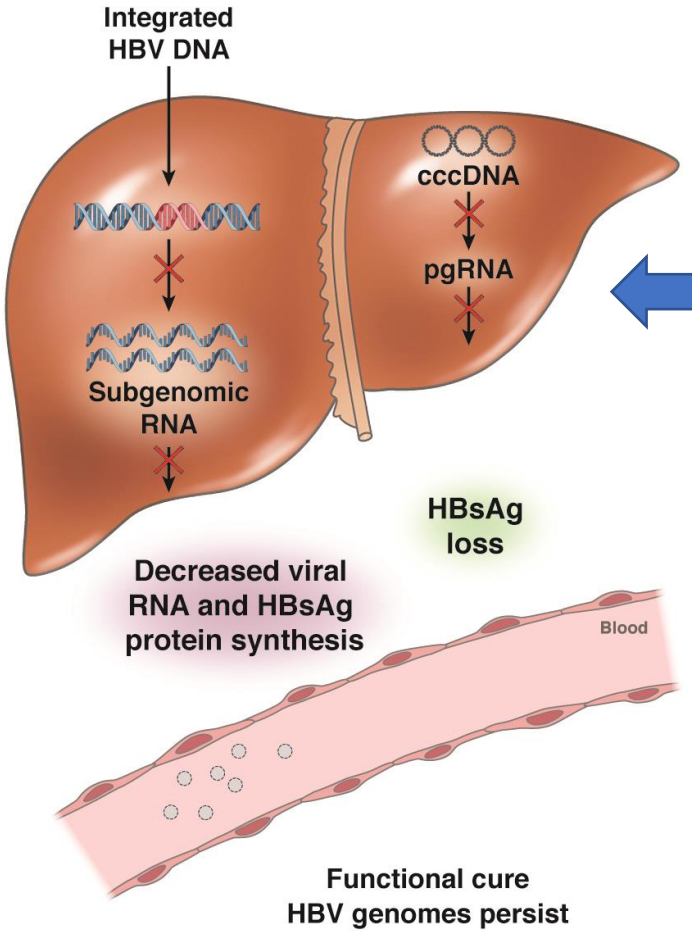
Huang Q Antimicrob Agents Chemother. 2020;64(11) Berke JM Antimicrob Agents Chemother. 2020;64(5).

