HBsAg Loss and Transaminase Flares: Therapeutic Implications for Functional Cure of HBV

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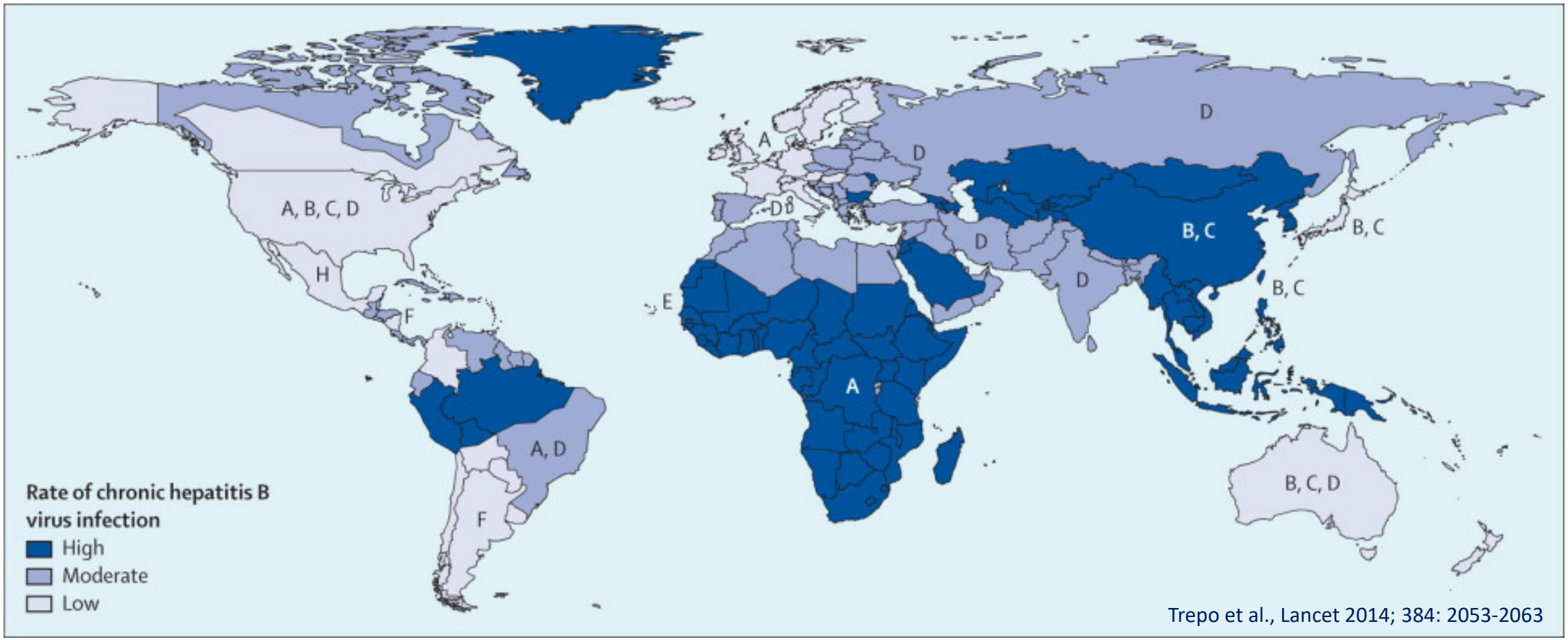
Disclosures

Employee and shareholder, Replicor Inc.

Global burden of chronic hepatitis B infection (CHB)

~300 million patients have HBV worldwide[1], 880,000 deaths annually[2]

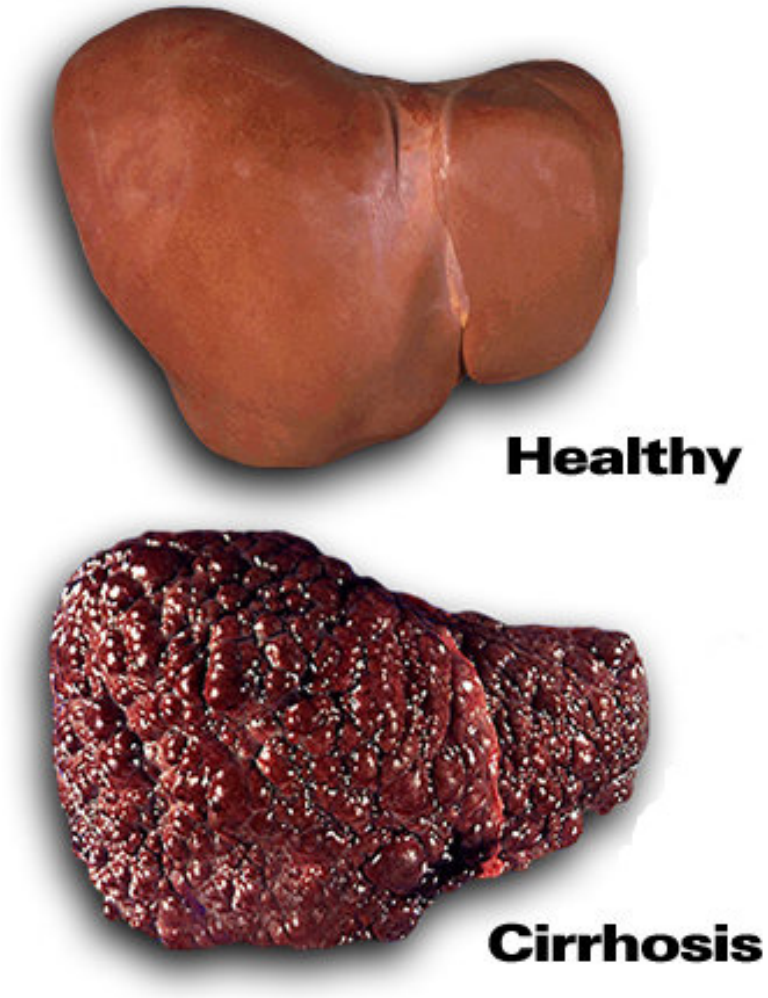


Trepo et al., Lancet 2014; 384: 2053-2063

True prevalence is higher! CHB can be asymptomatic for years…community based testing is lacking

1. Polaris observatory, Lancet Gastro Hepatol 2018; 3: 383-403
2. WHO, 2017

Why treat chronic HBV infection?



