Understanding HBsAg and Subviral Particles:
Key players in chronic HBV infection
Key targets in achieving functional cure of HBV

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July 16, 2020

Online Research Seminars

Disclosures

Shareholder and employee, Replicor Inc.

Inventor on all Replicor assigned patents.

Objectives

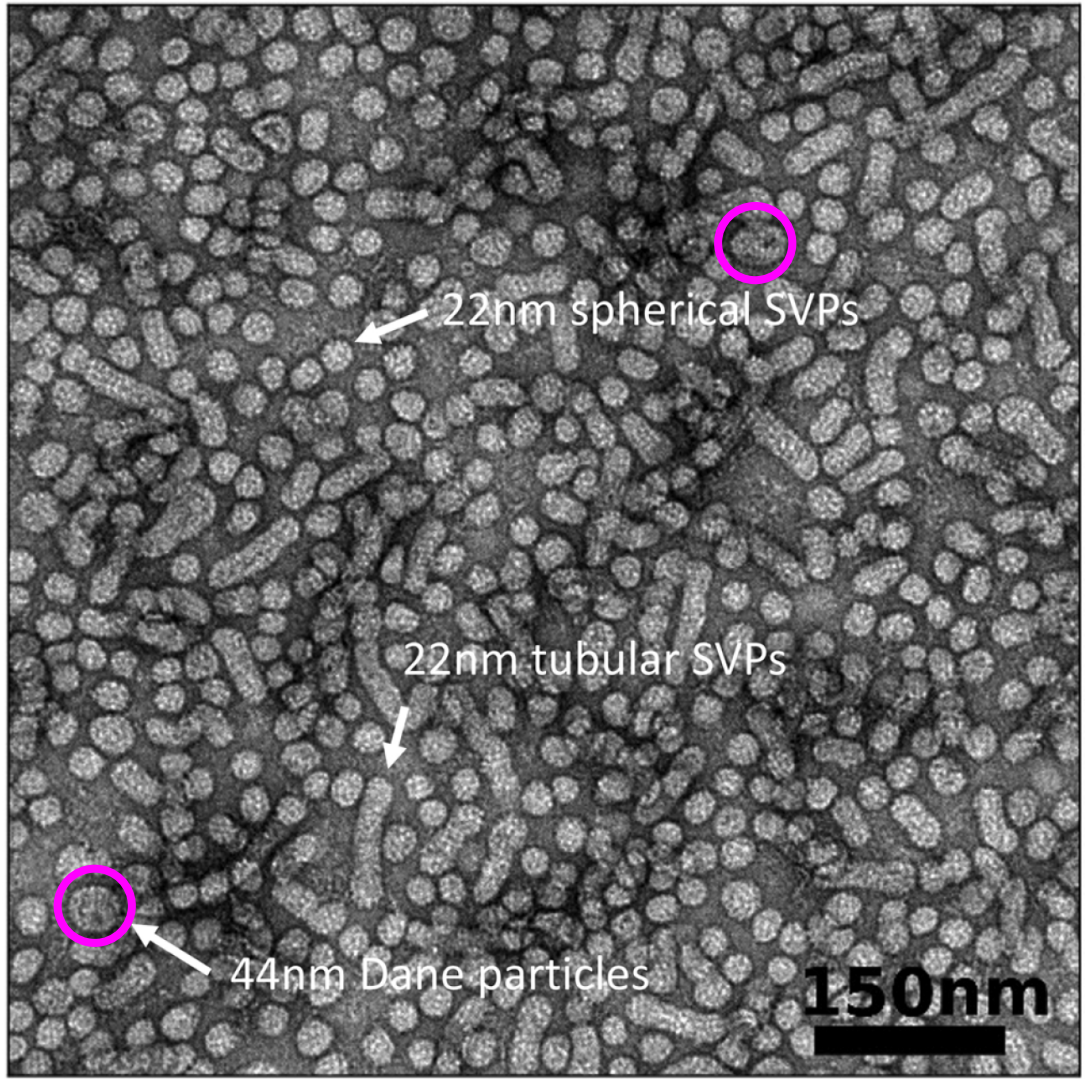
Understand the state of the art regarding particle production in HBV infection

Examine the impact of approved therapies on functional cure

Examine the ability of investigational therapies to target HBV subviral particles

Bring you up to date on nucleic acid polymers, their effects and clinical impact

What does HBV infection look like in the blood?



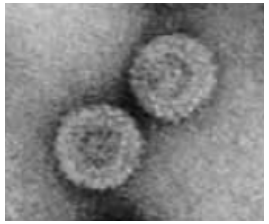
Infectious "Dane" particles comprise only a tiny fraction of circulating HBV particles

Cao et al., Vir Res. 2019; 259-90-96

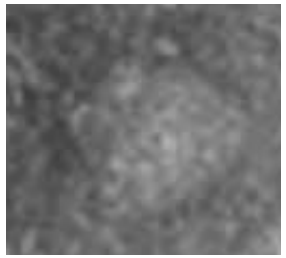
HBV particle morphology

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**

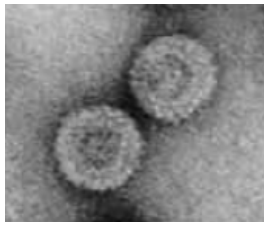


44nm



50-100 nm

(?)



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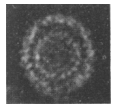

27nm

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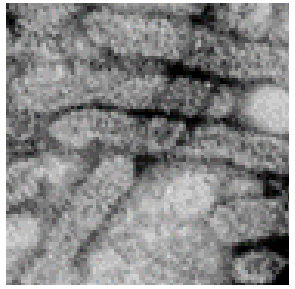
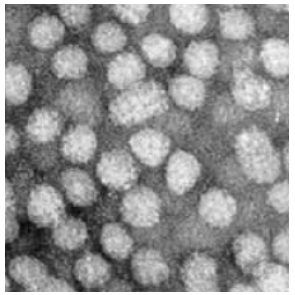
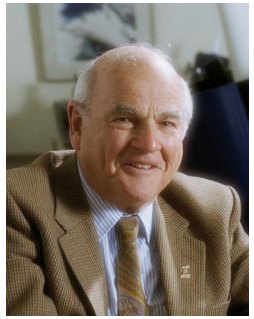


22nm



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

SVP were the first component of HBV discovered



Baruch Blumberg
Nobel Prize, Medicine
1976

Harvey Alter

A "New" Antigen in Leukemia Sera

The "Australia antigen" is found in the sera of some normal individuals from foreign populations. The total absence of the antigen from the sera of normal United States subjects and its relatively high frequency in acute leukemia suggests that the presence of the antigen may be of value in the diagnosis of early acute leukemia. Whether the antigen results from or precedes the leukemia process remains to be seen.

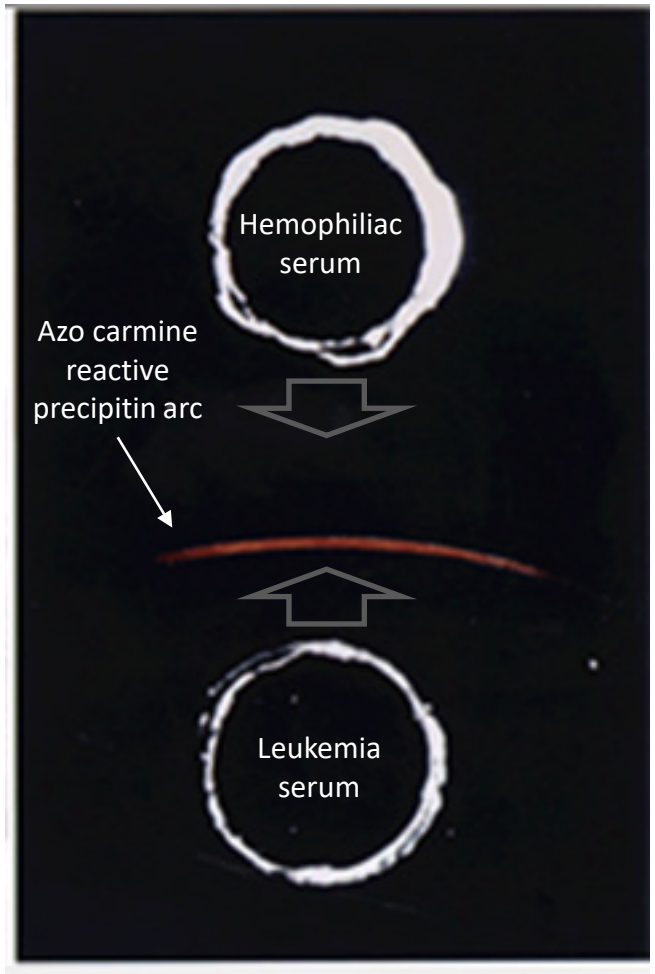
Baruch S. Blumberg, MD, Harvey J. Alter, MD, and Sam Visnich

Precipitin arc is driven by antigen-antibody precipitation between two serum samples

- Unusually reactive with azo carmine (protein)
  hemophiliac serum (resolved infection - HBsAg seroconversion)
  leukemia serum (chronic infection)
- First observed between hemophiliac serum and serum from an Australian aboriginal – the "Australian antigen"
- Most prevalent in hemophiliacs and Australian aboriginals

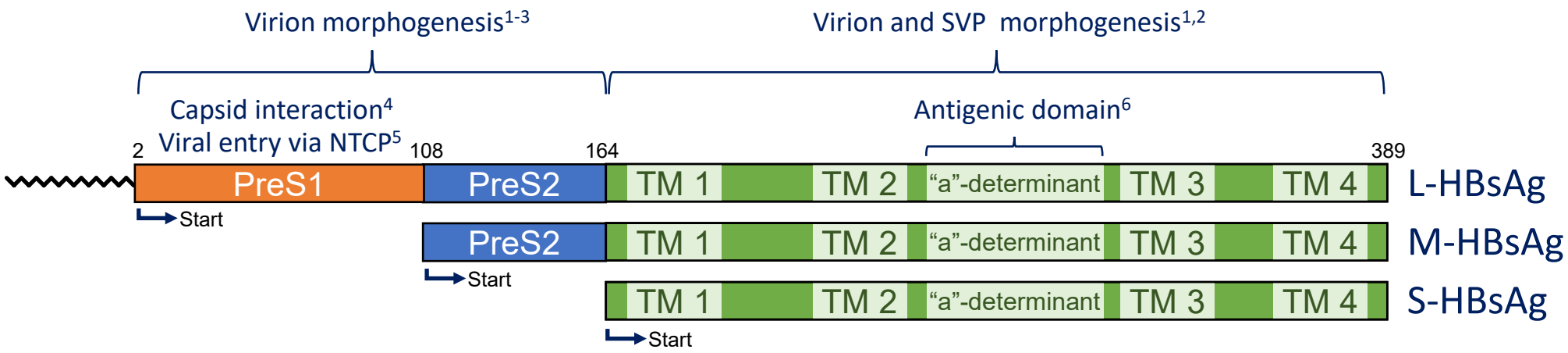
Antigen = HBsAg (SVP)!
Antibody = anti-HBs!

Ouchterlony double immunodiffusion

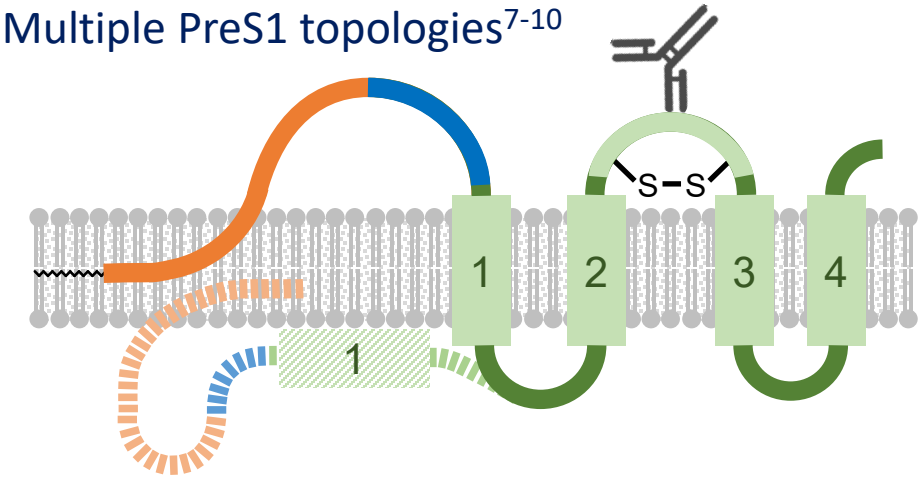


JAMA 1965; 191: 541-546

HBsAg isoforms and membrane interactions

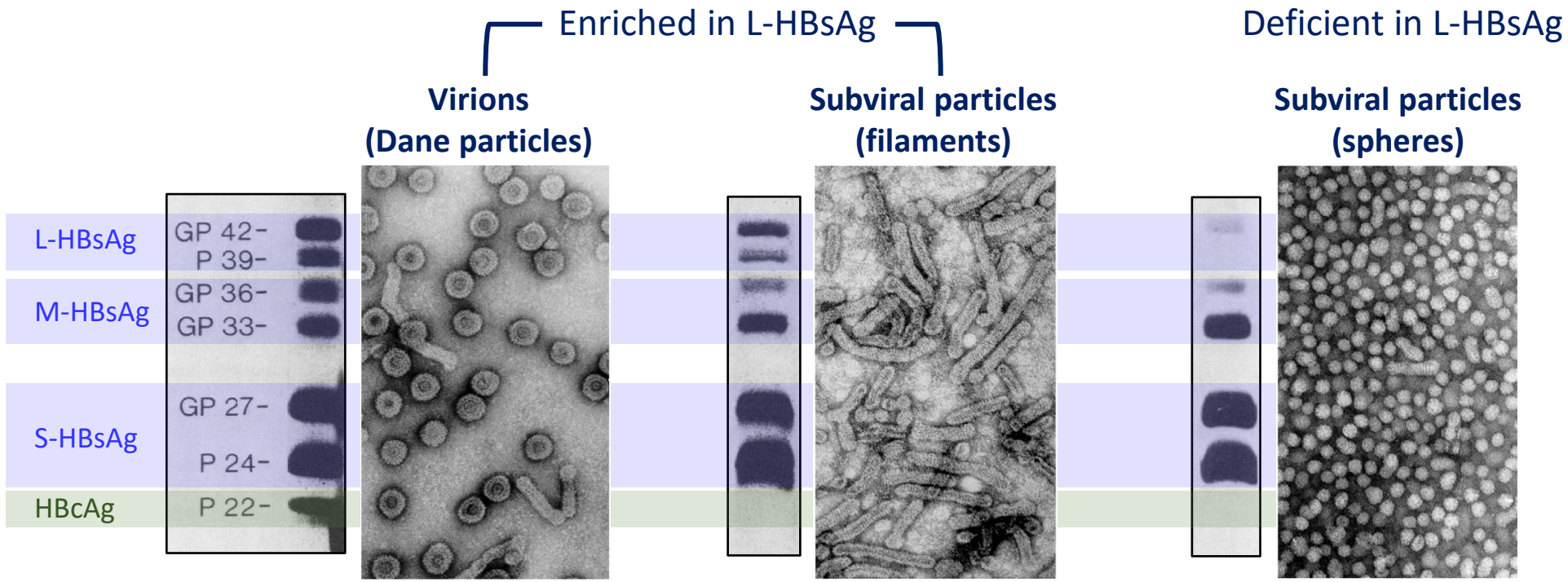


Multiple PreS1 topologies[7-10]