* Requires sensitive testing

HBV in the liver: integrated and replicative forms
HBsAg production

HBsAg: Not dependent on HBV replication

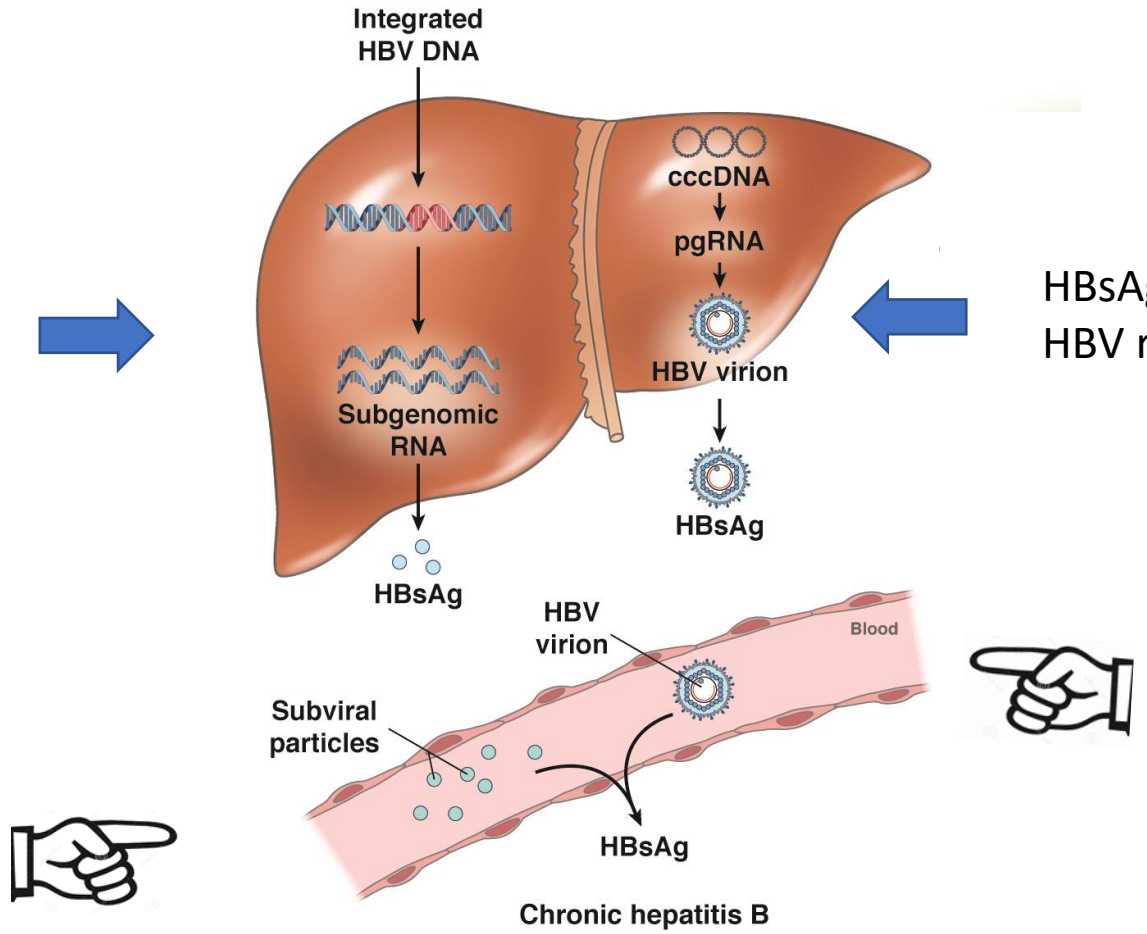




HBsAg from replicative

HBV in the liver: integrated and replicative forms
HBsAg production

HBsAg: independent of HBV replication

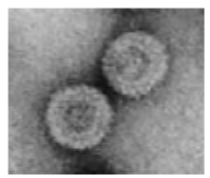


HBsAg: dependent on HBV replication

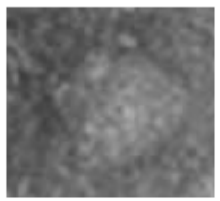
HBV particle morphology

Ratio to Dane particles and morphology by electron microscopy

Dane particles	Defective interfering particles (replication deficient)	Sem-infectious particles One or more viral proteins mutated or defective	Infectious virus not requiring viral qlycoprotein	Genome free virus	Envelope free virus	Genome and capsid free particles (produced independently from viral replication
1	HBV RNA virions[1] **0.001**	Quasispecies[2-4] **0.2-0.8**[5] (evolving during infection)	HBV exosomes[6-8] **Unknown**	"Empty" virions[1, 9] **100**	"Naked capsids" **Unknown**[10]	Subviral particles[1, 9, 11, 12] **10,000 – 100,000**

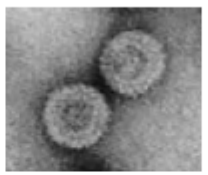


44nm



50-100 nm

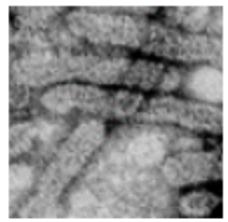
(?)



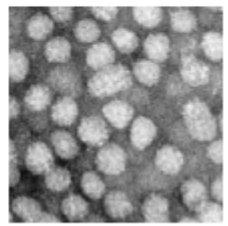
44nm

27nm

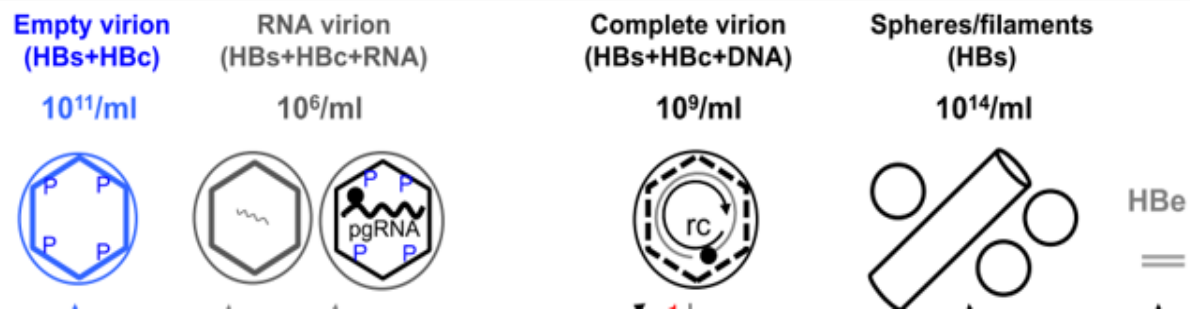
Ø22nm



22nm



1. Hu and Liu, Viruses 2017;9:56
2. Zhou et al., Sci Reports 2017;7:661
3. Aragri et al., J Inf Dis. 2016;213:1897-1905
4. Yang et al., J Clin Microbiol. 2015:53:2203-2214
5. Zhang et al., Clin Microbiol Inf. 2015:280-287
6. Yang et al., Cell Mol Immunol. 2017;14:465-475
7. Sukriti et al., J Viral Hep. 2019;26:211-214
8. Li et al., BioMed Res Int. 2019;2103943
9. Luckenbaugh et al., J Viral Hep. 2015;22:561-570
10. Stannard and Hodgkiss, J Gen Virol. 1979;45:509-514
11. Ganem and Prince, N Engl J Med 2004;350:1118-1129
12. Gerlich, Virol J. 2013;10:239



Vaillant https://dx.doi.org/10.1021/acsinfectdis0c00638

A functional cure remains a major challenge

- Even if measured by HBsAg seroclearance alone

Some of the challenges (and opportunities)

1. Replenishment and stability cccDNA: shuttle amplification pathway
2. cccDNA not rapidly degraded by current nucleoside analogue therapy
3. HBsAg production without replicative cycle:
    1. Random integration of HBV genome
        1. *Long reading frames identified*
        2. *Randomly scattered throughout human chromosomes*
4. High antigen load results in antigen specific immune dysfunction and T and B cell exhaustion

Werle-Lapostolle B, Bowden S, Locarnini S,. Gastroenterology. 2004;126(7):1750-1758

Definitions of cure

Functional cure defined:

- Sustained loss HBsAg
- Measurable end point
- Discontinuation
- Will require inhibition + control

Improved prognosis?

- Not complete eradication from the host
- Data to show reduction in outcomes?

A place for partial functional cure?