Chronic hepatitis

↓

Inflammation, altered liver function

↓

Scarring

↓

Cirrhosis

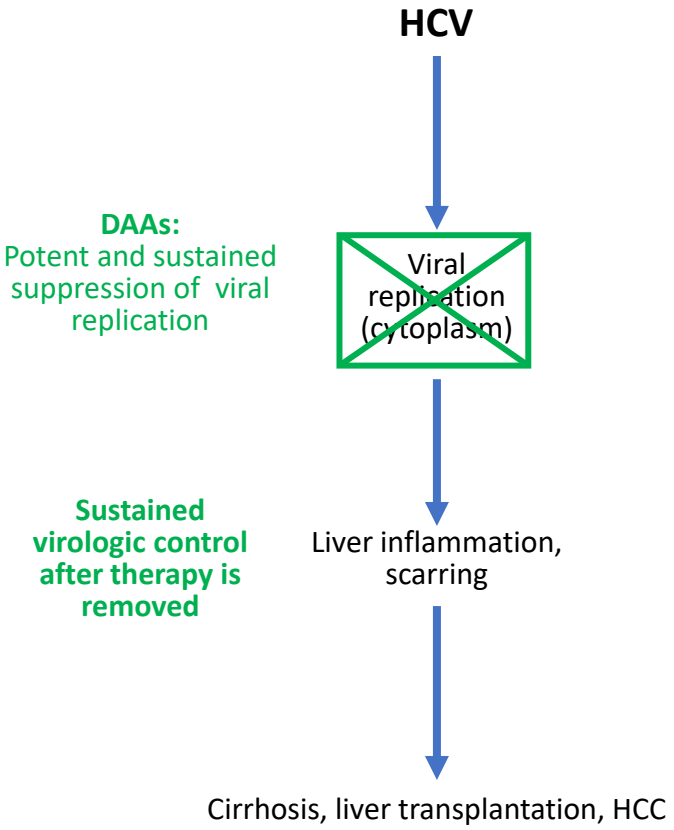
↓

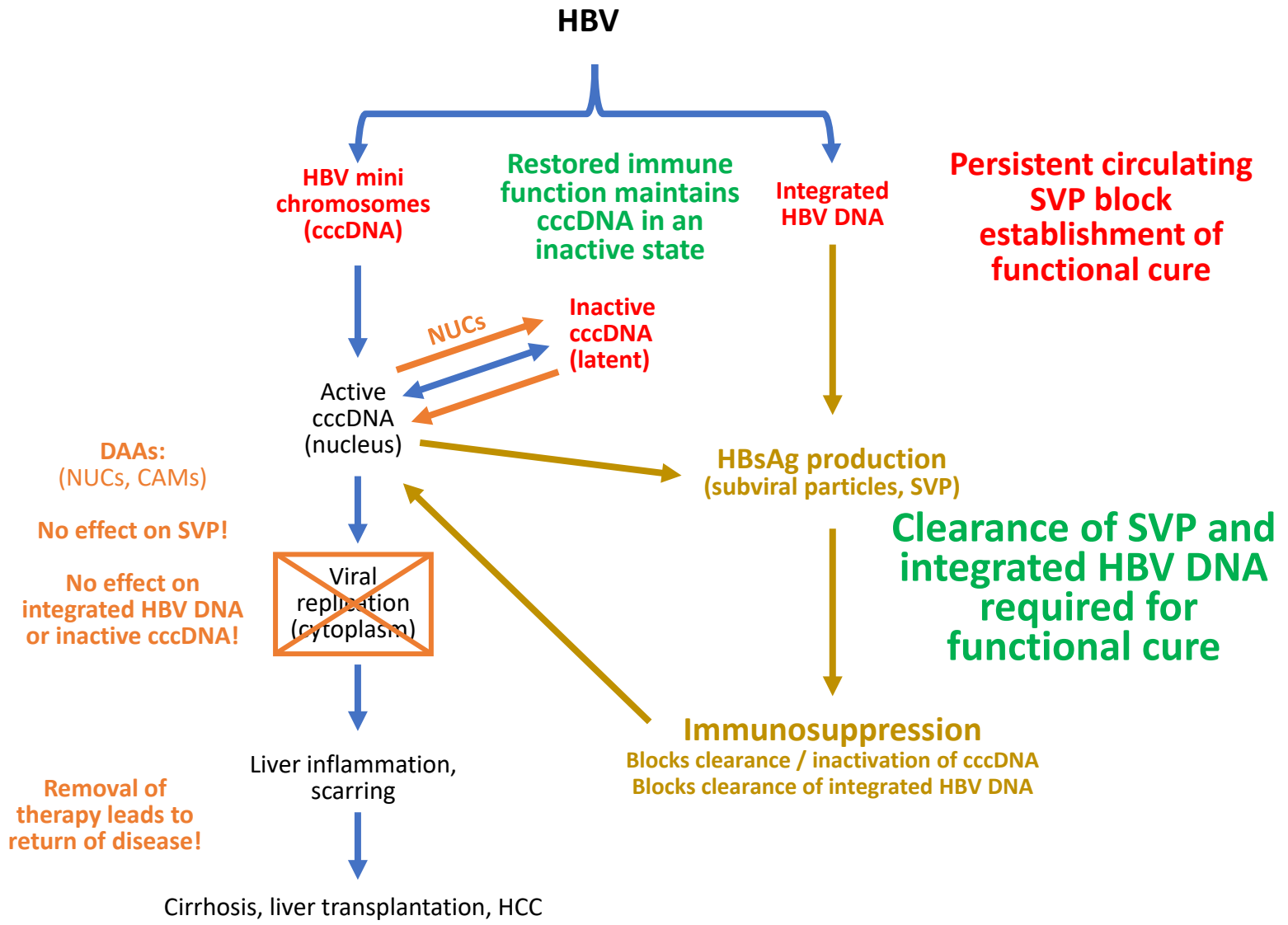
Liver decompensation or liver cancer

↓

Death

Lessons learned from HCV to not apply to HBV!





Production of SVP drives chronicity of HBV infection

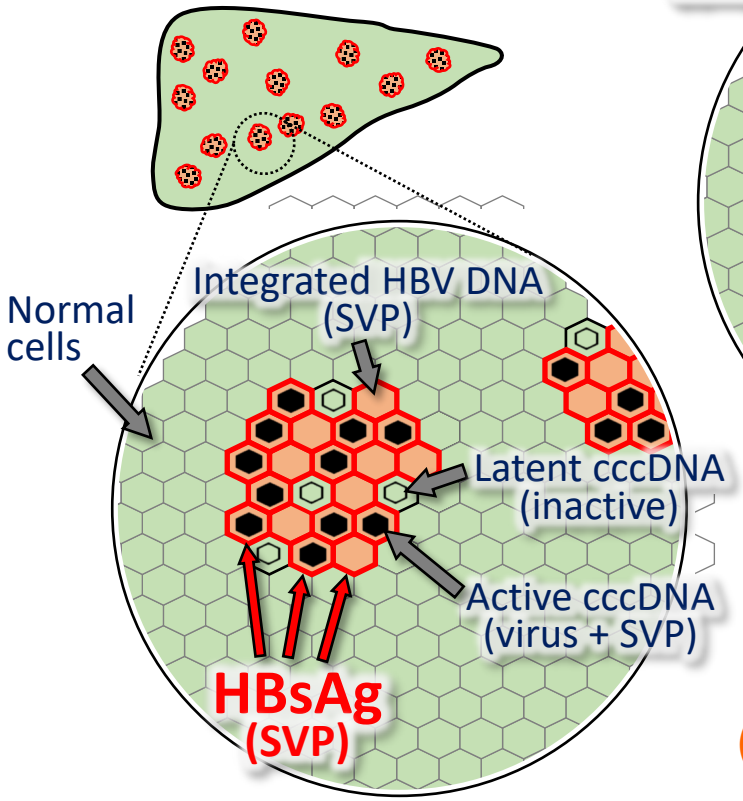
Immunoinhibitory properties of SVP (HBsAg)

Immune function	Target of inhibition	Effect observed
Innate HBsAg blocks inactivation of cccDNA	TLR function	*In vitro, in vivo*
	Cytokine signalling	*In vitro*, in humans
	Monocyte and macrophage function	*In vitro*
	Dendritic cell function	*In vitro*
	NK cell function	*In vitro, in vivo,* in humans
Adaptive HBsAg inhibits clearance of integrated HBV DNA	Sequester anti-HBs	*In vitro*
	HBV specific B-cell function	In humans
	HBV specific CD4+ T-cell function	In humans
	HBV specific T-cell tolerance	*In vitro, in vivo*
	HBV specific T-cell exhaustion	*In vivo*, in humans