1. Bruss World J Gastro. 2007; 7: 65-73
2. Patient et al., Cell Microbiol. 2009; 11: 1561-1570
3. Prange et al., Biol Chem. 1999; 380: 305-314
4. Bruss, Virus Res. 2004; 106: 199-209
5. Yan et al., J Virol. 2014; 88: 3272-3284
6. Brown et al., The Lancet 1984; 28: 184-187
7. Stirk et al., Intervirol. 1992; 33: 148-158
8. Bruss et al., EMBO J. 1994; 13: 2273-2279
9. Prange and Streeck, EMBO J. 1995; 14: 247-256
10. Lambert and Prange, PNAS 2003; 100: 5199-5204

Particle production in HBV



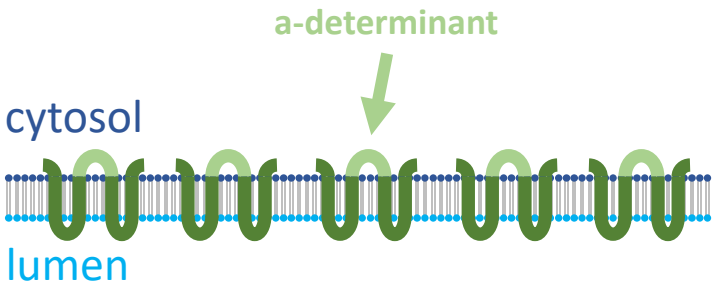
RATIO IN THE BLOOD **1** **10** **10,000 – 100,000**

Heermann et al., J Virol 1984; 52: 396-402
Ganem and Prince, N Engl J Med 2004; 350: 1118-1129
Gerlich, Virol J. 2013; 10: 239

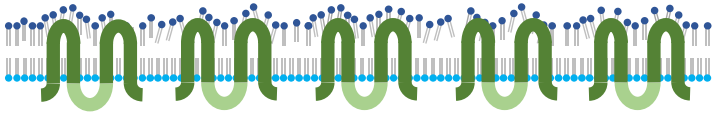
Lipid rearrangements in SVP

HBsAg translocation alters membrane structure in SVP

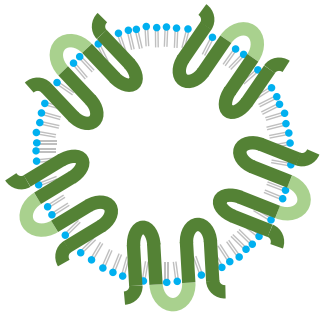
ER → **ERGIC** → **SVP**



HBsAg translocation[1-3]
lipid displacement



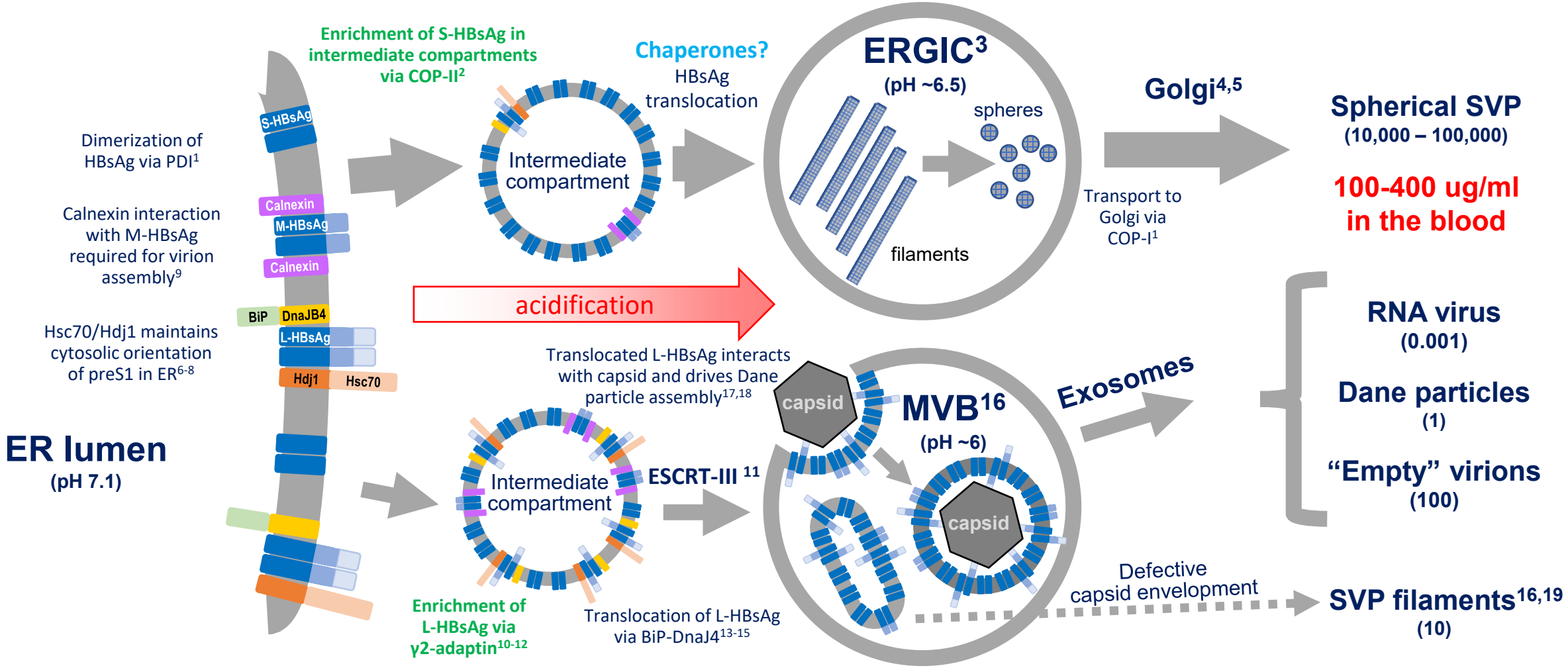
Cytosolic lipid shedding[4-6]



Reduced membrane fluidity[6]

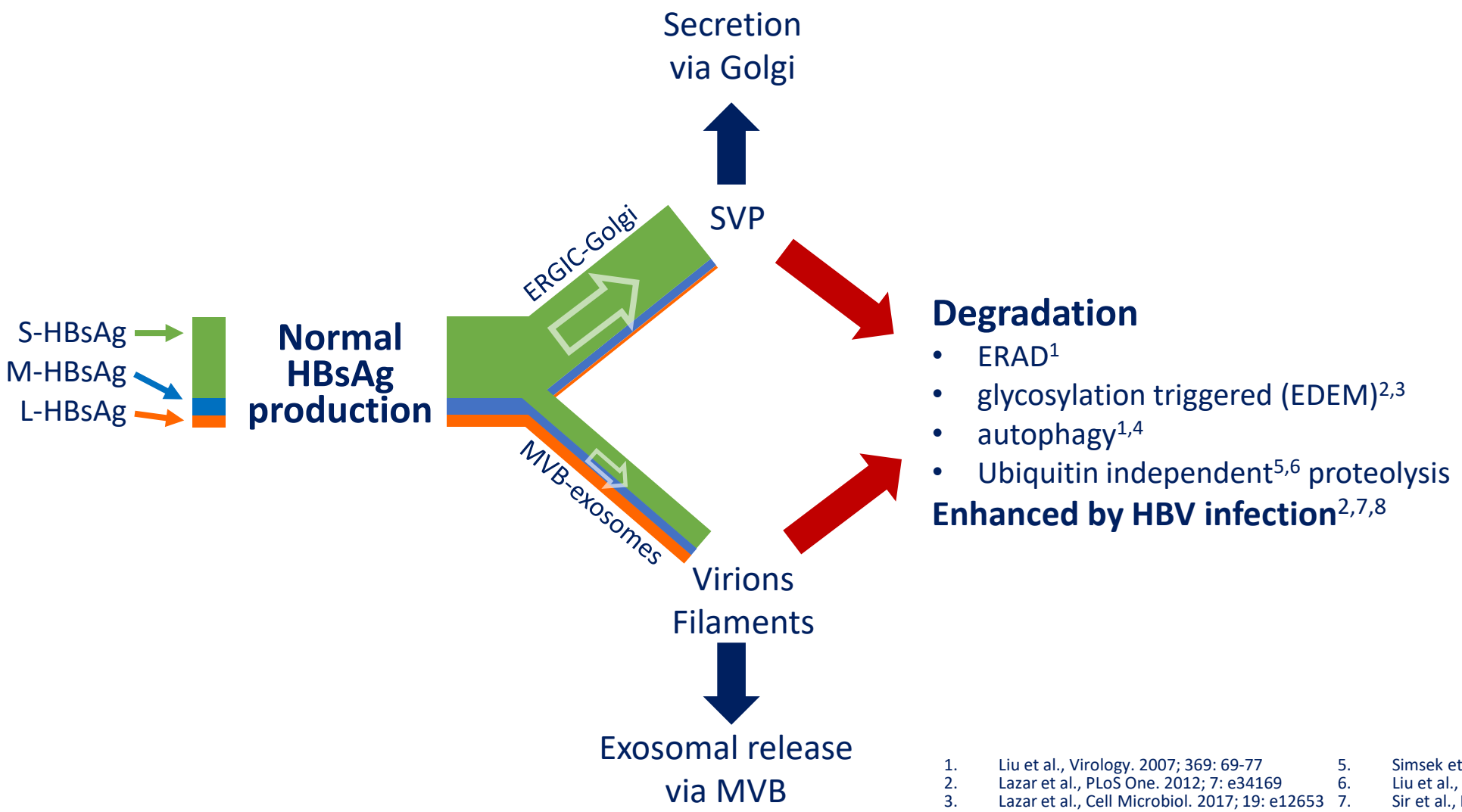
1. Simon et al., J Cell Biol. 1988; 107: 2163-2168
2. Eble et al., Mol Cell Biol. 1987; 7: 3591-3601
3. Eble et al., J Virol. 1990; 64: 1414-1419
4. Satoh et al., J Lipid Res. 1990; 31: 1293-1300
5. Sonveaux et al., Res Virol. 1995; 146: 43-51
6. Satoh et al., J Biochem. 2000; 127: 543-550

Discrete pathways of particle production in HBV



1. Huovila et al J Cell Biol 1992; 118:1305-1320
2. Zeyen et al., Cell Microbiol Epub Feb 4 2020
3. Patient et al., J Virol. 2007; 81: 3842-3851
4. Patzer et al., J Virol. 1984: 51: 346-353
5. Simon et al., Virology 1988; 166: 76-81
6. Prange et al., Biol Chem 1999; 380: 305-314
7. Lambert and Prange PNAS 2003; 100: 5199-5204
8. Wang et al., AAC 2010; 54: 2070-2177
9. Prange et al., Biol Chem. 1999; 380: 305-314
10. Hartman-Stuhler and Prange J Virol. 2001; 75: 5343-5351
11. Lambert et al., J Virol. 2007; 81: 9050-9060
12. Jurgens et al., Nat Chem Biol. 2013; 9: 540-547
13. Awe et al., FEBS Lett 2008; 582: 3179-3184
14. Ostapchuk et al., EMBO 1994; 13: 1048-1057
15. Bruss et al., EMBO 1994; 13: 2273-2279
16. Watanabe et al., PNAS 2007; 104: 10205-10210
17. Bruss, Virus Res. 2004; 106: 199-209
18. Pastor et al., Sci Rep. 2019; 9: 16178
19. Jiang et al., J Virol. 2016; 90: 3330-3341

Intracellular cycling of HBsAg



1. Liu et al., Virology. 2007; 369: 69-77
2. Lazar et al., PLoS One. 2012; 7: e34169
3. Lazar et al., Cell Microbiol. 2017; 19: e12653
4. Lin et al., Prot Cell. 2019; 10: 60-66
5. Simsek et al., J Virol. 2005; 79: 12914-12920
6. Liu et al., PLoS One. 2011; 9: e24477
7. Sir et al., PNAS 2010; 107: 4383-4388
8. Li et al., J Virol. 2011; 85: 6319-6333

Effects of HBsAg isoforms on hepatotoxicity

Increased production / accumulation of S-HBsAg

- No effect on morphogenesis / secretion[1-3]
- No observed hepatoxicity *in vivo*[1]