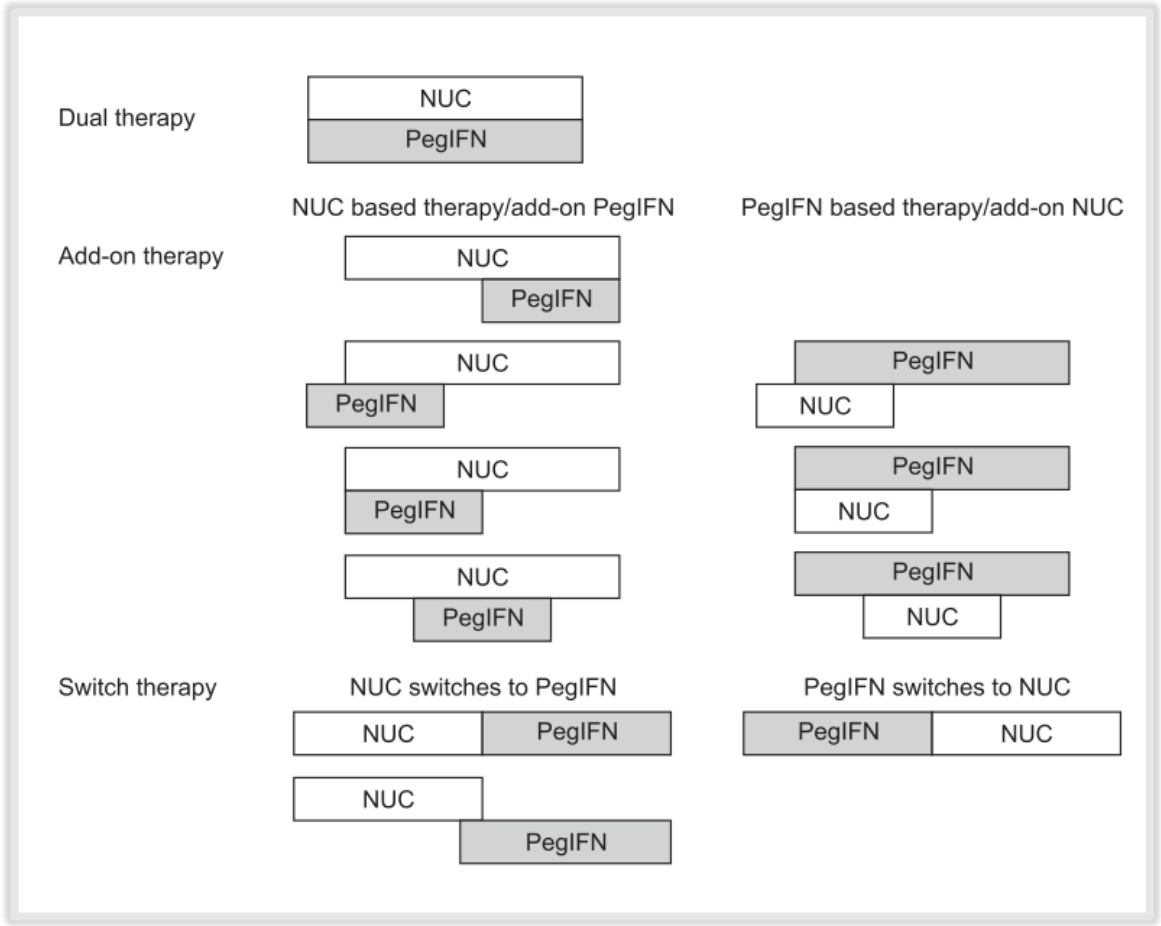
- Decline in HBsAg to lower levels after finite treatment.
- Patients remain HBsAg positive
- Reset to a low replicative state?
- How low is a low HBsAg level?
  - In HBeAg positive
  - HBeAg negative?
- Reach absolute level or log decline?
- Improved patient prognosis?

Cornberg M Journal of hepatology. 2020;72(3):539-557

Current therapies for cure

Nucleoside analogues and interferon combinations

Cessation of nucleoside analogues



Su et al Gut and Liver 11: 590-603 2017

Cessation of nucleoside analogues

- Two aims to this approach:
  - To enter HBsAg-positive low replicative state
  - Or to trigger HBsAg loss
  - Off treatment HBsAg loss: 4-10% (20%)
  - HBsAg range associated with HBsAg loss: < 100 U/L to < 1000 U/l
- Unanswered questions
  - Lower concentrations of HBsAg favour HBsAg loss
  - Ethnic influence on response?
  - Requires more precise information
    - Biomarkers
    - Immune dysfunction and likelihood of restoration
  - Timing between TDF and ETV

Berg T Journal of hepatology. 2017;67(5):918-924. Garcia-Lopez EASL Digital conference 2020; Geneva Van Bommel EASL Digital Conference 2020; Geneva. Hall S. EASL Digital Conference 2020; Geneva.