Vaillant, ACS Inf Dis 2020; Dec 10 doi: 10.1021/acsinfecdis.0c00638

Functional cure of chronic HBV infection

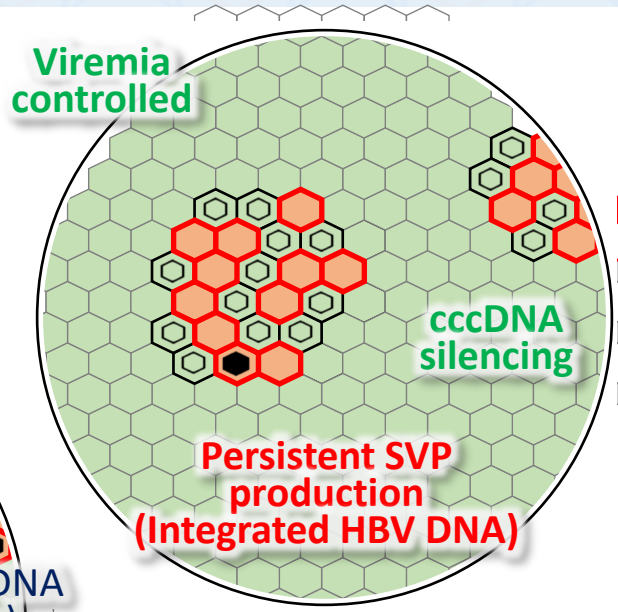
Chronic HBV



HBsAg blocks immune control

Progression of liver disease

Risk of HCC



Current Therapy

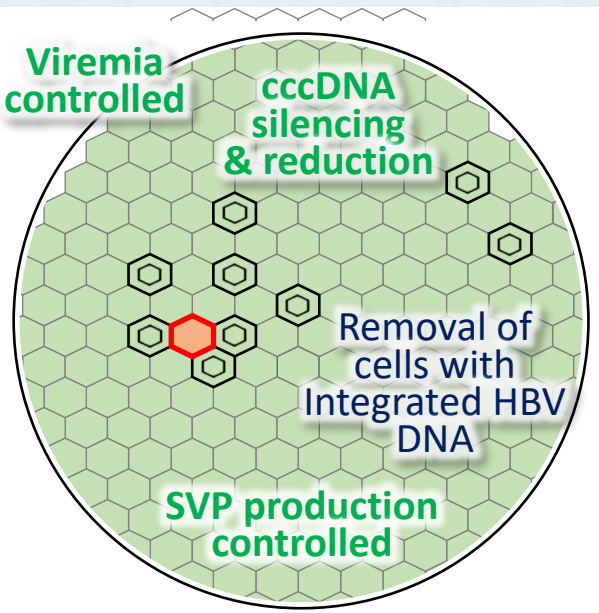
(NUCs)

No immune control

Life long therapy (viral rebound if removed)

Controlled progression of liver disease

Risk of HCC (late initiation of therapy)



Functional Cure

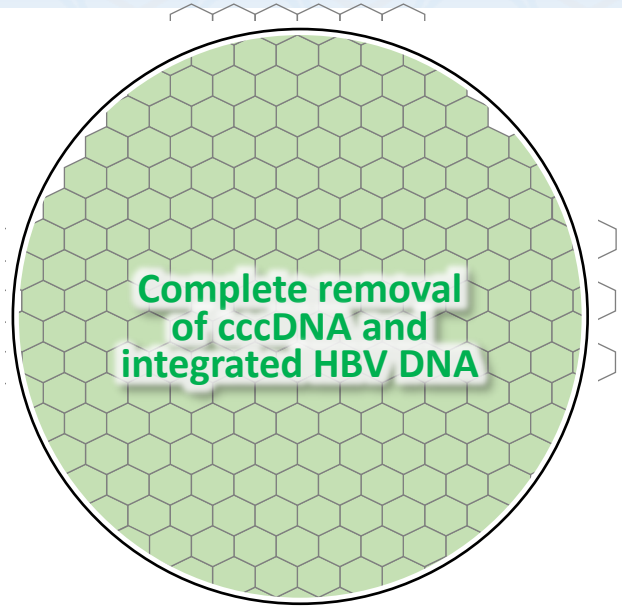
Restoration of immune control

Finite therapy

Reversal of liver disease

Reduced risk of HCC

48 weeks NUCs + pegIFN:
9% functional cure (GT A)
0% functional cure (GT D)



True Cure

No immune control required

Finite therapy

Reversal of liver disease

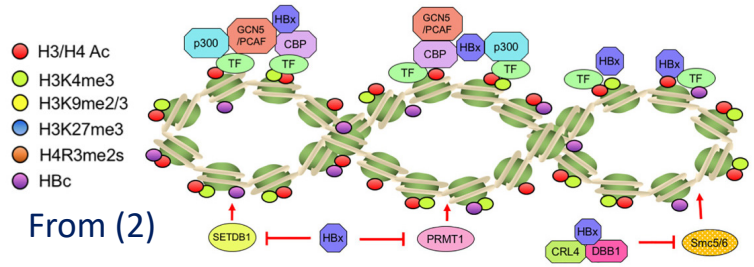
Reduced risk of HCC

Persistence of latent cccDNA is a high barrier

Vaillant, ACS Viruses 2021; 13: 745

Latent cccDNA – the barrier to true cure

Inside the nucleus, the HBV genome (cccDNA) is chromatinized – the HBV minichromosome



From (2)

Euchromatic form – uncondensed (active)[1,2]

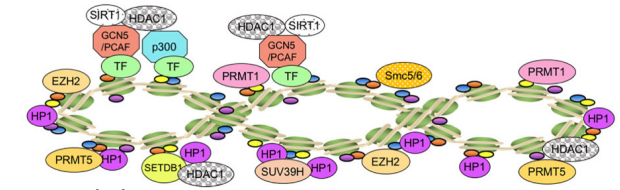
Rapid turnover (1-4 weeks)[3,4] → **rapid fixation of escape mutants to NUCs, CAMs and RNAi/antisense**

Activity can be suppressed by innate immunity[5-9]

Epigenetically regulated condensation[8-10]

Transition[11]

HBx regulated decondensation[12,13]



From (2)

Heterochromatic form – condensed (inactive / latent)[1,2]

Very slow turnover
Insoluble - bound to nuclear scaffold[14,15]
Immunologically silent

Effective barrier to sterilizing cure

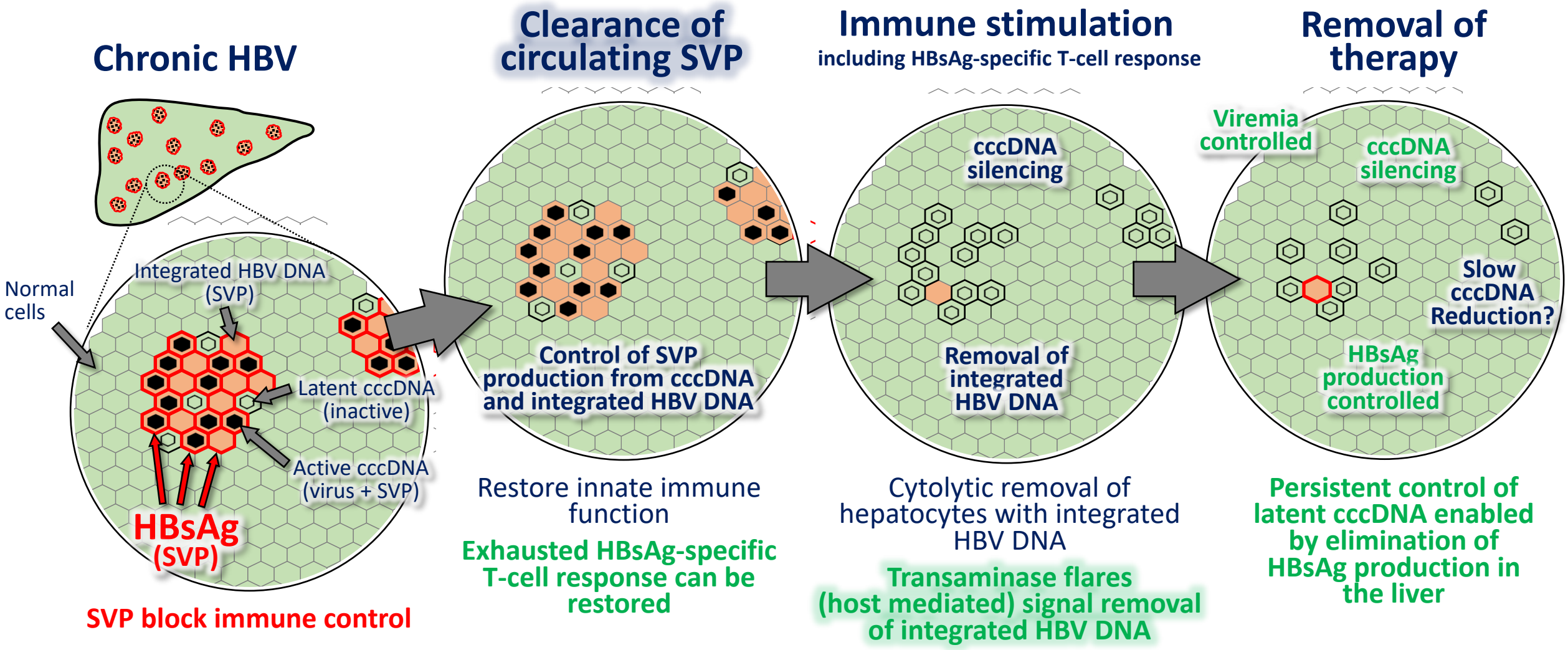
Persists in resolved[10] and occult[11] HBV infection – reactivated by immunosuppression[16-19]