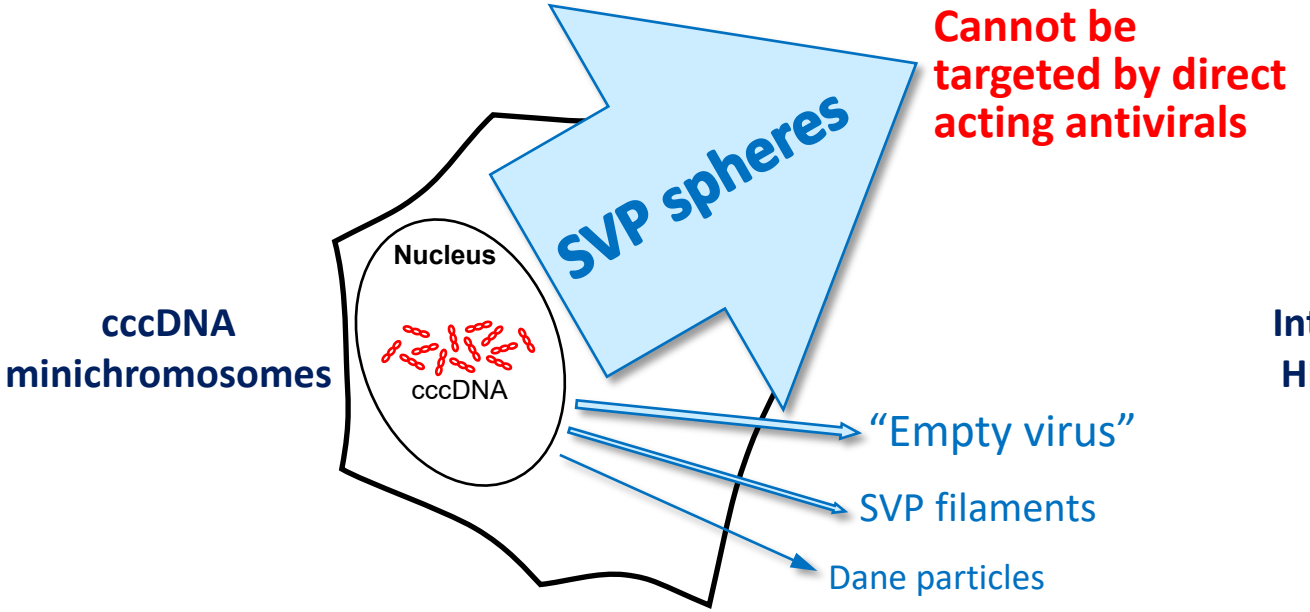
Increased production / accumulation of L-HBsAg

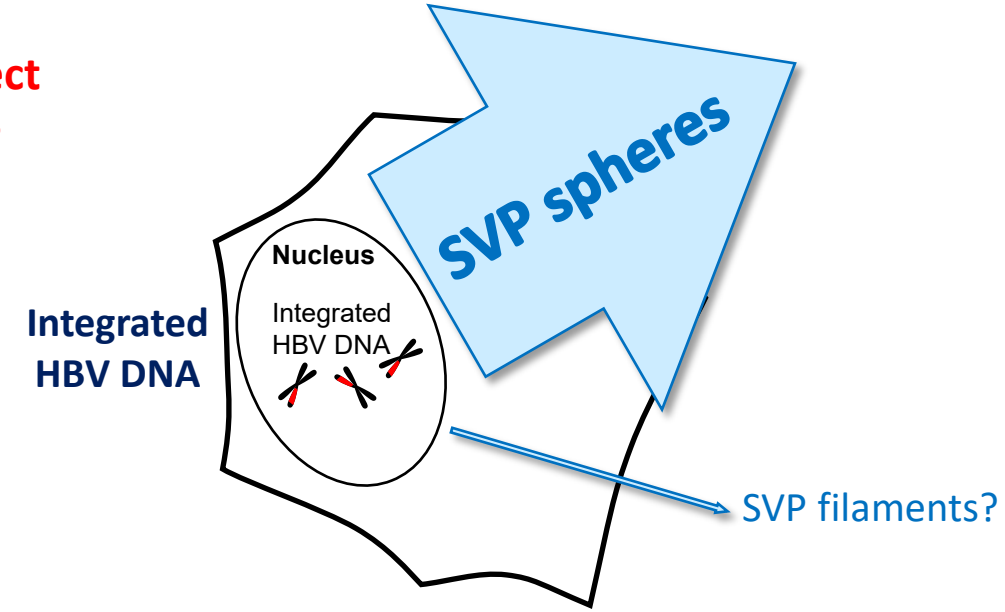
- Inhibition of SVP morphogenesis – intracellular accumulation of SVP filaments[1,4,5]
- Hepatotoxicity *in vivo* – ground glass hepatocytes, HCC[6-9]

1. Chisari et al., J Virol. 1986; 60: 880-887
2. Ou and Rutter, J Virol. 1987; 61: 782-786
3. Persing et al., Science 1986; 234: 1388-1391
4. Molnar-Kimber et al., J. Virol. 1988; 62: 407-416
5. Patient et al., J Virol. 2007; 81: 3842-3851
6. Chisari et al., PNAS 1987; 84: 6909-6913
7. Xu et al., J Virol. 1997; 71: 7387-7392
8. Chisari et al., Cell 1989; 1145-1156
9. Gilles et al., Hepatol. 1992; 16: 655-63

HBsAg production is difficult to target

Sources of circulating HBsAg





Occurs early in HBV infection[1]
Only produces HBsAg efficiently[1,2]
Plays a role in HCC[2,3]
Present in HBeAg positive and negative infection[4]
Cannot be targeted by direct acting antivirals
Cannot be targeted by innate immunity

1. Tu et al., Viruses 2017; 9: 75
2. Yang et al., J Cancer 2018; 9: 3225-3235
3. Podlaha et al., PLoS One 2019; 14: e0220376
4. Budinska et al., Emerg Microb Inf. 2018; 7: 142

(In)fidelity in viral replication

A commonly evolved mechanism for immune evasion by viruses?

Viral family	Genome and capsid free particles	Ratio to virus	Function
Herpesviridae (HSV)	L-particles, PREPs[1-4]	1000 - 10000	**Immune evasion**
Retroviridae (HIV)	Nef/Gag-containing exosomes[5,6]	1-10	**Immune evasion**
Hepadnaviridae (DHBV / HBV)	Subviral particles[7-10]	10,000 – 100,000	**Immune evasion**

1. Heilingloh and Krawczyk, Front Microbiol. 2017: 8: 2565
2. Kalamvoki and Deschamps, Virol J. : 63
3. Szilagyi and Cunningham, J Gen Virol. 1991; 72: 661-668
4. Dargan et al., J Virol. 1995;69: 4924-4932
5. Raymond et al., AIDS Res Hum Retrovir. 2011; 2: 167-178
6. Madison and Okeoma, Viruses 2015; 7: 4093-4118
7. Hu and Liu, Viruses 2017; 9: 56
8. Luckenbaugh et al., J Viral Hep. 2015; 22: 561-570
9. Ganem and Prince, N Engl J Med 2004; 350: 1118-1129
10. Gerlich, Virol J. 2013; 10: 239

Inhibition of immune function by SVP

HBsAg: the immunoinhibitory "Swiss Army Knife" of HBV

Inhibition of innate immunity[1-11]
Inhibition of monocyte and macrophage function[5,12,13]
Inhibition of dendritic cell function[3,4,14,15]
Inhibition of NK cell function[16]
Inhibition of HBV specific B-cell function[17,18]
Inhibition of HBV specific CD4+ T-cell function[19]
HBV specific T-cell tolerance[20-21]
HBsAg specific CD8+ T-cell exhaustion[22-25]
Sequester anti-HBs[26]

HBsAg displays all the characteristics of a multifunctional checkpoint inhibitor

May be driven by the plasma membrane altering properties of SVP[27,28]

1. Wu et al., Hepatol. 2009; 49: 1132-1140
2. Xu et al., Mol Immunol. 2009; 46: 2640-2646
3. Shi et al., PLoS One 2012; 7: e44900
4. Aillot et al., Antimicr Agents Chemoth. 2018; 62: e01741-17
5. Wang et al., J Immunol. 2013; 190: 5142-5151
6. Lebossé et al., J Hepatol. 2017; 66: 897-909
7. Xu et al., Biochem Biophys Res. 2016; 473: 219-233
8. Real et al., Sci Rep. 2016; 6: 24865
9. Jiang et al., J Viral Hep. 2014; 21: 860-872
10. Liu et al., J Hepatol. 2015; 62: 1015-1023
11. Zanetti et al., J Immunol. 2016; 197: 356-367
12. Vanlandschoot et al., J Gen Virol. 2002; 83: 1281-1289
13. Jochum et al., J Virol. 1990; 64: 1956-1963
14. Op den Brouw et al., Immunol. 2008; 126: 280-289
15. Woltman et al., PloS One 2011; 6: e15324
16. Yang et al., Int Immunopharmacol. 2016; 38: 291-297
17. Tout et al., J Immunol. 2018; 201: 2331-2344
18. Burton et al., J Clin Invest. 2018; 128: 4588-4603
19. Kim et al., Sci Rep. 2020; 10: 1835
20. Loriat et al., Int Immunol. 2003; 15: 1125-1136
21. Fang et al., J Immunol. 2015; 195: 4873-4883
22. Mueller and Ahmad, PNAS 2009; 106: 8623-8628
23. Wherry et al., Nat Rev Immunol. 2015; 15: 486-499
24. Reignat et al., J Exp Med. 2002; 195: 1089-1101
25. Le Bert et al., Gastroenterol. 2020 epub Apr 14
26. Rydell et al., Virology 2017; 509: 67-70
27. Simons and Toomre, Nat Rev Mol Cell Biol. 2000; 1: 31-39
28. Koberlin et al., Curr Op Cell Biol. 2016; 39: 28-36

Clinical impact of SVP clearance

With NUCs: HBsAg clearance <1% per year of therapy[1]

- more likely in GTA[2]
- associated with transaminase flares[3,4]
- allows withdrawal of NUC therapy with sustained virologic control or functional cure[5-8]

With pegIFN: HBsAg clearance ~6% with 48 weeks of therapy[9]
With pegFN + NUCs: ~10% with 48 weeks of therapy[9]

- more likely in GTA[9,10]
- more likely in HBeAg positive infection[9]
- virologic control more likely with lower baseline HBsAg[11]

- HBsAg loss on therapy predicts functional cure[12-15]
- Strong HBsAg response (> 3.5 $\log_{10}$ IU/mL from baseline) predicts functional cure[14]
- Poor HBsAg response (< 1 $\log_{10}$ IU/mL from baseline) predicts futility[16-18]

- Strong HBsAg declines / clearance are frequently accompanied by transaminase flares[19,20]

1. Chevaliez et al., J Hepatol. 2013; 58: 676-683
2. Marcellin et al., J Hepatol. 2014; 61: 1228-1237
3. Wong et al., Liv Int. 2018; 38: 1760-1769
4. Jeng et al., J Viral Hep. 2018; 25: 421-428
5. Liang et al., Ailment Pharmacol Ther. 2011; 34: 344-352
6. Chan et al., Antiviral Ther. 2011; 16: 1249-1257
7. Lee et al., Clin Mol Hepatol. 2016; 22: 382-389
8. Chen et al., J Viral Hep. 2018; 25: 590-597
9. Marcellin et al., Gastroenterol. 2016; 150: 134-144
10. Brunetto et al., J Hepatol. 2013; 59: 1153-1159
11. Wang et al., Sci Rep. 2016; 6: 29605
12. Wiegand et al., Antiviral Ther. 2008; 13: 547-554
13. Moucari et al., Hepatol. 2009; 49: 1151-1157
14. Marcellin et al., Alimen Pharmacol Ther. 2016; 44: 957-966
15. Ahn et al., Dig Dis Sci. 2018; 63: 3487-3497
16. Brunetto et al., Hepatol. 2009; 49: 1141-1150
17. Rijckborst et al., Hepatol. 2010; 52: 454-461
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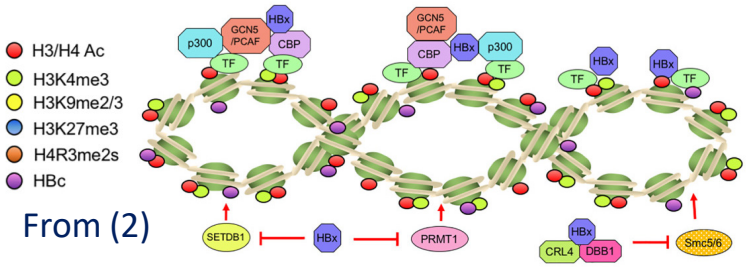
19. Nagaoka et al., Hepatol Res. 2016; 46: E89-E99
20. Yano et al., Biomed Rep. 2017; 7: 257-262

Latent cccDNA

cccDNA is chromatinized – the HBV minichromosome



From (2)

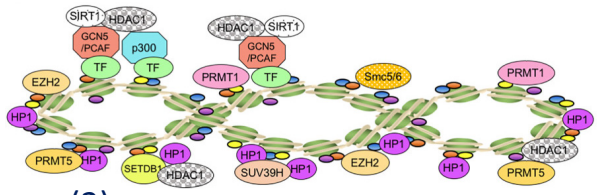
Euchromatic - uncondensed

Transcriptionally active[1,2]
Rapid turnover (1-4 weeks)[3,4]
Activity can be suppressed by innate immunity[5-9]

Epigenetically regulated condensation[8-10]

Transition[11]

HBx regulated decondensation[12,13]



From (2)

Heterochromatic – condensed - insoluble

Transcriptionally inactive[1,2]
Bound to nuclear scaffold[14]
Very slow turnover
Difficult to target

Barrier to sterilizing cure

Latent cccDNA persists in resolved[10] and occult[1] HBV infection.

Immunosuppression can reactivate HBV infection from functional cure[15-18]

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. Loomba and Jiang, Gastroenterol. 2017; 152: 1297-1309
16. Wang et al., Hematologica 2019; 104: 435-443
17. Zhang et al., J Immunother Canc. 2019; 7: 322
18. Kuo et al., Sci Rep. 2020; 10: 2456

Clinical impact of cccDNA inactivation / clearance

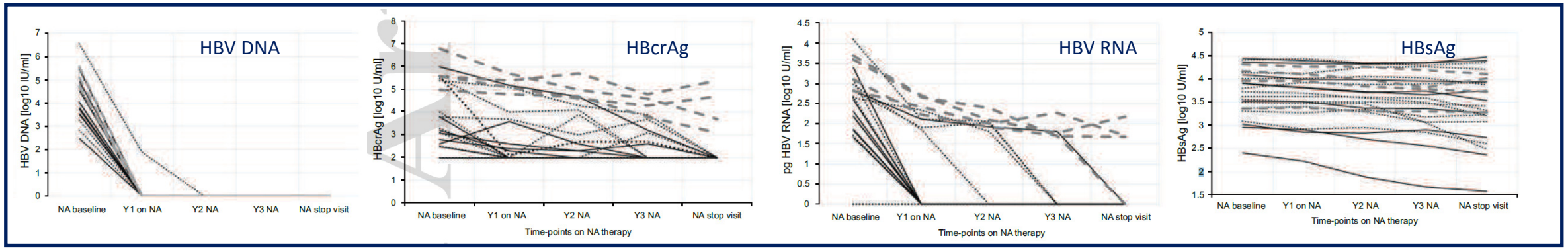
CAMs: potent effects on HBV RNA and HBV DNA - expected antiviral effects based on mechanism[1]

negligible effects on cccDNA to date[1-4] (HBcrAg)

what is the role of reinfection in cccDNA maintenance in humans (?)