Two main strategies for finite functional cure

1. A reduction in HBsAg expression
   - Ultimate HBsAg seroclearance
   - Subtext: immunological clearance?

2. Deepening inhibition of HBV replication

HBsAg knockdown strategies

Newer compounds

Interfere with:
Translation of HBsAg
Assembly HBsAg

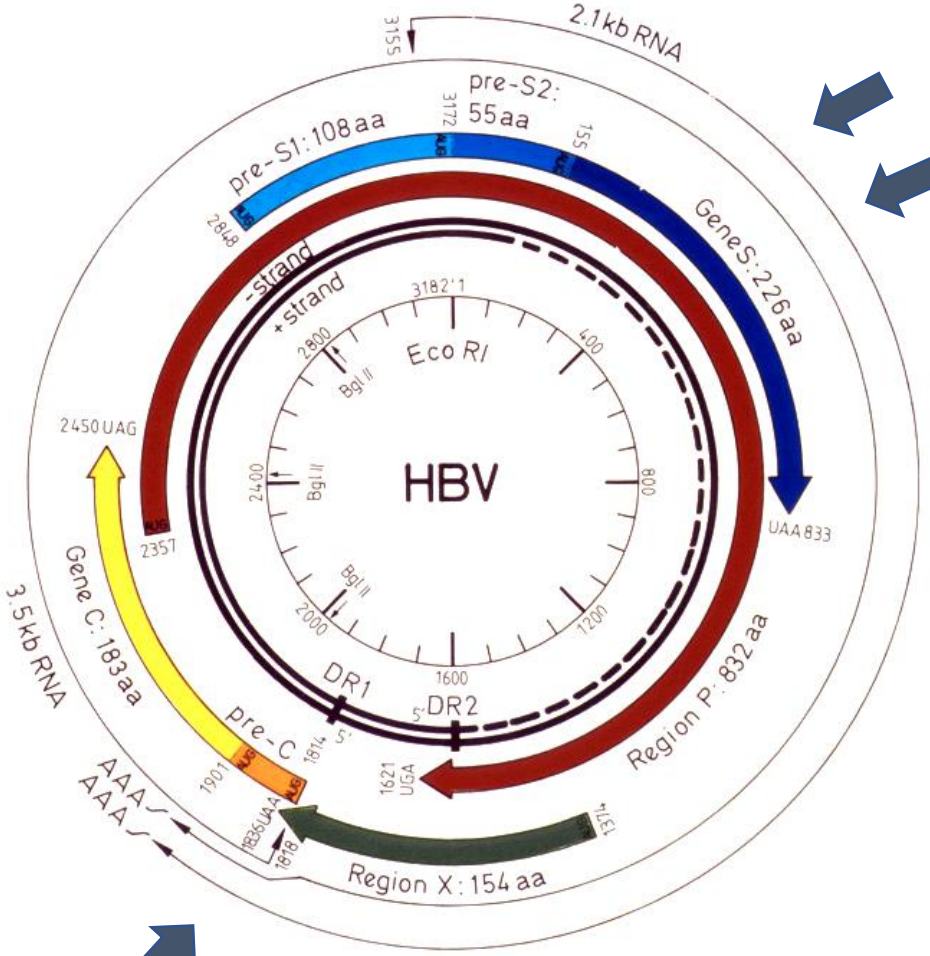
Reduce HBsAg directed to subviral particles as well as complete virions

Mechanisms

- HBsAg derived from integrated viral genomes:
- Hitherto relatively inaccessible source of HBsAg
- RNAi
  - Interfere with translation
  - siRNA's, locked nucleic acids and antisense oligonucleotides
- siRNAs
  - Target transcripts cause destruction by RISC/Ago2 complex
- Second generation ASO
- NAPs and STOPs
  - Class of amphipathic phosphorothioate oligonucleotides
  - Selectively inhibit HBsAg particle assembly and hence secretion
- Entry inhibitors and monoclonal antibodies

Gane E. EASL Digital Conference 2020; Geneva Yuen MF. Digital Conference 2020; Geneva Boulon R, Antiviral Res. 2020:104853

The HBV Genome and trigger locations



Dicerna

Aligos

Arrowhead

Arrowhead X

Arbutus

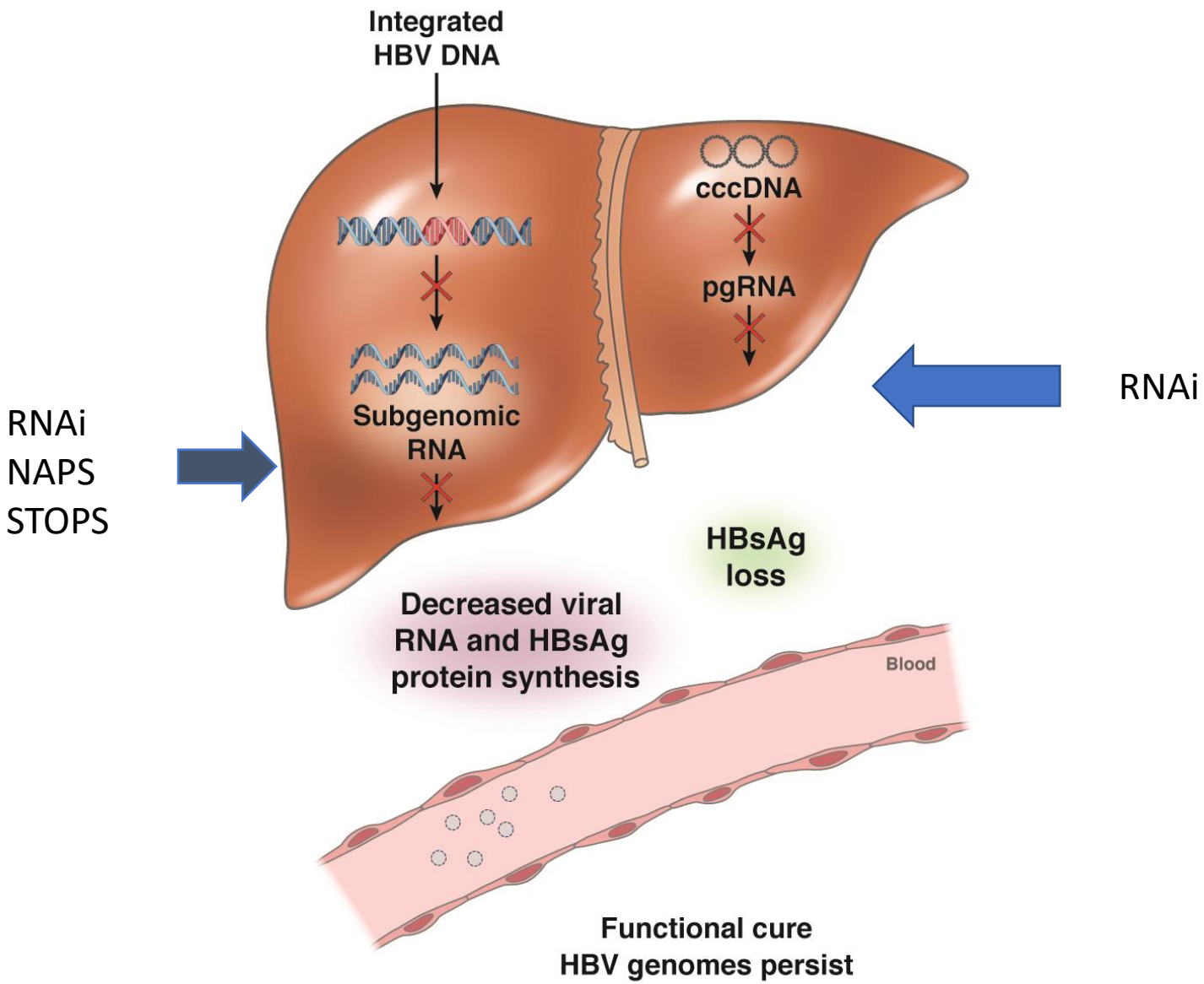
GSK

Vir

(Roche ASO)