Persists despite biopsy negative for cccDNA during NUC therapy

↳ **Rapid rebound with NUC withdrawal in the presence of HBsAg[20]**

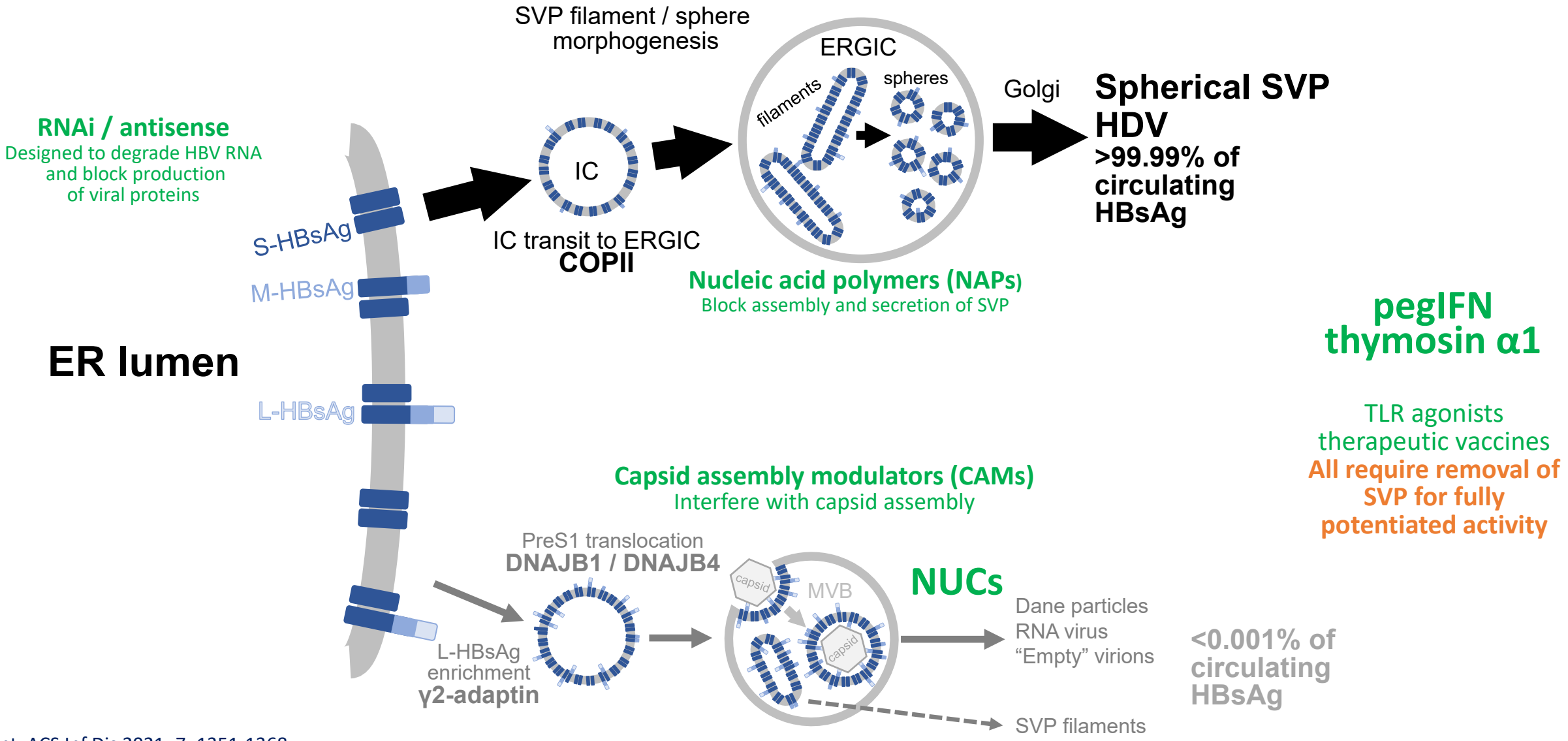
1. Levrero et al., J Hepatol. 2009; 51: 581-592
2. Hong et al., Hepatol. 2017; 66: 2066-2077
3. Huang et al., Hepatol. 2020; epub Mar 19
4. Yuen et al., Hepatol. 2018; 68: 46A
5. Lucifora et al., Science 2014; 343: 1221-1228
6. Xia et al., Gastroenterol. 2016; 150: 194-205
7. Li et al., Sci Rep. 2017; 7: 12715
8. Liu et al., PLoS Path. 2013; 9: e1003613
9. Palumbo et al., PLoS One 2015; 10: e0142599
10. Bloom et al., Genes 2018; 9: 207
11. Troperger et al., PNAS 2015; 112: E5715-E5724
12. Rivière et al., J Hepatol. 2015; 63: 1093-1102
13. Hensel et al., Epigenetics & Chromat. 2018; 11: 34
14. Cam et al., Cell 2009; 136: 610-614
15. Chen et al., Nuc Acids Res 2016; 44: 6482-6492
16. Loomba and Jiang, Gastroenterol. 2017; 152: 1297-1309
17. Wang et al., Hematologica 2019; 104: 435-443
18. Zhang et al., J Immunother Canc. 2019; 7: 322
19. Kuo et al., Sci Rep. 2020; 10: 2456
20. Lai et al., JHEP Rep. 2020; 2: 100112

The path to functional cure



Vaillant, ACS Viruses 2021; 13: 745

Investigational approaches to achieving functional cure of HBV



Vaillant, ACS Inf Dis 2021; 7: 1351-1368

What is “potent” HBsAg (SVP) reduction?

0.5 - 1 log_{10} IU/mL reduction from baseline → **Should not be described as “potent”!**

- Rare with CAMs in the absence of NUCs
- Common with NUCs and pegIFN and RNAi
- Consistent with inactivation of cccDNA
- Abundant circulating SVP still present
- Predicts clinical futility for achieving functional cure[1-3]

> 4 log_{10} IU/mL reduction from baseline → **Potent SVP clearance predicting functional cure[4-7]**

- **Associated with strong therapeutic transaminase flares and HBsAg loss**[8-15]
- Allows withdrawal of NUC therapy with sustained virologic control or functional cure[16-19]

- NUCs: <1% per year of therapy[20]
  more likely in GT A[21]

- PegIFN: 6% with 48 weeks of therapy[22]
- PegIFN + NUCs: 9% with 48 weeks of therapy[22]
  Mostly restricted to GT A, more likely with HBeAg positive infection[22,23]

- Rarely observed with RNAi or antisense

- **pegIFN + NUCs + NAPs: 70% with 48 weeks of therapy[24]**
  GT A, GT C and GT D, HBeAg positive or negative[24,25], HBV / HDV co-infected[26]