NUCs: no direct effect on HBV RNA[5] but indirect effects on cccDNA via stimulation of innate immunity[6-13]

Example of effects during TDF[14]



HBsAg persists during NUC therapy despite cccDNA inactivation/clearance[14, 15]

NUC removal with undetectable cccDNA but persistent HBsAg leads to rapid viral rebound in humans[15]

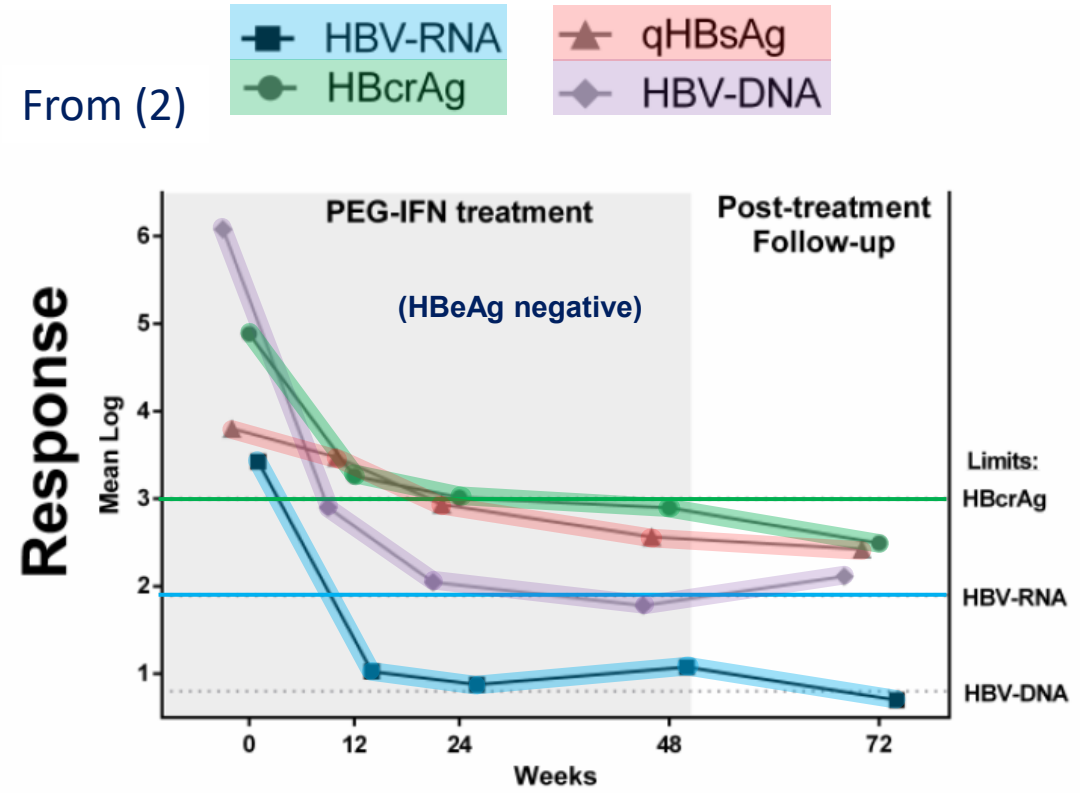
Reactivation of latent cccDNA made possible by persistently circulating HBsAg?

1. Lahlali et al., Antimicrob Agents Chemother. 2018; 10: e00835-18
2. Yuen et al., Gastroenterol. 2019; 156: 1392-1403
3. Yuen et al., Lancet Gastro Hepatol. 2020; 5: 152-166
4. Zoulim et al., Gastroenterol. 2020 epub Apr 25
5. Lam et al., Antimicrob Agents Chemother. 2017; 61: e00680-17
6. Melchjorsen et al., J Acquir Immune Defic Sndr. 2011; 57: 265-275
7. Kmoníčková et al., Eur J Pharmacol. 2006; 530: 179-187
8. Potměšil et al., Eur J Pharmacol. 2006; 540: 191-199
9. Lee et al., PNAS 2003; 100: 6646-6651
10. Shibata et al., Int Immunol. 2016; 28: 211-22
11. Davenne et al., Eur J Immunol. 2020; 50: 56-62
12. Murata et al., Gut 2018; 67: 362-371
13. Kurihara et al., Antiviral Ther. 2018; 23: 239-248
14. Carey et al., Hepatol. 2019 epub Nov 7
15. Lai et al., JHEP Rep. 2020; 2: 100112

Clinical impact of cccDNA inactivation / clearance

PegIFN: direct effect on cccDNA via stimulation of innate immunity[1]

From (2)



Early declines in HBV RNA and HBcrAg to < LLOQ

HBsAg response disconnected from cccDNA response
(HBV DNA, HBV RNA and HBcrAg)

HBsAg persistence, HBV DNA rebound in follow-up
Virologic control but not functional cure

1. Belloni et al J Clin Invest. 2012; 122: 529-537
2. Farag et al., Clin Inf Dis. 2020; epub Jan 8

Approaches to targeting SVP
(from cccDNA and integrated HBV DNA)

GalNAc RNAi / antisense

GalNAc –ASGPR driven hepatocyte delivery[1,2], designed to target cleavage of HBV RNA from cccDNA or integrated HBV DNA
RNAi: monthly SC injection, antisense: weekly SC injection, **injection site reactions in 15-27% of participants[3,4]**

But be careful with interpretation of effects in humans…..

Target RNA cannot be completely degraded (a known pharmacologic limitation of RNAi and antisense[5])

Single point mutations prevent targeting by antisense or RNAi[6]

- **numerous preexisting quasispecies and rapid cccDNA turnover favor the rapid expansion of escape mutations**
- **not modelled in *in vitro* or *in vivo* systems**

Cleavage of NAc sugar from GalNAc occurs early after administration

- **allows accumulation in Kupffer cells (TLR reactive)**

dsRNA are immunostimulatory

- reactivity is sequence independent
- impossible to completely remove without blocking RISC loading (RNAi effect)[7]
- **includes activation of TLR3, RIG-I, MDA5 and NLRX-1[8-11]**

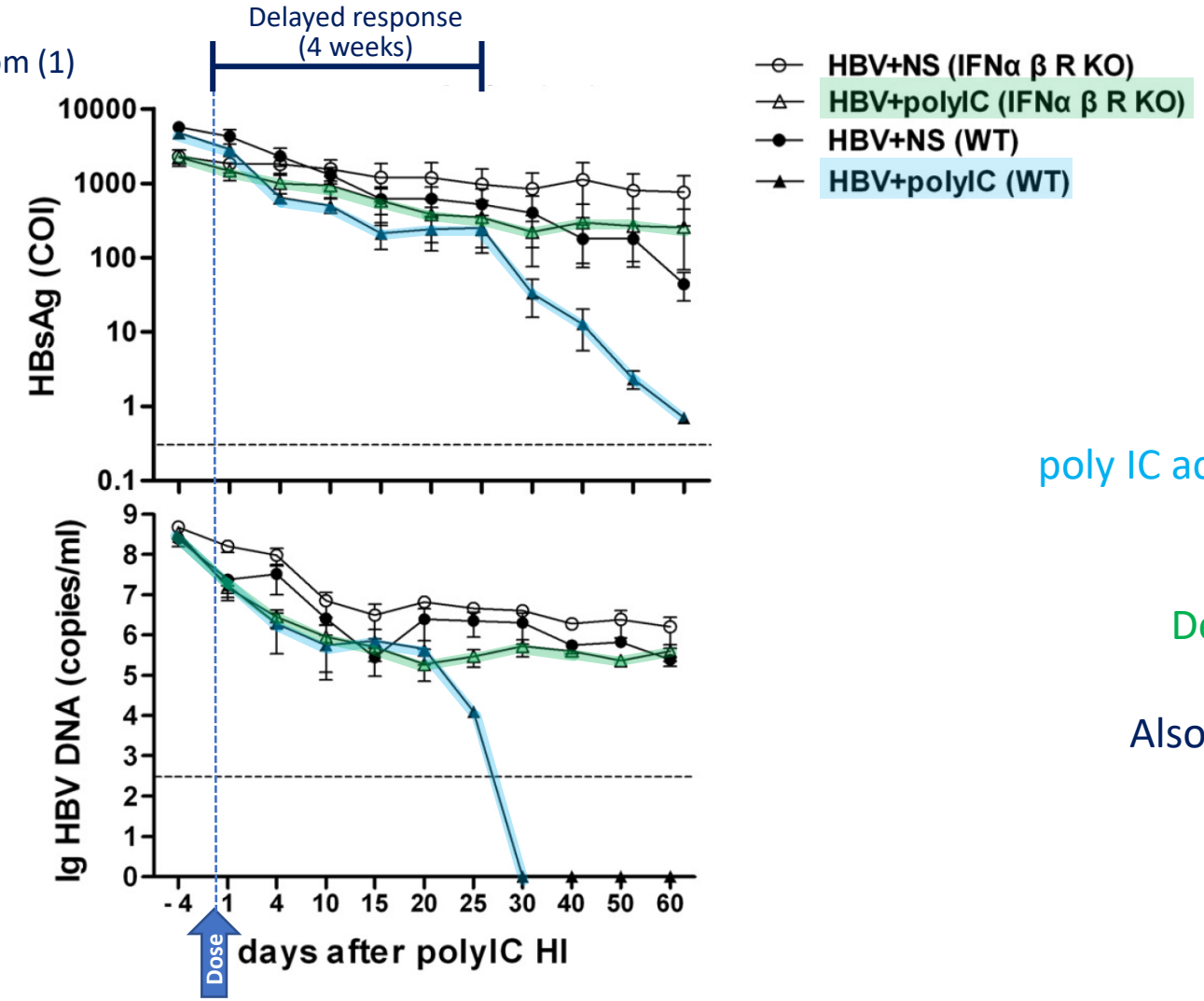
TLR3 stimulation has antiviral activity in HBV[12-15]

1. Prakash et al., Nuc Acid Res. 2014; 42: 8796-8807
2. Nair et al., J Am Chem Soc. 2014; 136: 16958-16961
3. Springer and Dowdy, Nuc Acids Res. 2018; 28: 109-118
4. Tsimikas et al., NEJM 2020; 382: 244-255
5. Dowdy, Nat Biotech. 2017; 35: 222-229
6. Vickers et al., J. Biol. Chem. 2003; 278: 7108-7118
7. Leuschner et al., EMBO Reports 2006; 7: 314-320
8. Zhou et al., Innate Immun 2013; 19: 184-192
9. Hong et al., Immunity 2012; 36: 337-347
10. Meng and Lu, Front. Immunol. 2017; 8: 331
11. Robbins et al., Hum. Gene Ther. 2008; 19: 991-999
12. Wu et al., Hepatol. 2007; 46: 1769-1778
13. Real et al., Sci Rep. 2016; 6: 24865
14. Zhang et al., Front Immunol. 2018; 9: 2921
15. Lucifora et al., Sci Rep. 2018; 8: 5390

Antiviral effects of TLR-3 activation by dsRNA on HBV

Poly I:C – a dsRNA with sequence independent activation of TLR-3

From (1)



HDI HBV mouse model

poly IC achieves multilog reduction in HBsAg and HBV DNA in mice[1]

Dependent on interferon pathway

Also observed in DHBV infected ducks[2]

1. Wu et al., J Virology 2014; 88: 10421-10431
2. Ijichi et al., J Med Virol. 1994; 43: 161-165

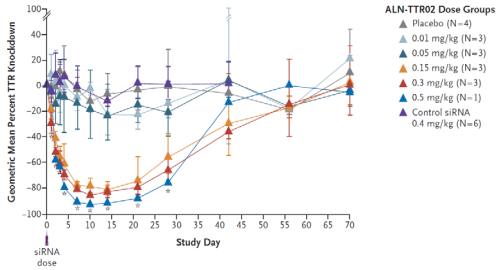
The consistent pharmacological signature of RNAi

Liver targets

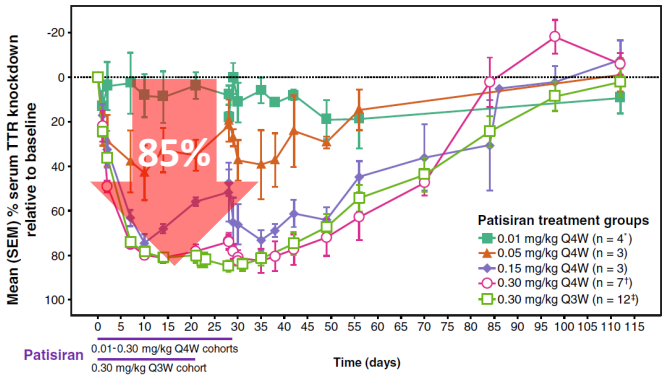
Saturation at 0.15-0.30 mg/kg/month (LNP siRNA - Patirisan)

Coelho et al., NEJM 2013; 369: 819-829
Suhr et al., Orphanet J. Rare Dis. 2015; 10: 109