Arrowhead) JNJ-3989: Wooddell, EASL 2018 Vir2218: WO 2020/036862 A1
GSK-836: US 9,145,558 B2 RG6346 (Roche/Dicerna siRNA): WO 2019/079781 AB-729: WO 2018/191278 A2

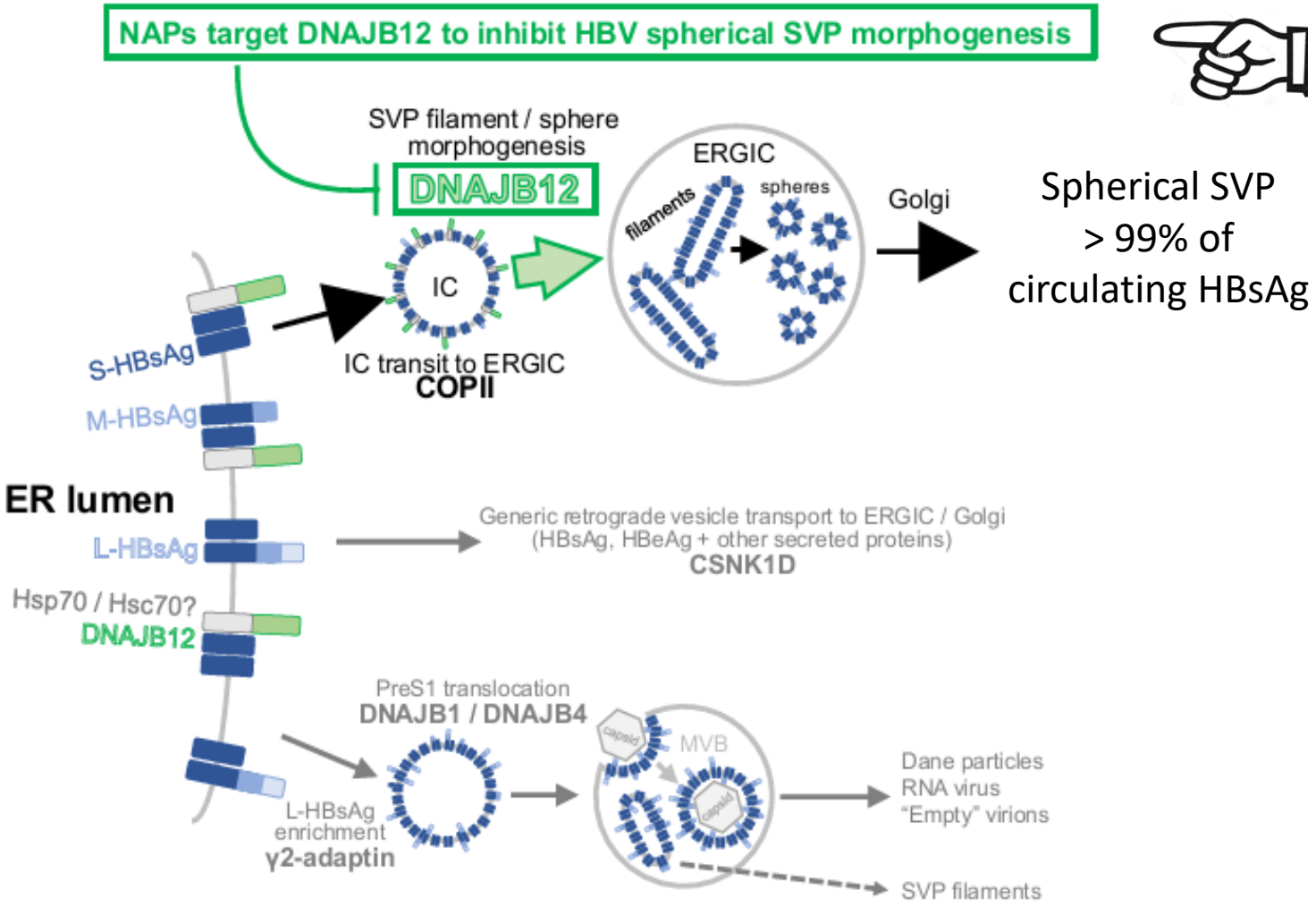
RNAi and NAPS/STOPS



HBsAg knock down: Questions and requisites?

- Reduce HBsAg protein (assembly, chaperoning) of subviral particles as well as complete HBV virions?
- RNA interference and nucleic acid polymers reduce HBsAg concentrations on treatment- some persistence observed.
  - Hepatocyte cytolysis or apoptosis may be required to obtain sustained declines in HBsAg concentrations or HBsAg seroclearance?
  - (immunological clearance ALT proxy)
  - Precipitous response required?
  - Immunomodulatory strategy applicable?
  - Effect on HBV replication and cccDNA? (pre-S1 HBsAg capsid interaction)
  - What explains the range and heterogeneity of decline (HBV genotype?)
  - What isoforms and particles are reduced?