1. Brunetto et al., Hepatol. 2009; 49: 1141-1150
2. Rijckborst et al., Hepatol. 2010; 52: 454-461
3. Sonneveld et al., Heaptol. 2013; 58: 872-880
4. Wiegand et al., Antiviral Ther. 2008; 13: 547-554
5. Moucari et al., Hepatol. 2009; 49: 1151-1157
6. Marcellin et al., Alimen Pharmacol Ther. 2016; 44: 957-966
7. Ahn et al., Dig Dis Sci. 2018; 63: 3487-3497
8. Wong et al., Liv Int. 2018; 38: 1760-1769
9. Jeng et al., J Viral Hep. 2018; 25: 421-428
10. Nagaoka et al., Hepatol Res. 2016; 46: E89-E99
11. Yano et al., Biomed Rep. 2017; 7: 257-262
12. Hall et al., J Hepatology 2020;73: S69
13. Choi et al., J Hepatology 2020; 73: S866
14. Farag et al., J Hepatology 2020; 73: S877
15. Bazinet et al., J Viral Hepatitis 2021: epub Feb 8
16. Liang et al., Ailment Pharmacol Ther. 2011; 34: 344-352
17. Chan et al., Antiviral Ther. 2011; 16: 1249-1257
18. Lee et al., Clin Mol Hepatol. 2016; 22: 382-389
19. Chen et al., J Viral Hep. 2018; 25: 590-597
20. Chevaliez et al., J Hepatol. 2013; 58: 676-683
21. Marcellin et al., J Hepatol. 2014; 61: 1228-1237
22. Marcellin et al., Gastroenterol. 2016; 150: 134-144
23. Brunetto et al., J Hepatol. 2013; 59: 1153-1159
24. Bazinet et al., Gastroenterology 2020; 158: 2180-2194
25. Al-Mahtab et al., PLoS One 2016; 11: e0156667
26. Bazinet et al., Lancet Gastro Hepatol 2017; 2: 877-889

Transaminase flares:
A key step in establishing functional cure

Transaminase flares are driven by hepatocyte death

ALT / AST: hepatocytes throughout the liver
GGT: hepatocytes lining the sinusoidal epithelium

Non-viral hepatitis (i.e. NASH): generalized loss of functional hepatocytes -> **reduced liver function (↑bilirubin, ↓albumin, ↑INR)**

Acute viral hepatitis: loss of functional hepatocytes with spread of infection -> **reduced liver function**

Chronic viral hepatitis: liver function acclimates and is maintained in steady state with chronic infection

flares signal immune mediated clearance of infected (non-functional) hepatocytes
no change in liver function
when viremia is suppressed, flares are always associated with improved virologic status

NUCs: HBeAg seroconversion, reduction of circulating HBsAg
pegIFN: SVP clearance, HBsAg seroconversion, functional cure

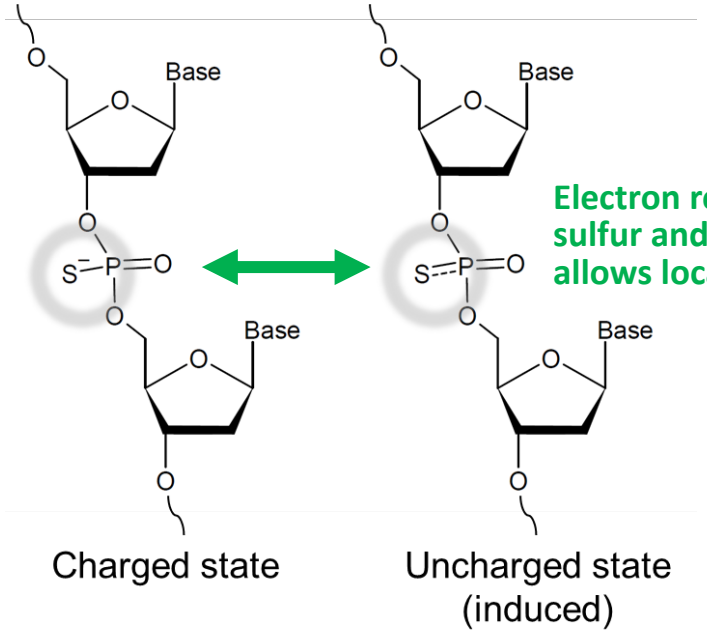
Vaillant, ACS Viruses 2021; 13: 745

Current clinical effects of investigative therapies for HBV

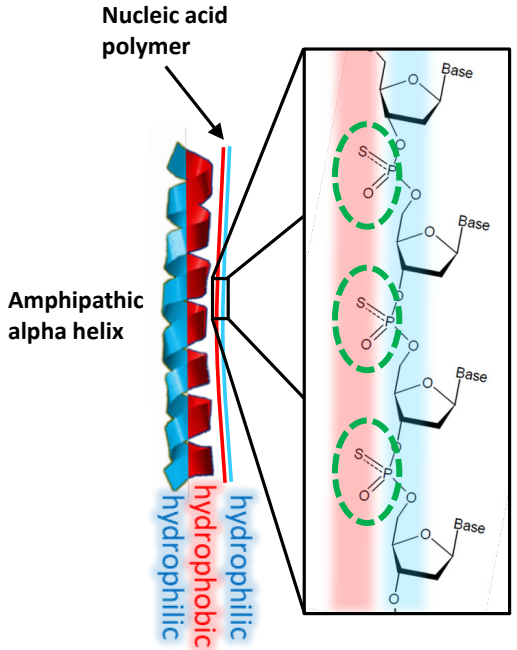
Therapy	Milestones during therapy			Outcome	
	Clearance of SVP	Removal of integrated HBV DNA (transaminase flares)	Silencing of cccDNA	Partial cure	Functional cure
NUCs	1% per year of therapy	Rare	**Yes** ETV and TDF / TAF also stimulate innate immunity	Rare	Rare
NUCs + pegIFN	6%	Rare In patients clearing SVP	**Yes**	~20%	9% Genotype A
RNAi	**No** Presence / evolution of escape mutants	Rare	**Yes** All dsRNA stimulates innate immunity via TLR3	?	**No**
Antisense	**No** Presence / evolution of escape mutants	**No**	**No**	**No**	**No**
CAMs	**No**	**No**	**No**	**No**	**No**
NAPs	**Yes**	**Yes**	**Yes**	**39%** (all genotypes)	**39%** (all genotypes)

Nucleic acid polymers (NAPs)

Oligonucleotides with sequence independent activity



Electron resonance between sulfur and phosphorous allows local hydrophobicity



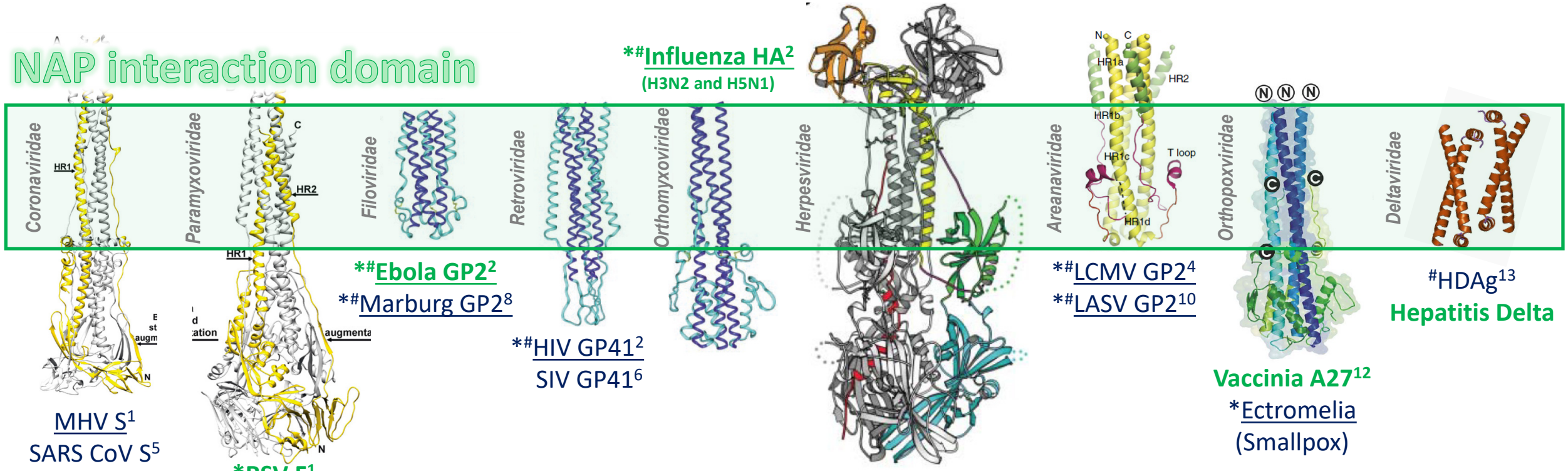
Electron resonance drives annealing of NAPs to the hydrophobic face of uncomplexed amphipathic alpha helices