Single dose



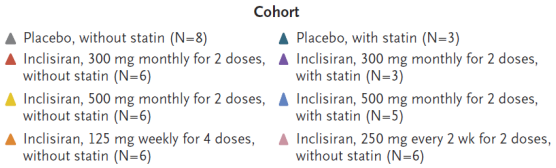
Multi dose



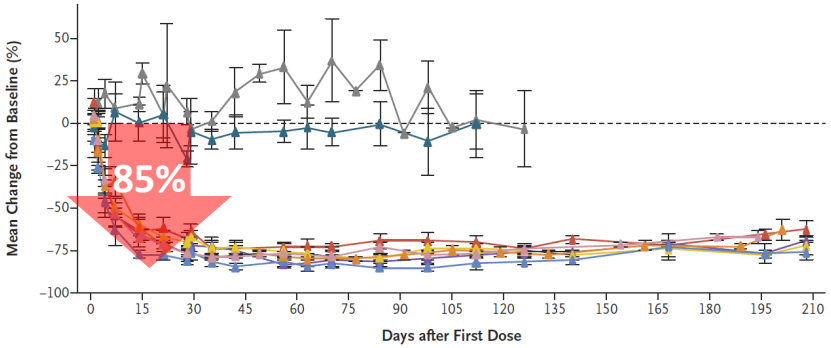
Transthyretin
(amyloidosis)

Saturation at 125mg/week (GalNAc siRNA - Inclisiran)

Fitzgerald et al., NEJM 2016; 376: 41-51



Multi dose



PCKS9
(hypercholesterolemia)

RNAi achieves rapid 1 $\log_{10}$ reduction in protein within 2 weeks

Reduction is saturated– even with repeated dosing

Target RNA can not be completely cleared with RNAi approach

JNJ-3989 (ARO-HBV)

Two GalNAc-RNAi compounds (X + S triggers) + NUC (ETV) + CAM (JNJ-6379)

LP4 – AASLD 2019

HBsAg response during 1st 4 weeks

- absent (7/12 participants)
- << 1 $\log_{10}$ from baseline (12/12 participants)

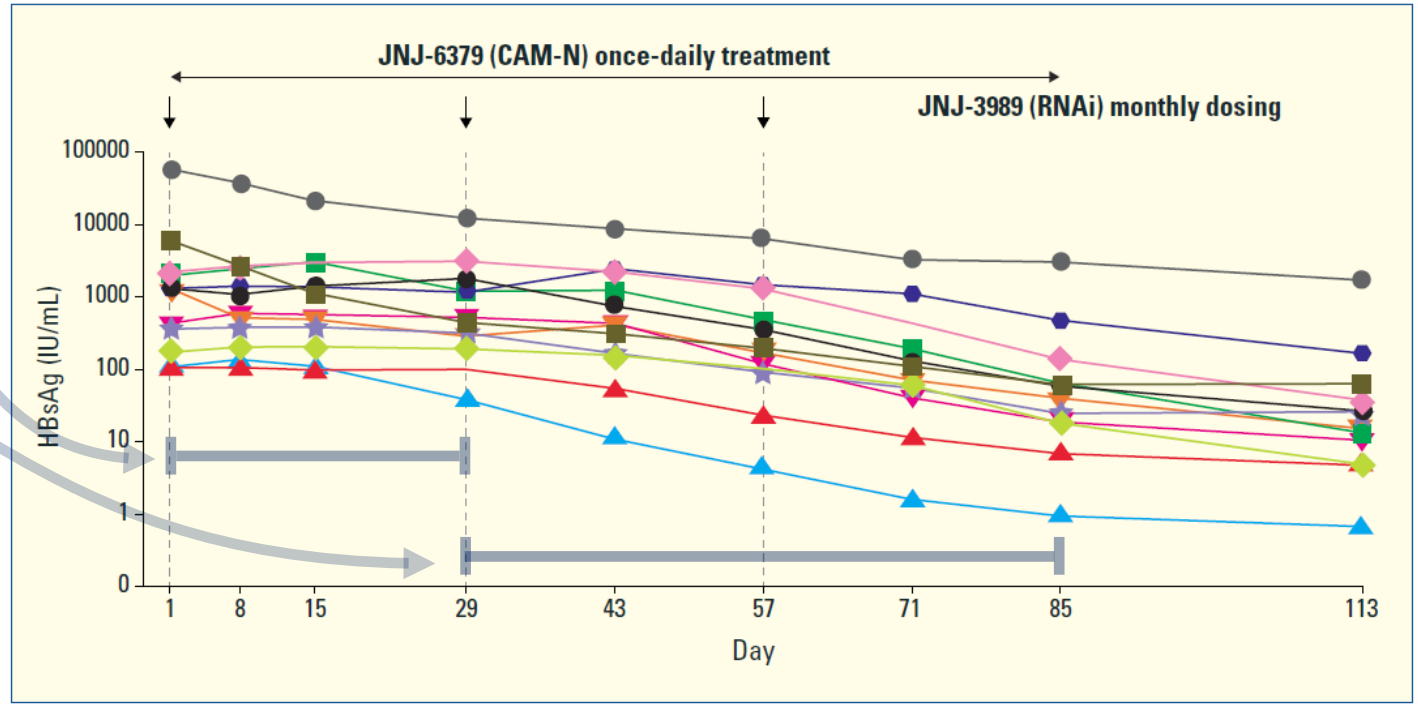
Delayed HBsAg response > 4 weeks

- 12/12 participants

Inconsistent with RNAi effect
HBsAg turns over rapidly (½ life 1-6 days)[1,2]

Consistent with TLR3 stimulation
observed with poly I:C in mice

Figure 1: Individual Changes in HBsAg Levels over Time with JNJ-3989, JNJ-6379 and NA Treatment.



Observation of upper respiratory tract infections consistent with TLR3 stimulation in the lung[3-5]

Also observed for ARC-520 (LNP), ARO-AAT (GalNAc) and ALN-AAT (GalNAc)

Increased cytokine responses consistent with TLR3 activation observed with ARC-520[6-9]

1. Loomba et al., Clin Inf Dis. 2019; 69: 542-545
2. Shekhtman et al., Sci Rep. 2020; 10: 7837
3. Sköld et al., Blood 2012; 120: 768-777
4. Murray et al., Am J Resp Care Med. 2008; 178: 1227-1237
5. Stowell et al., Respir Res. 2009; 10: 43
6. Schluep et al., Clin Pharmacol Drug Dev. 2017; 6: 350-360
7. Yang et al., Am J Heart Circ Physiol 2006; 291: H2334-H2343
8. Dia et al., FASEB J 2015; 29: 4978-4988
9. Kumar et al., Immunology 2005; 117: 11-21

VIR-2218 (ALN-HBV02)

One GalNAc-RNAi compound (X trigger)

Vir SEC filing March 2020

HBsAg response during 1st 4 weeks

- Absent (1/6 participants)
- << 1 log_{10} from baseline (4/6 participants)

Delayed HBsAg response > 4 weeks

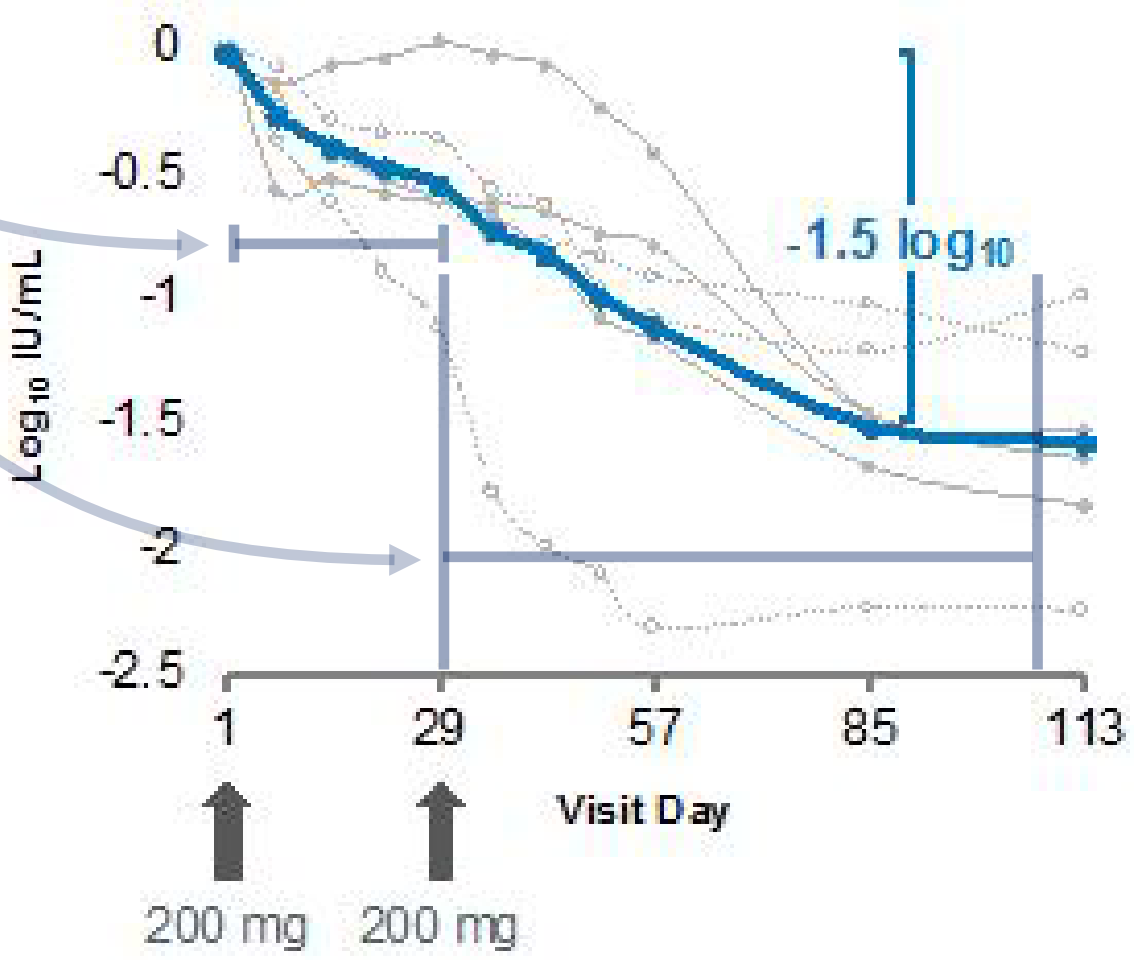
- 6/6 participants

HBsAg response inconsistent with RNAi mechanism

HBsAg response consistent with TLR 3 simulation

Grade 3 upper respiratory tract infection reported!
(likely TLR3-mediated)

HBsAg reduction from baseline



AB-729

One GalNAc-RNAi compound (X trigger)

Arbutus website

http://www.arbutusbio.com/portfolio/ab-729-galnac-rnai.php

HBsAg response during 1st 4 weeks

- Absent (3/6 participants)
- << 1 $\log_{10}$ from baseline (2/6 participants)

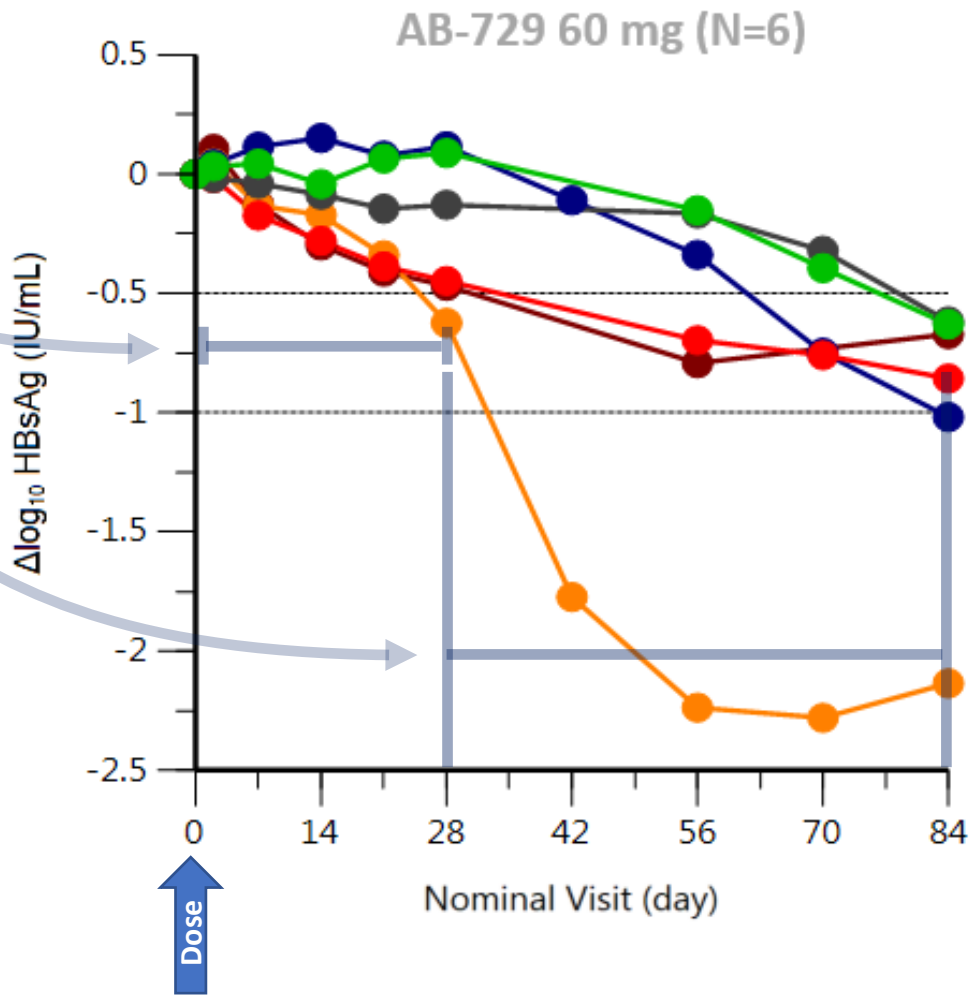
Delayed HBsAg response > 4 weeks

- 6/6 participants

HBsAg response inconsistent with RNAi mechanism

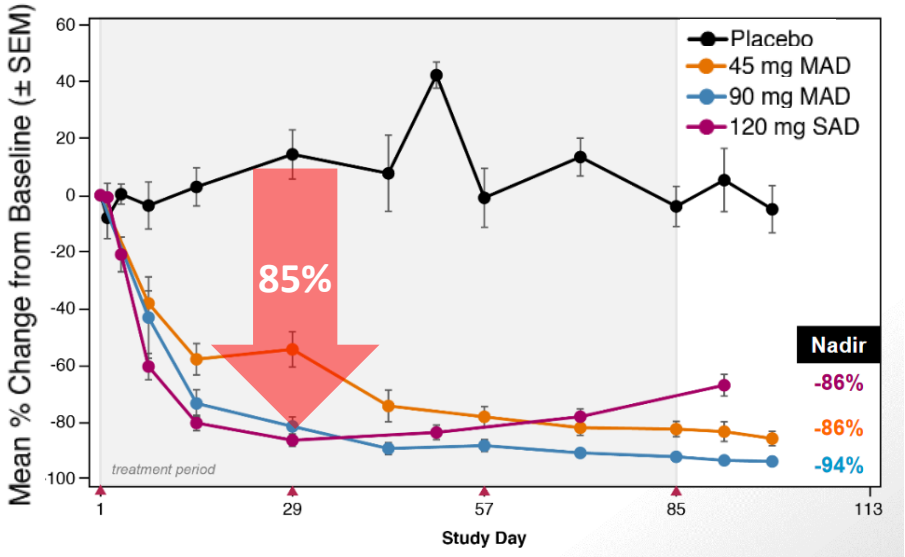
HBsAg response consistent with TLR3 stimulation