NAP target DNAJB12: inhibition of spherical SVP HBsAg morphogenesis



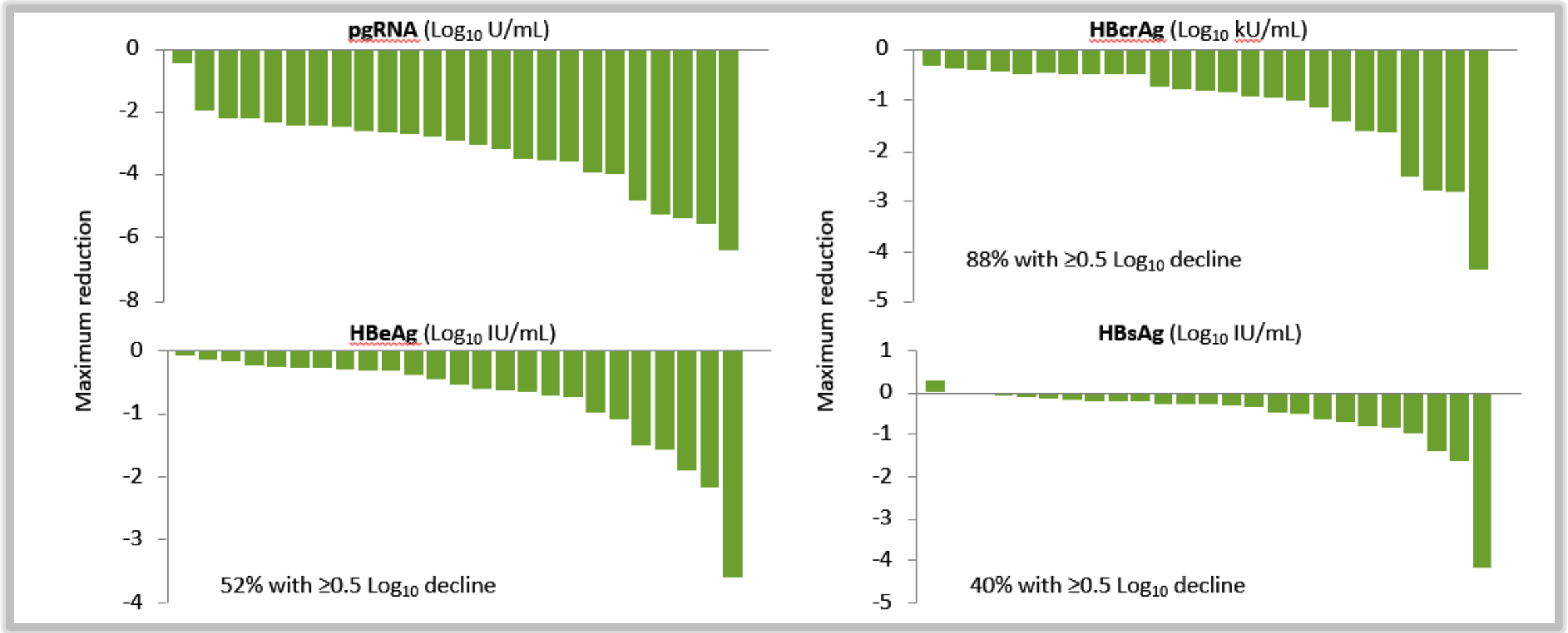
Boulon et al AASLD 2020 LP42

Inhibitors of HBV replication: CAMS

- Two major classes
  - Class A (aberrant) core protein aggregates
  - Class N (normal, but empty capsids)
- Clear effects on HBV replication
  - Further check point targeted
  - Deepen inhibition (shut down) HBV replication
  - Interference with capsid assembly and inhibition of pgRNA encapsidation (primary mechanism)
    - Reduction in serum HBV RNA
    - Ultrasensitive DNA tests show deeper inhibition of HBV replication

Effect of hepatitis B core inhibitor ABI-H0731 + nucleoside analogue

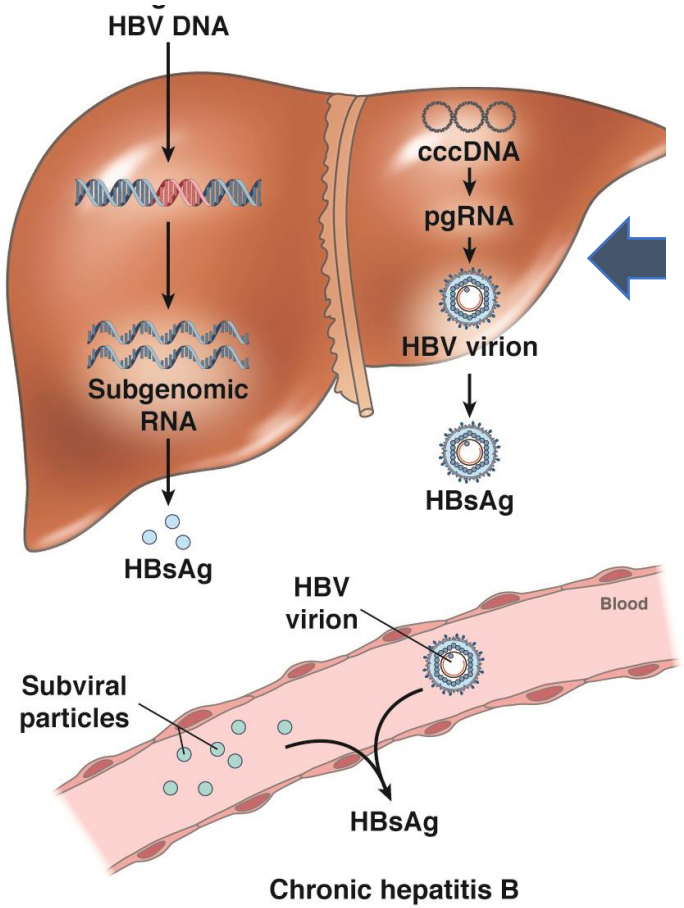
Patients with HBeAg positive HBV



HBV viral transcripts in treatment-naive patients
(change from baseline for individuals)

Yuen M-F, et al. EASL dILC2020. #LBP30

CAMS: mechanism and effect on HBV markers



CAMS

Reduce pgRNA and DNA
Much less effect on HBsAg

HBsAg

- Relatively limited decline 0.5_{10} log HBsAg concentrations
- In HBeAg positive nucleoside analogue naïve patients after 24 weeks
- No dominant effect on HBsAg
- In particular HBsAg derived from integrated viral genomes?
- **Lower reduction of HBsAg in**
  - HBeAg positive nucleoside analogue suppressed
  - HBeAg negative naïve and nucleoside analogue suppressed patients
  - Population-dependent reductions in HBsAg mainly due to the reduction of HBsAg from Dane particles?