Vaillant. ACS Inf Dis 2019; 10: 675-687

Broad-spectrum activity of NAPs in viruses with class 1 fusion proteins

Conserved amphipathic α-helices provide a common antiviral target for NAPs

NAP interaction domain



MHV S[1]
SARS CoV S[5]
MERS CoV S[10]
Human CoV S[11]

*RSV F[1]
SIV-5 F[6]
*PIV-3 F[7]

*#Ebola GP2[2]
*#Marburg GP2[8]

*#HIV GP41[2]
SIV GP41[6]

*#Influenza HA[2]
(H3N2 and H5N1)

No equivalent structures in viral proteins for HBV

*HSV gB[3]
*CMV gB[9]

*#LCMV GP2[4]
*#LASV GP2[10]

Vaccinia A27[12]
*Ectromelia
(Smallpox)

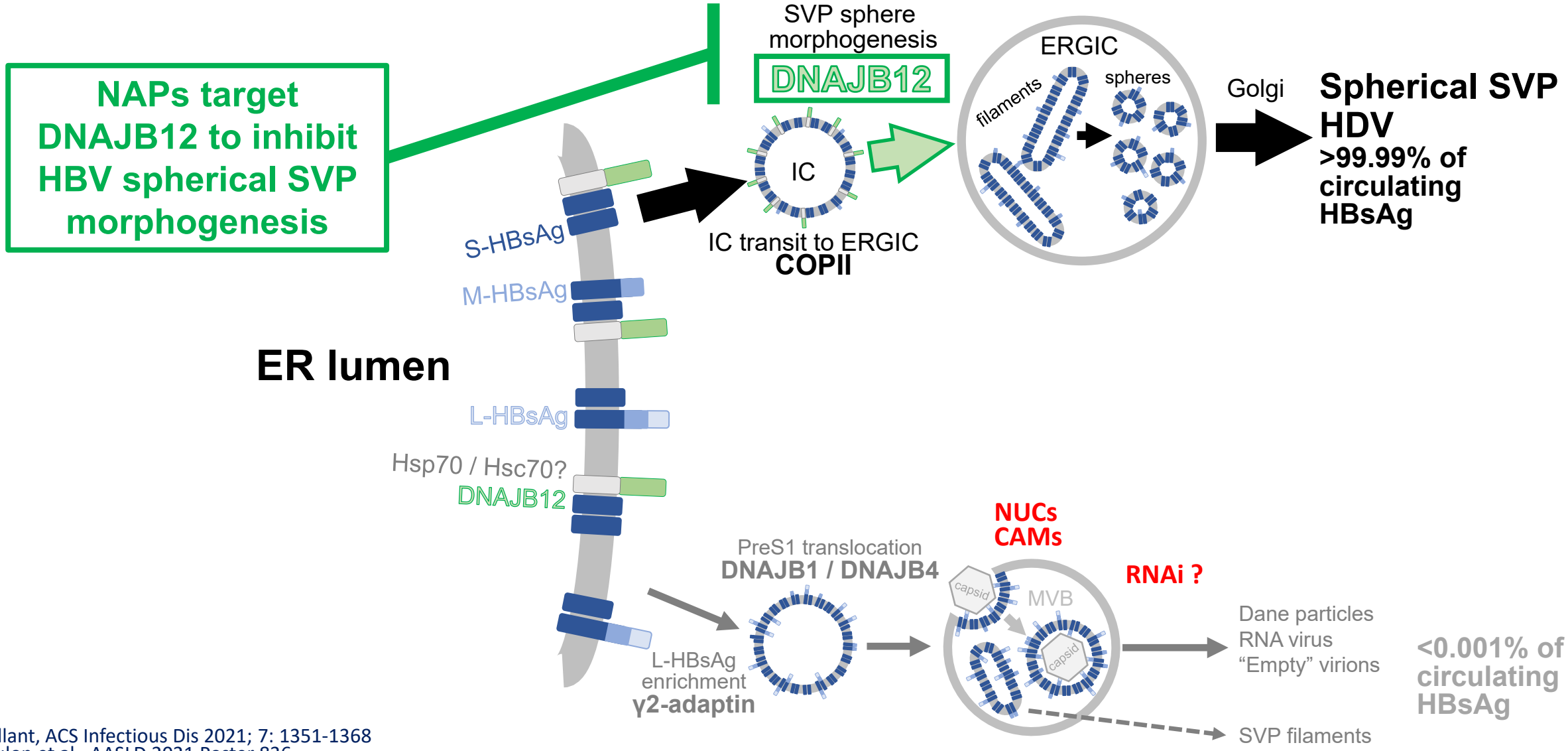
#HDAg[13]
Hepatitis Delta

Vaillant Antiviral Res 2016; 133: 32-40
Vaillant. ACS Inf Dis 2019; 10: 675-687

*SAR consistent with hydrophobic α-helical interaction with NAPs
#NAP/glycoprotein interaction validated
In vitro activity
***In vivo* activity – active against liver / lung / spleen viral infections**
(consistent with demonstrated accumulation of NAPs in liver, lungs and spleen)

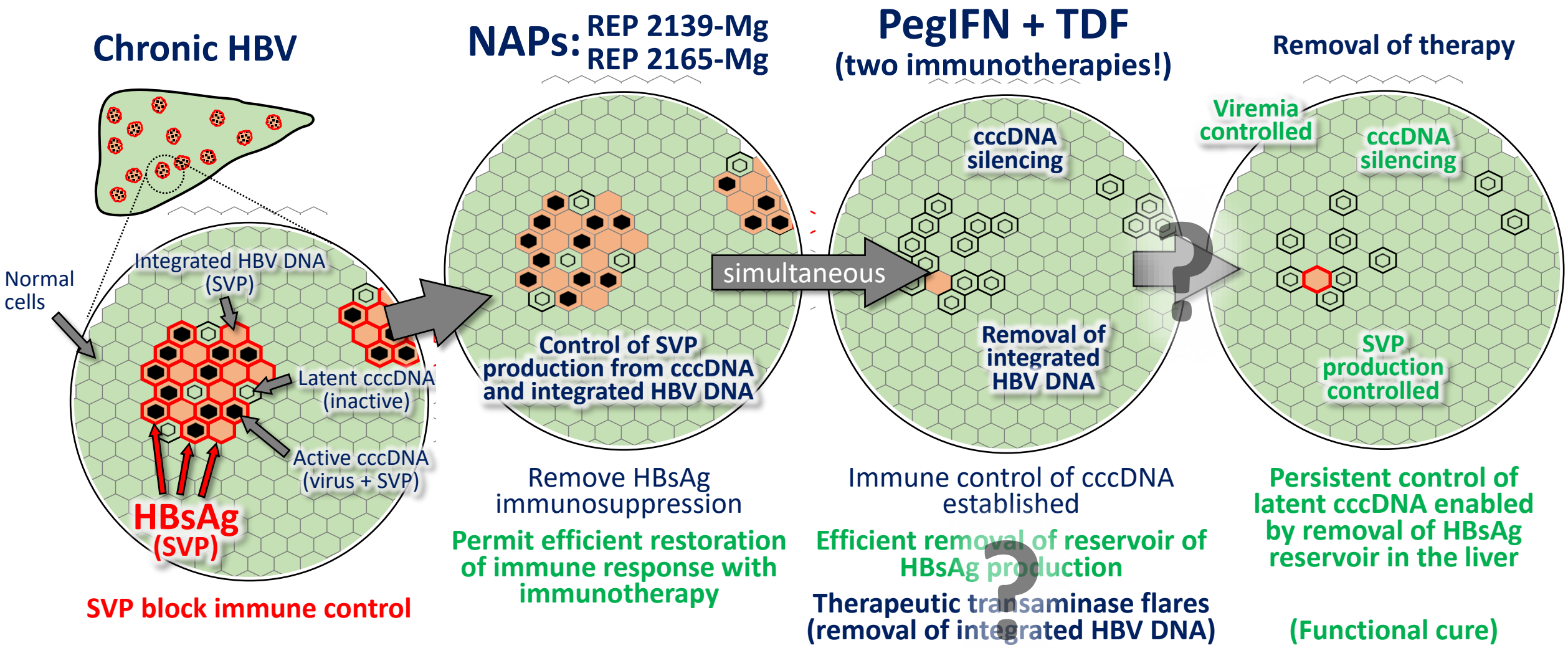
Crystal structures
1. Walls et al., PNAS 2017; 114: 11157-11162
2. Malashkevich et al., PNAS 1999; 96: 2262-2667
3. Heldwein et al., Science 2006; 313: 217-220
4. Hastie et al., Nat Struc Biol 2016; 23: 513-521
5. Lamb and Jardetzky Curr Op Struc Biol 2007; 17: 427-436
6. Eckert and Kim Annu Rev Biochem 2001; 70: 777-810
7. Smith et al., Prot Engineering 2002; 15: 365-371
8. Koellhoffer et al., Biochem 2012; 51: 7665-7675
9. Chandramouli et al., Nat Comm 2015; 6: 8176
10. Zhang et al., Front Microbiol 2019; 10: 1829
11. Tortorici et al., Nat Struct Mol Biol 2019; 26: 481-489
12. Chang et al., PLoS Pathogens 2012; 9: e1003563
13. Zuccola et al., Structure 1988; 6: 821-830