HBsAg reduction from baseline



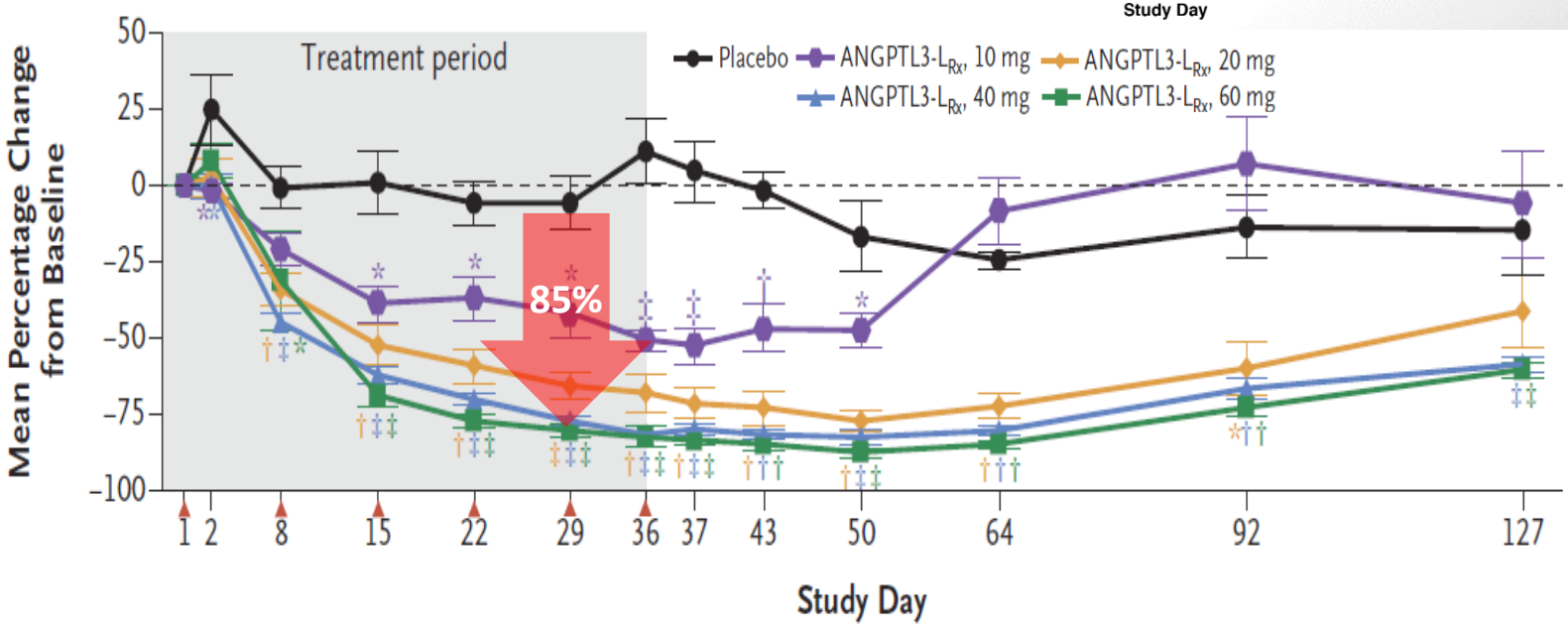
The consistent pharmacological signature of GalNAc-antisense

AKCEA / IONIS TTR-L_{Rx}
GalNAc antisense for transthyretin
https://akceatx.com/wp-content/uploads/Tsimikas_EuATTR_Tegsedi-Beyond-FINAL.pdf



Early saturation of effect (85% reduction in target protein) within 2-4 weeks

AKCEA / IONIS ANGPTL3-L_{Rx}
GalNAc antisense for angiopoietin-like 3
Graham et al., NEJM 2017; 377: 222-232



To GalNAc or not to GalNAc….

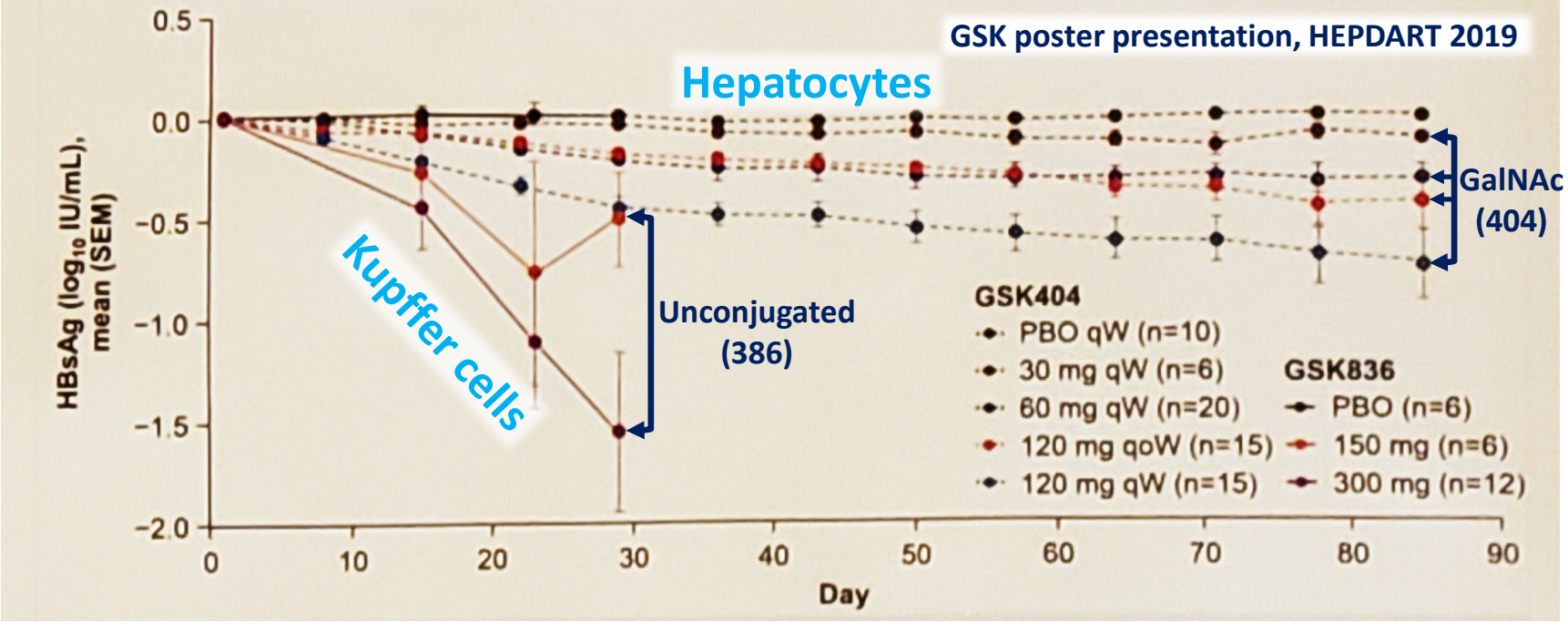
GSK 3389404 (HBV-L_{Rx}) versus GSK 3228836 (HBV_{Rx})

Cryptic class B CpG
(TLR9 stimulation)

Enriched in hepatocytes → GalNAc-GCAGAGGTGAAGCGAAGTCG **GSK 3389404 (GSK 404)**

Enriched in Kupffer cells → GCAGAGGTGAAGCGAAGTCG **GSK 3228836 (GSK 836)**
(TLR reactive)

2'MOE 2'MOE



Minor, gradual HBsAg response with GalNAc ASO
(Hepatocyte targeting)
Inconsistent with GalNAc-antisense effect

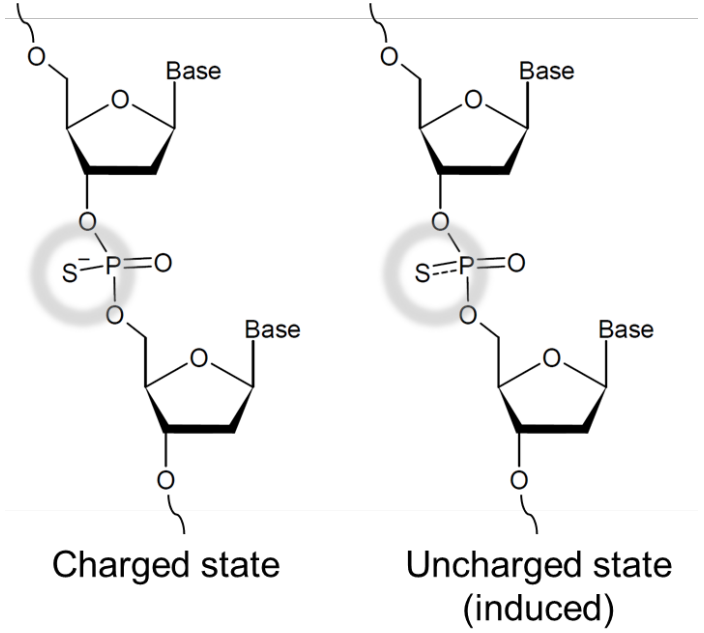
Stronger (but more variable) HBsAg response with unconjugated ASO
(Kupffer cell targeting)
Consistent with TLR9 stimulation of innate immunity

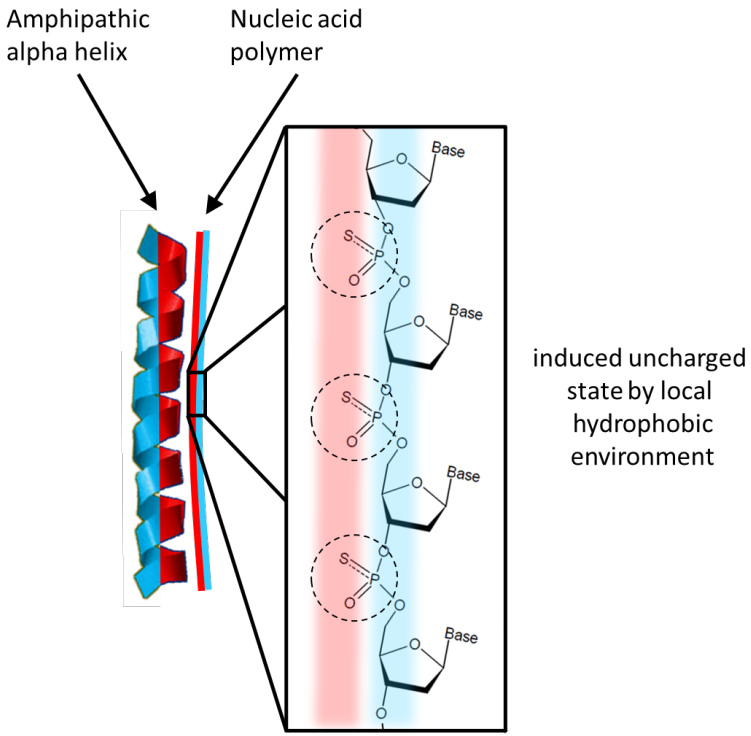
Development of GSK 404 (GalNAc) halted
(GSK Annual Report 2019)

Nucleic acid polymers (NAPs)

Oligonucleotides with sequence independent activity

Anneal to the exposed hydrophobic face of an uncomplexed amphipathic α-helix





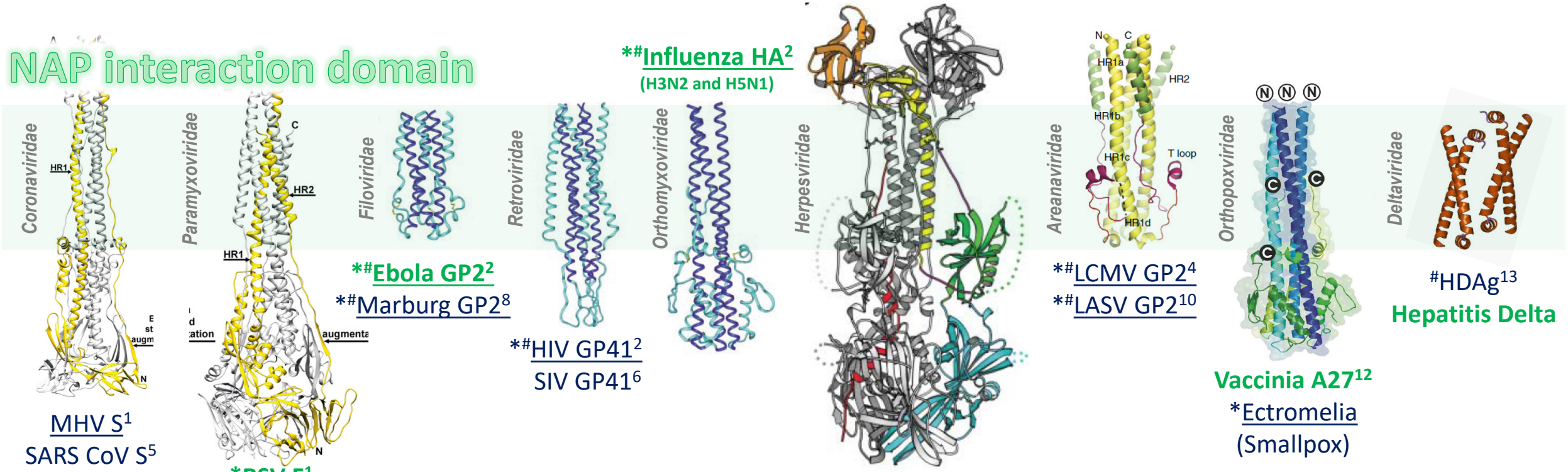
Broad spectrum antiviral activity

HBV, HDV, HCV, HSV, CMV, RSV, PI-3, LCMV, HIV, Influenza, Ebola, Marburg, Lassa Fever, Coronaviruses, prion disease + others