Reductions of PgRNA from baseline

- Most significant in HBeAg-positive nucleoside analogue naive patients.
- HBV RNA concentrations are low at baseline in HBeAg negative cohorts

Yuen MF The lancet Gastroenterology & hepatology. 2020;5(2):152-166 Janssen H. EASL Digital Conference 2020; Geneva.

Fung EASL Digital conference 2020; Geneva Yuen MF. EASL Digital Conference 2020; Geneva.

CAMS: some problems

- Require longer term use: 48 weeks
  - Some problems include
  - Emergence of signature mutations
  - Core and capsid intracellular accumulation?
  - Other potential toxicities?
  - Difficulty in identifying DILI from immunologically (potentially beneficial response) ALT elevations

Combination antiviral therapies

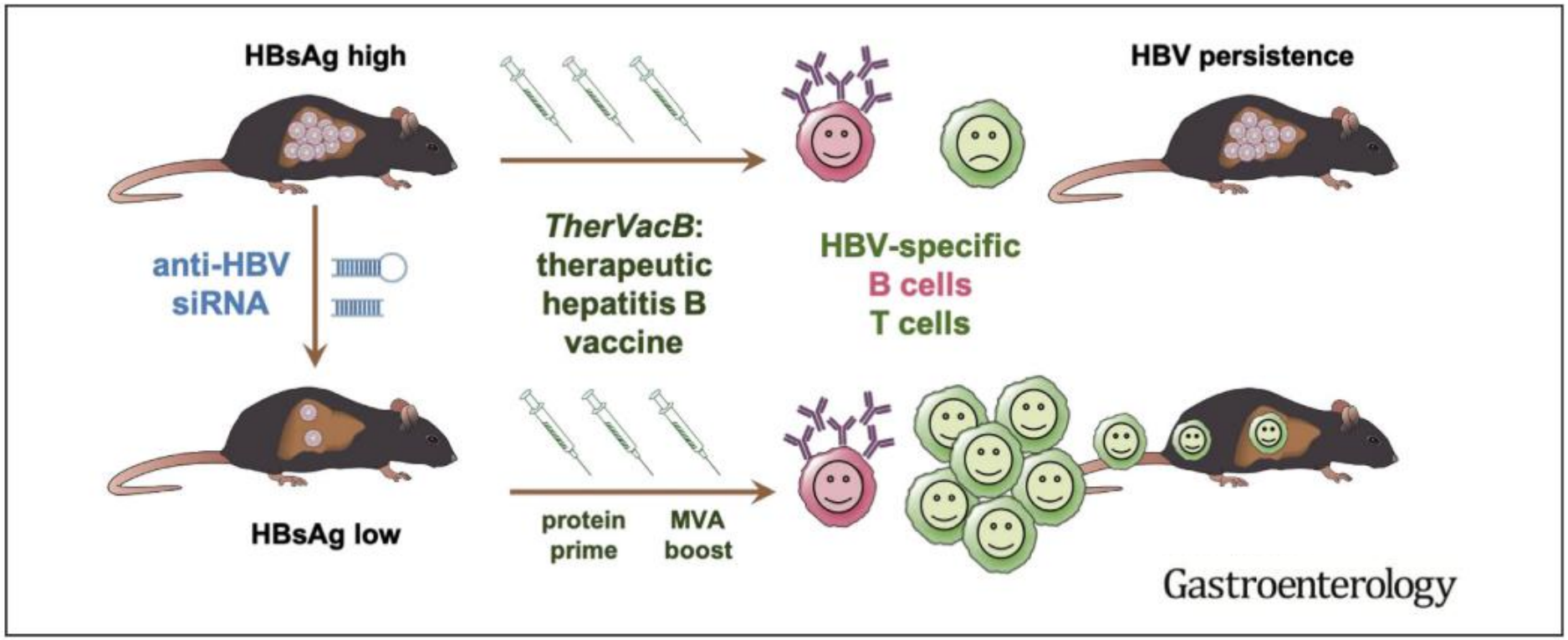
- RNA interference being studied in combination with capsid assembly modulators
  - Many programs in the offing
  - Outcomes will provide data in 2021

Immunomodulatory therapy

- Lagged behind antiviral therapy?
- Multiple immunomodulatory agents
  - Check point inhibitors
  - Therapeutic vaccines
  - Immunological engineered cells to enhance T and B cell recognition and cytokine stimulation as well as (pathogen receptor agonists)
- Agonists such as selgantolimod (GS 9688, an oral TLR8 agonist) intensively studied.
- Expectations to date have been unrealistic?
- Increase in the multilayered impaired and dysfunctional immune response
- Perhaps more likely and feasible after a reduction in host antigen burden
  - Addition of immunotherapy (PEG IFN TRL agonists thymosin alpha after reduction in HBsAg and host antigen burden

Gane E. EASL Digital Conference 2020; Geneva.

Anti-HBV siRNA + therapeutic HBV vaccine strategy



Michler T Gastroenterology. 2020;158(6):1762-1775 e1769.