Nucleic acid polymers (NAPs): a unique molecular mechanism in HBV

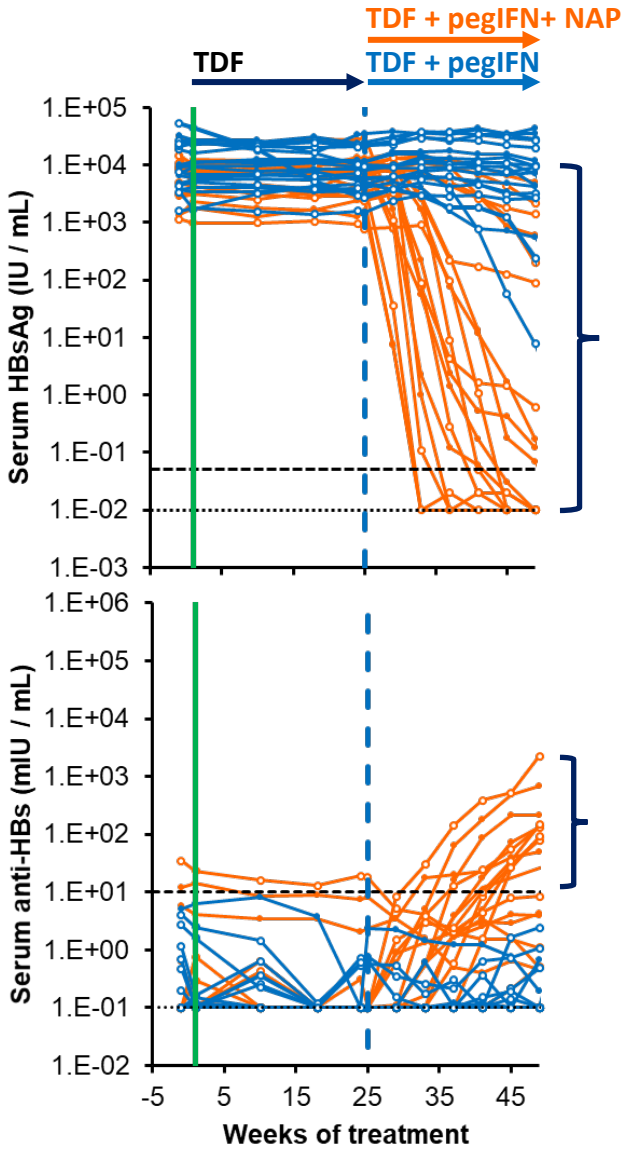


Vaillant, ACS Infectious Dis 2021; 7: 1351-1368
Boulon et al., AASLD 2021 Poster 836

REP 401 study:
Putting the pieces of the puzzle together



REP 401 study:
NAPs dramatically improve response to TDF + pegIFN



Up to 8 $\log_{10}$ decline from baseline (to < 0.005 IU/mL)
Potent HBsAg decline

Rapid HBsAg seroconversion

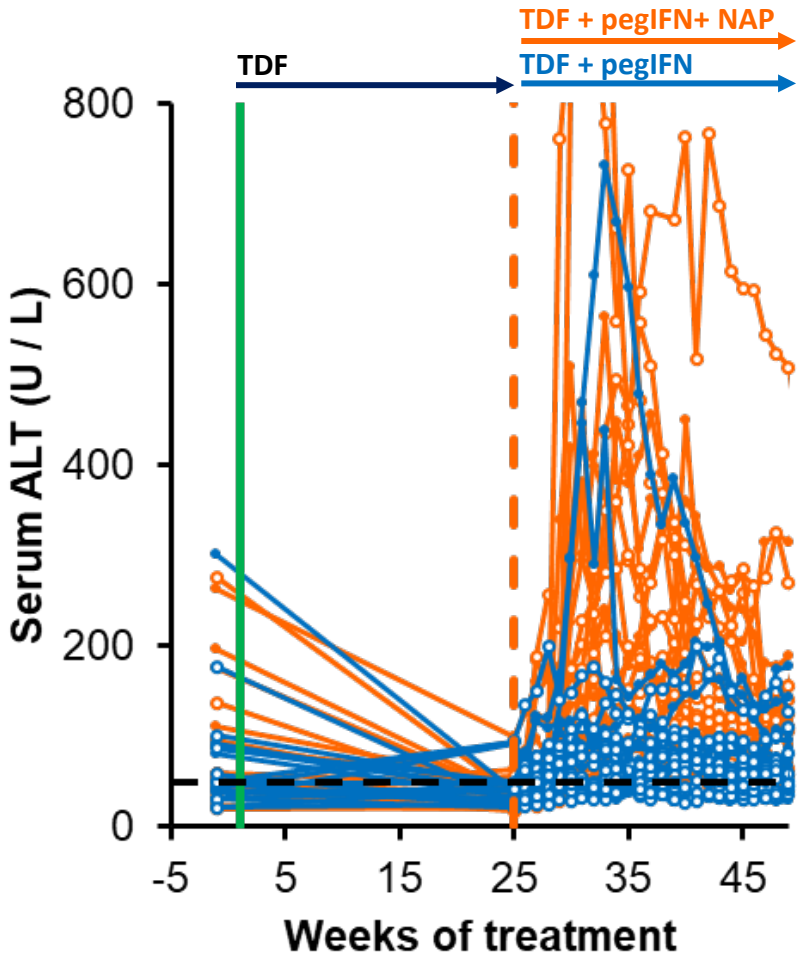
NAP monotherapy:
REP 2055 = REP 2139
Up to 7 $\log_{10}$ HBsAg reduction at 12 weeks
HBsAg seroconversion
Low rates of HBV functional cure

NAPs + TDF + pegIFN
HBsAg < 0.005 IU/mL (60%)
HBsAg seroconversion
Inactivation of cccDNA
Host mediated transaminase flares (95%)
High rates of HBV functional cure (39%)
No further therapy required in 78% of patients

GT D functional cure rate
TDF + pegIFN = 0%
(Marcellin et al, Gastroenterology 2016; 150: 134-144)
NAPs + TDF + pegIFN = 39%

Bazinet et al., Gastroenterol. 2020; 158: 2180-2194
Al-Mahtab et al., PLoS One; 2016; 11: e0156667

REP 401 study:
NAPs dramatically improve response to TDF + pegIFN



Dramatic increase in host mediated transaminase flares[1]

- occur in 95% of participants[2]
- no alteration in liver function / asymptomatic[2]
- correlated with functional cure (when HBsAg is also < 1 IU/mL)[2]
- Signals the removal of cccDNA and integrated HBV DNA[3]

Consistent with the safe and beneficial nature of host mediated transaminase flares during therapy with approved agents, even in cirhottics[4]