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



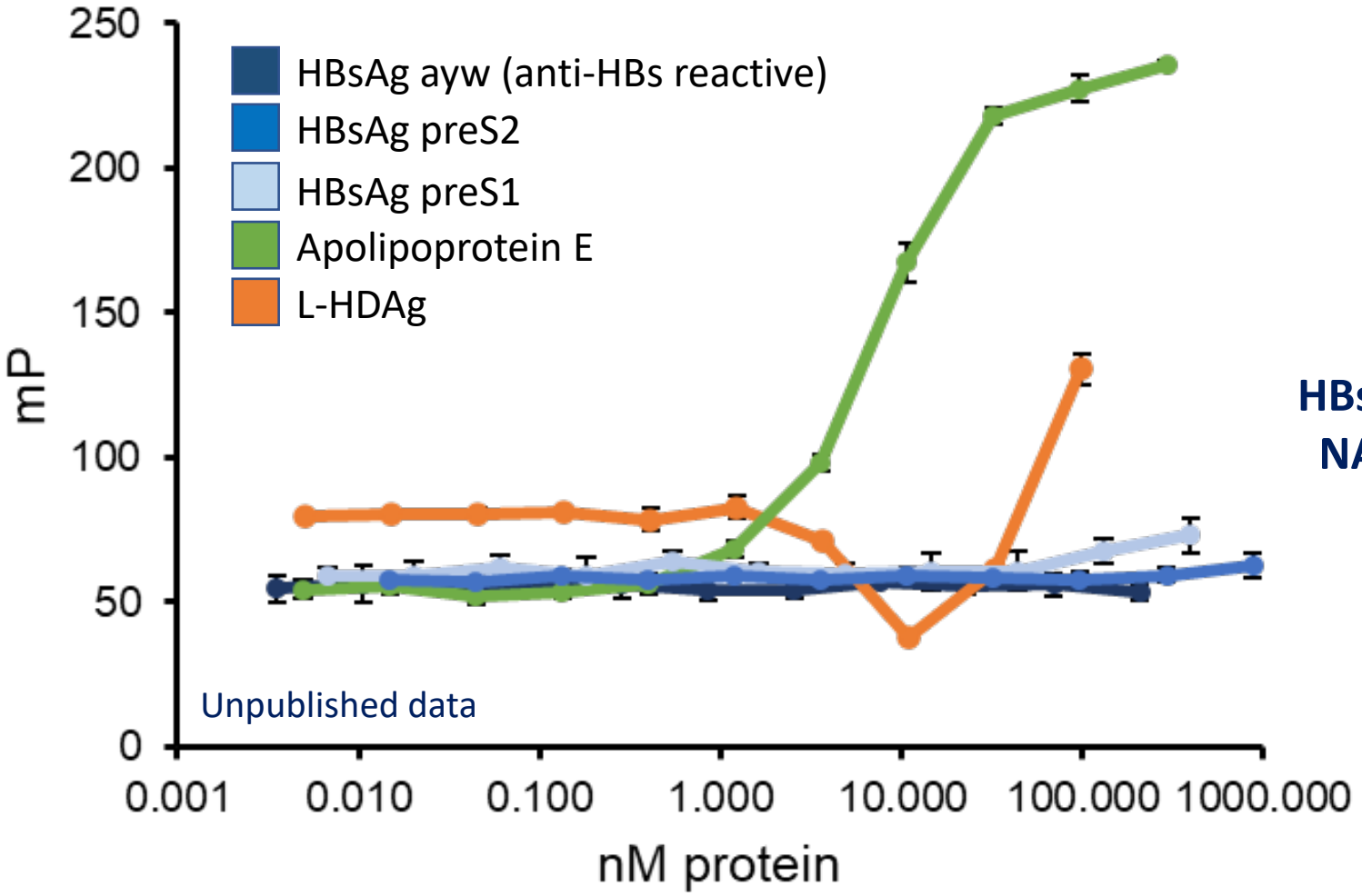
No equivalent structure in viral proteins for HBV and HCV

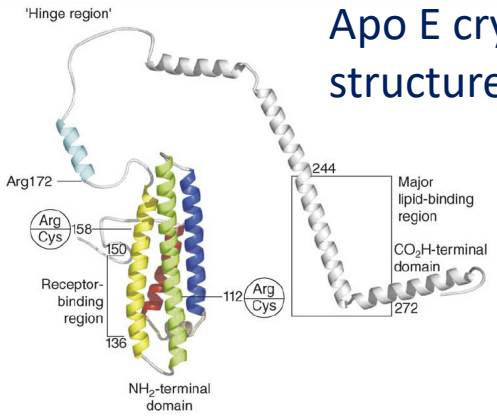
Vaillant Antiviral Res 2016; 133: 32-40
Vaillant. ACS Inf Dis 2019; 10: 675-687

*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

Selected NAP - protein interactions





Apo E crystal structure[1]

HBsAg does not contain amphipathic α-helices
NAPs do not interact with any HBV proteins[2]

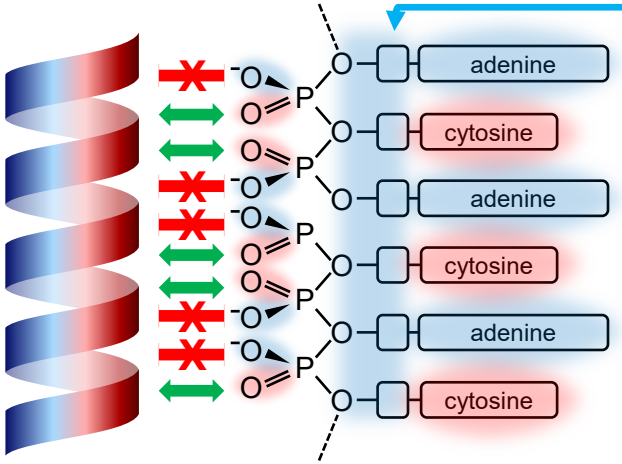
1. Hatters et al., TIBS 2006; 31: 445-454
2. Bazinet et al., Lancet Gastro Hepatol. 2017; 2: 877-889

Charge distribution driving NAP activity

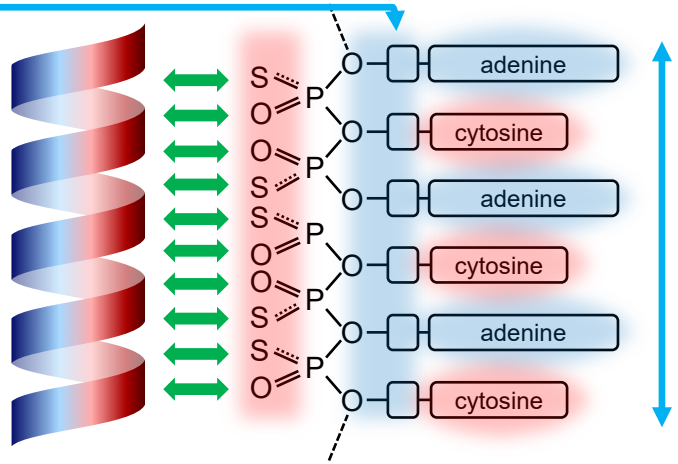
Phosphodiester (inactive) NAPs | **Phosphorothioate (active) NAPs**

Sugar modification does not impact antiviral activity[1]

Activity is sequence independent[1]



Planar stacking (π-π) of aromatic bases induces dipoles[2,3]



Activity is length dependent[1]

Strong electronegativity of oxygen prevents electron resonance with phosphorous
Cooperative hydrophobic interactions blocked

Weak electronegativity of sulfur allows electron resonance (⋯)with phosphorous
Cooperative hydrophobic interactions
(equivalent with R or S stereoisomer)

Activity is phosphorothioate dependent[1]

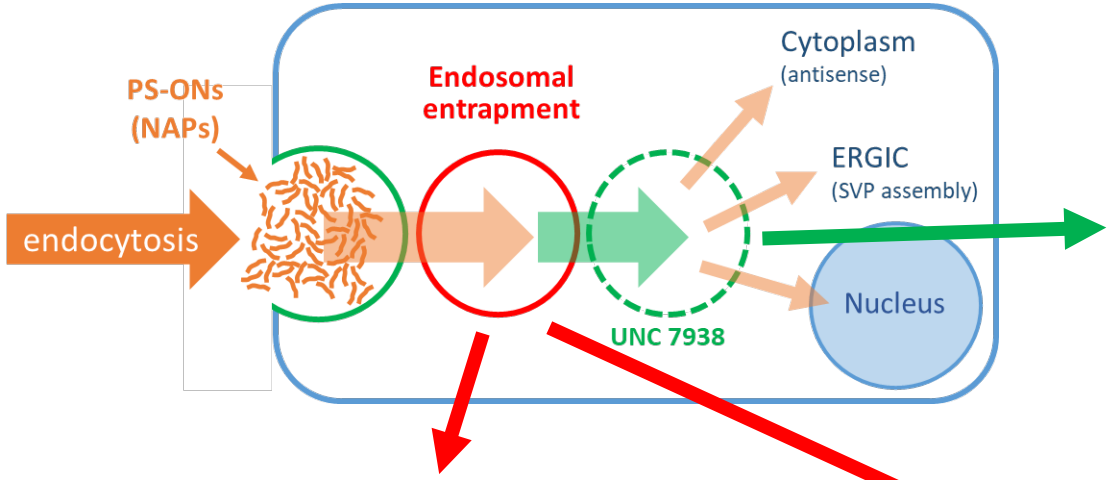
hydrophobic hydrophilic

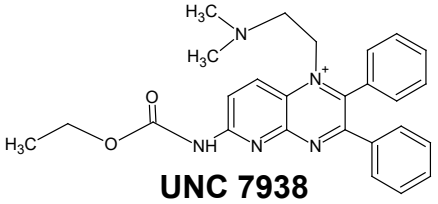
1. Vaillant. ACS Inf Dis 2019; 10: 675-687
2. Guckian et al., J AM Chem Soc. 2000; 122: 2213-2222
3. Mignon et al. Nucleic Acids Res 2005; 33: 1779-1789

Developing a suitable *in vitro* system for mechanistic studies

Restoration of endosomal release of PS-ONs with UNC 7938[3]

(small molecule that restores endosomal release of PS-ONs *in vitro*)





UNC 7938:

- Preserves PS-ON uptake and trafficking observed *in vivo*.
- **Well suited for PS-ON IC_{50} determination and optimization.**
- Does not alter HBV life cycle.

Endosomal entrapment of NAPs occurs *in vitro*

1. Akhtar et al. Nuc Acids Res. 1991;19:5551-5559
2. Koller et al. Nuc Acids Res. 2011;39:4795-4807
3. Yang et al. Nuc Acids Res. 2015;43:1987-1996

No post entry activity of NAPs *in vitro* with standard treatment!

Guillot et al. PLoS ONE 2017; 12: e0179697
Beilstein et al. J Virol. 2018; 92: e01416-17
Blanchet et al., Antiviral Res. 2019; 165: 97-105

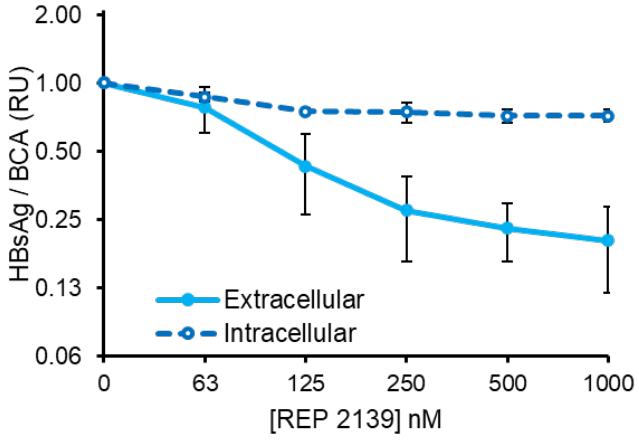
Restoration of endosomal release of PS-ONs with UNC 7938

NAP treatment of HepG2.2.15 cells

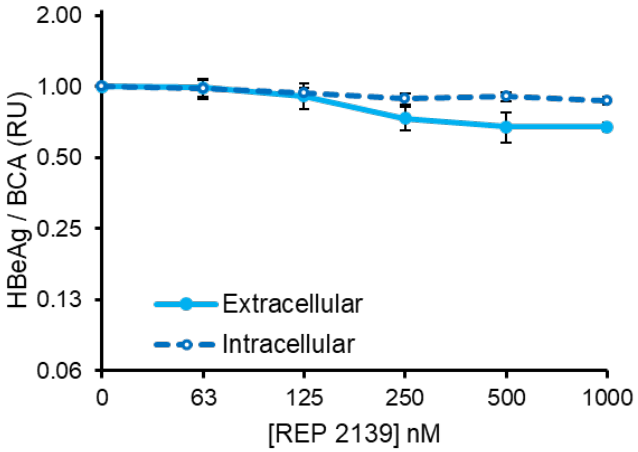
Inhibition of HBsAg secretion

Intracellular HBsAg reduction with NAP treatment

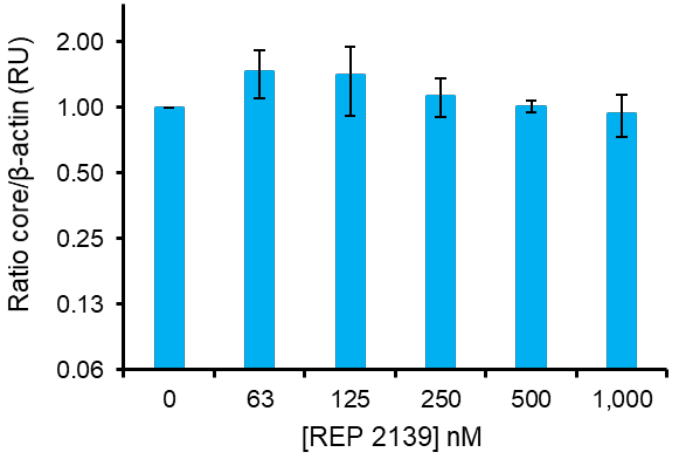
Intrahepatic HBsAg and cccDNA clearance *in vivo*[1,2]