Other potential strategies

- CRISPr-Cas9 genome editing nucleases.
  - Target and cleave cccDNA or specific genes (X)
  - Not feasible for integrated viral genomes
- Epigenetic silencing
  - Reduce gene transcription
  - Targeting HBx to modulate its association with cccDNA:
  - (promote degradation) and decrease transcription
- Neutralising monoclonal antibody

Terminology of HBV and treatment indications

EASL HBV Guidelines, J Hepatol 2017

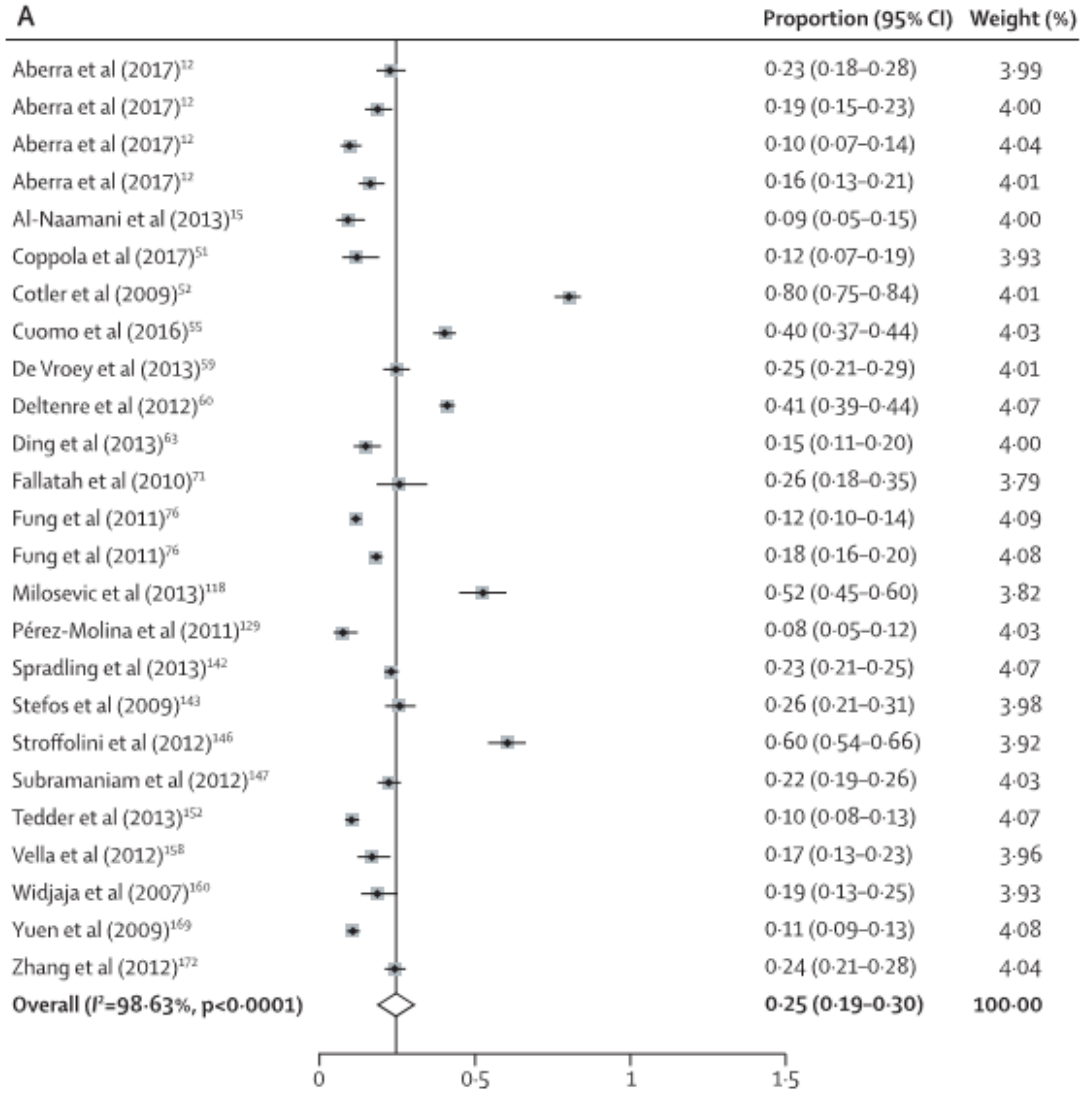
New terminology	HBeAg negative Chronic *infection*	HBeAg positive Chronic *infection*	HBeAg positive Chronic hepatitis	HBeAg negative Chronic hepatitis
Old terminology	***Inactive carrier***	***Immune tolerant***	***HBeAg-positive CHB***	***HBeAg-negative CHB***
HBsAg	Low	High	High/Intermediate	Intermediate
HBeAg	Negative	Positive	Positive	Negative
HBV DNA	<2,000 IU/mL*	>10^7 IU/mL	10^4-10^7 IU/mL	>2,000 IU/mL
ALT	Normal	Normal	Elevated	Elevated**
Liver disease	None	None/minimal	Moderate/severe	Moderate/severe
Disease progression	No, very low	Low	**Moderate to high**	**Moderate to high**
Treat	Not indicated	Not indicated***	**Indicated**	**Indicated**

Knock down HBsAg
Immune clearance
Cells expressing HBsAg

Shut down replication
Knock down HBsAg
Restore "inert"
T cell response

Shut down replication; knock down HBsAg
Immune clearance: heterogeneity of T cell response:

Estimates of proportion eligible: health care facilities



Tan et al Lancet Gastro Hep 2021: 6: 106-119

Conclusions: how and when

- Getting it right:
  - Right combinations and sequential strategies
  - Define outcomes as treatments evolve
- Use newer biomarkers and develop others
  - (HBsAg derivation; integration signatures)
  - Tool to identify a reduction in the pool of infected cells
  - Immunological signatures: Horses for courses:
  - Different regimens for different HBV phenotypes
- Toxicities and interactions: to be evaluated
- Affordability: cost effective application