1. Bazinet et al., Gastroenterol. 2020; 158: 2180-2194
2. Bazinet et al., J Viral Hep 2021; 28: 817-825
3. Bazinet et al., Hepatol Comm 2021; 28: 817-825
4. Vaillant, Viruses 2021; 131: 745

REP 401 study:
Outcomes after removal of all therapy

Completed treatment and 24-48 weeks of follow-up		36
Clinical response	Normal ALT	89%
	Normal liver median stiffness	56%
HBsAg response	< 1000 IU/mL	72%
	< 1 IU/ml	50%
	≤ LLOQ (0.05 IU/mL)	42%
	Seroconversion	53%
HBV DNA response	≤ 2000 IU/mL	78%
	Target not detected (TND)	47%
Virologic response	Partial cure (Inactive HBV) (HBV DNA ≤ 2000 IU/mL, normal ALT)	39%
	Functional cure (HBsAg < LLOQ, HBV DNA TND, normal ALT)	39%
	Clinical benefit, no therapy required (low risk of progression, reduced risk of HCC)	**78%**

→ **Reversal of liver inflammation / fibrosis**

All with:
- **HBsAg < 0.005 IU/mL (ARCHITECT® NEXT)**
- **No HBsAg immunocomplexes**
- **HBV RNA target not detected**
- **HBcrAg < LLOQ**

Efficient silencing of cccDNA

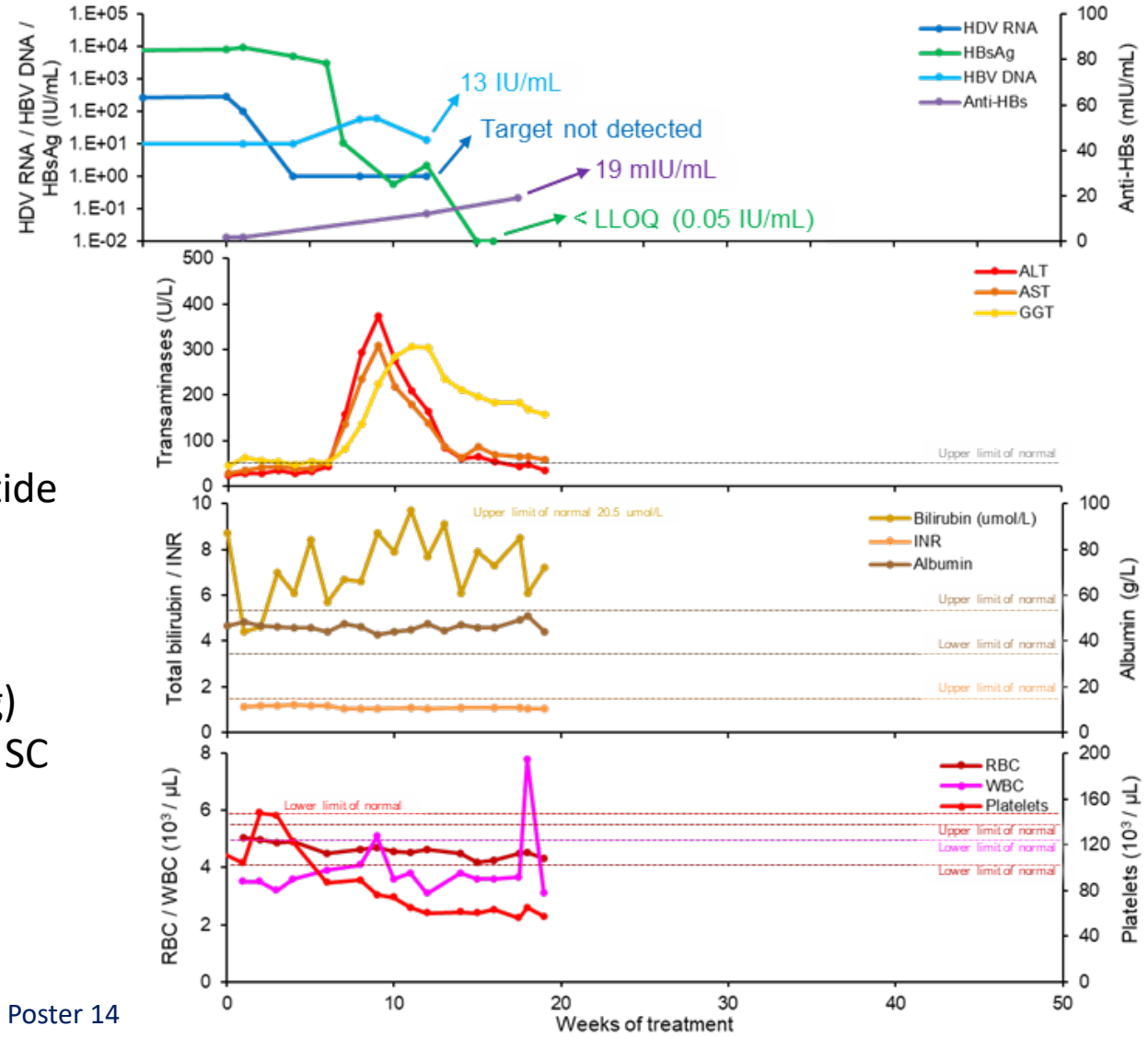
Removal of integrated HBV DNA

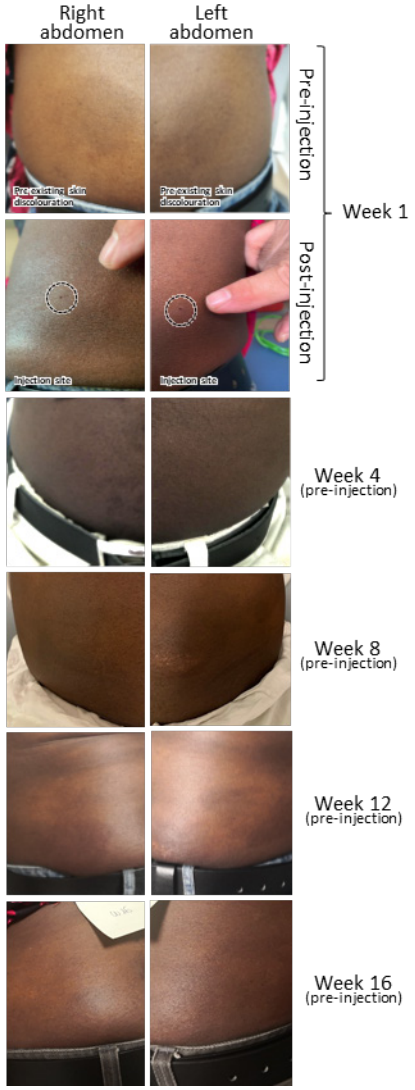
Bazinet et al., Gastroenterol. 2020; 158: 2180-2194
Bazinet et al., Hepatol Comm 2021, 5: 1873-1887

Transition of REP 2139-Mg to subcutaneous administration

Senegalese
51 years old male
HBV / HDV (GT3)
Previous failure on:
- TDF + pegIFN
- TDF + pegIFN + bulvertide

Currently receiving:
- TDF
- low dose pegIFN (90ug)
- REP 2139-Mg (250mg) SC (once every week)





Bourlière et al., AASLD 2021 Late Breaking Poster 14

Summary

Subviral particles (SVP): > 99.99% of circulating HBsAg
Prevent immune control and function of immunotherapy
Removal during therapy is essential for functional cure
Poorly targeted by direct acting antivirals (NUCs / CAMs / RNAi)

Key to Functional Cure

Integrated HBV DNA: Bulk of SVP production in HBeAg negative infection
HBsAg specific T-cell response is required to target efficiently
Therapeutic transaminase flares signal removal of integrated HBV DNA from the liver

Key to Functional Cure

NAPs: Efficient targeting of SVP production allows efficient, host-mediated clearance of HBsAg
Creates a permissive environment for efficient action of immunotherapy

T-cell focused immunotherapy is critical (pegIFN, thymosin alpha1)
When used in combination with NAPs:
high rates of asymptomatic host-mediated transaminase flares
high rates of functional cure, silencing of cccDNA and removal of integrated HBV DNA

Successful transition to SC administration