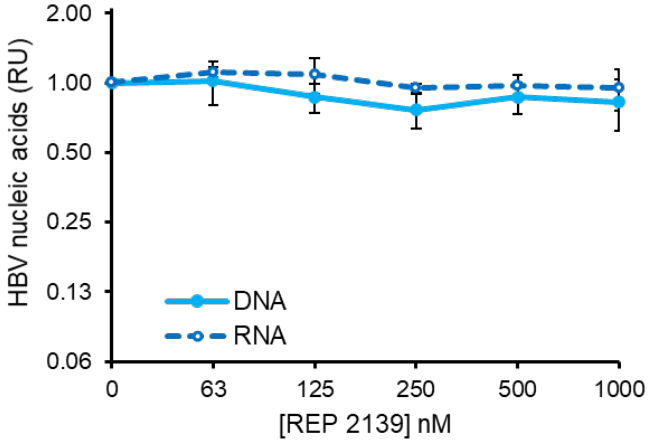
No effect on HBeAg

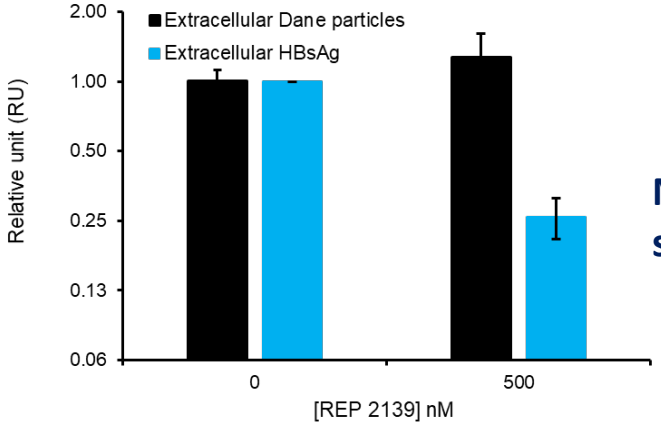


No effect on intracellular HBcAg



No effect on cellular HBV DNA or HBV RNA



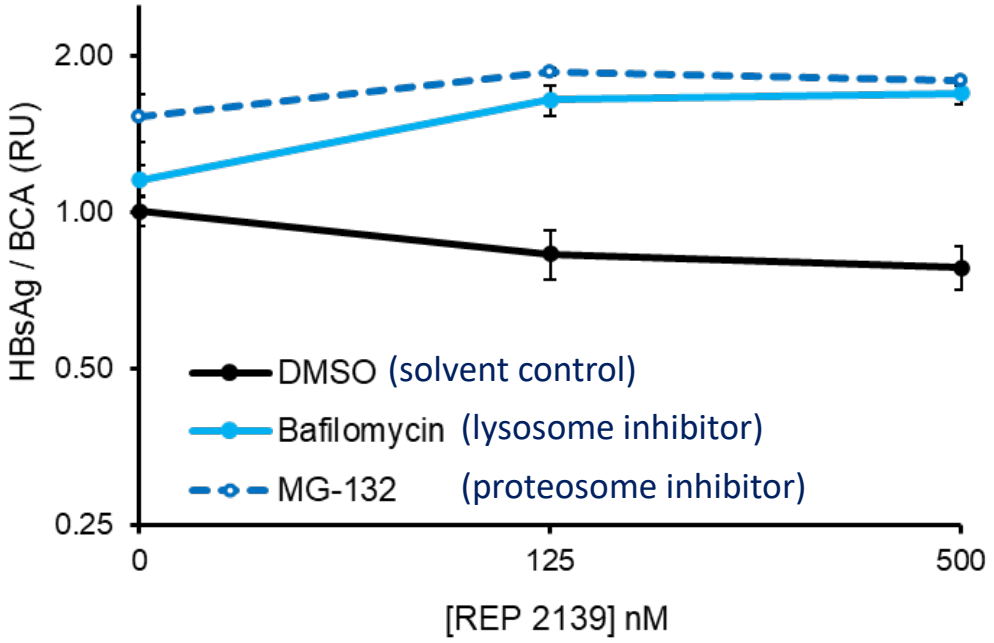


No effect on Dane particle secretion

Boulon et al., Antiviral Res. 2020 epub June 22

1. Noordeen et al., PLoS One 2015; 10: e0140909
2. Quinet et al., Hepatol. 2018; 67: 2127-2140

Intracellular proteolysis of HBsAg with NAPs



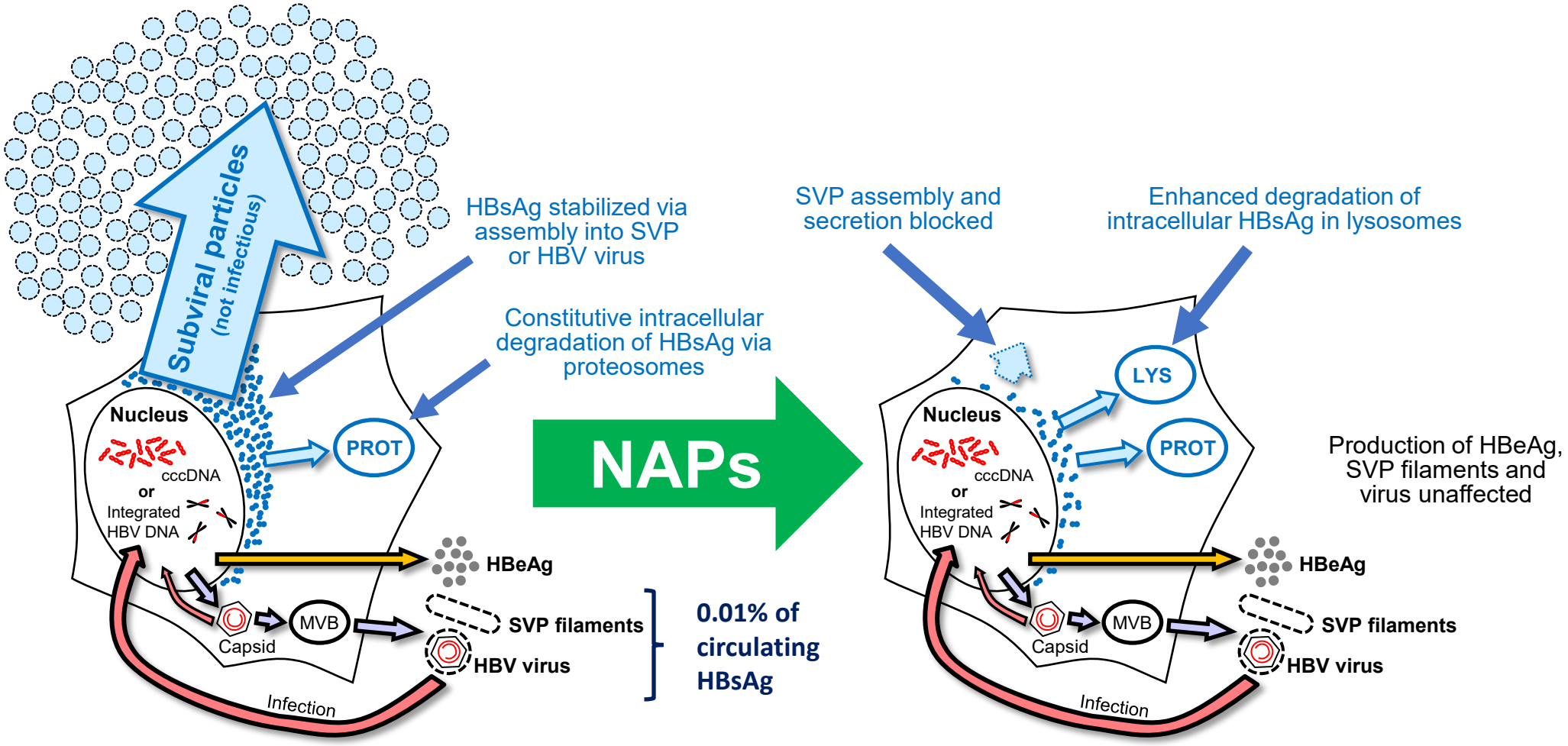
Decline in intracellular HBsAg with REP 2139

Inhibition of SVP assembly leads to HBsAg proteolysis via constitutive degradation pathways

Lysosomal degradation appears to play a greater role in intracellular HBsAg clearance in the presence of NAPs

Boulon et al., Antiviral Res. 2020 epub June 22

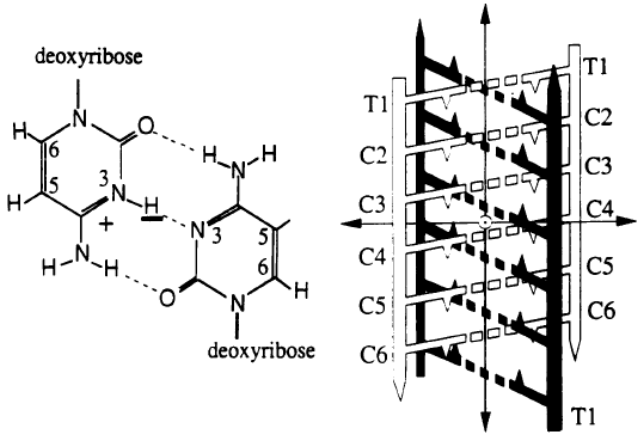
Antiviral effects of NAPs



Boulon et al., Antiviral Res. 2020 epub June 22

REP 2031: a key NAP identifying the location of post-entry NAP activity in HBV

NAP	REP 2055	REP 2031
NAP chemistry	**40mer PS DNA**	
Sequence	**poly AC**	**poly C**
Activity against HIV[1], HSV[2], CMV[3,4], LCMV[5], HCV[6], prion disease[7]	equal	
Post entry activity in HBV *in vitro*[8,9]	**potent**	absent / weak
Antiviral activity against HBV *in vivo*[10]	**potent**	absent



Polypyrimidines (i.e poly cytidine) undergo tetramerization at acidic pH[11,12]

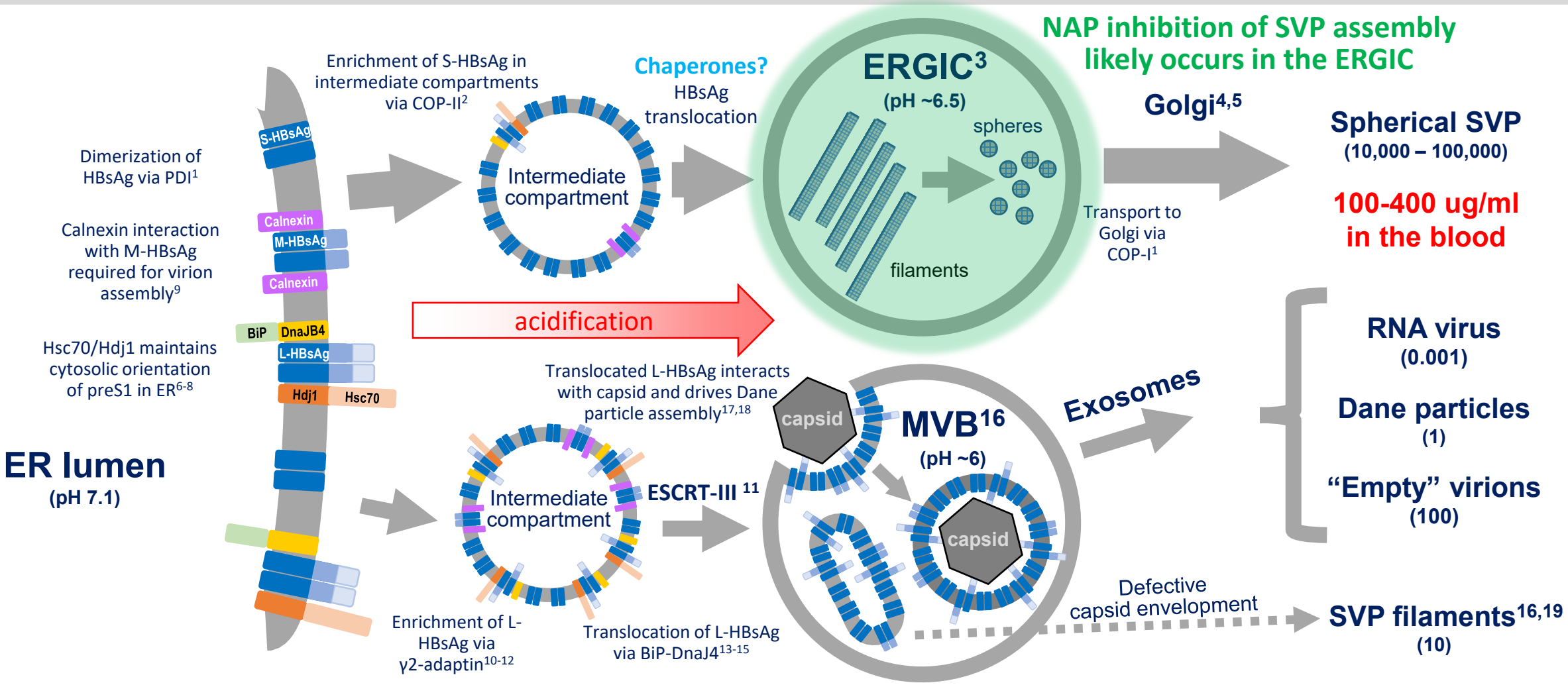
Loss of ability to target exposed amphipathic alpha helices

Tetramerization inhibited by doping with purine nucleotides (adenosine)[13]

i.e. REP 2055

1. Vaillant et al., AAC 2006; 50: 1393-1401
2. Guzman et al., Antiviral Ther 2007; 12: 1147-1156
3. Bernstein et al., AAC 2008; 52: 2727-2733
4. Cardin et al., Virol J 2009; 6: 214
5. Lee et al., Virology 2008; 372: 107-117
6. Matsumura et al., Gastroenterol 2009; 137: 673-681
7. Kocisko et al., AAC 2006; 50: 1034-1044
8. Noordeen et al., AAC 2013; 57: 5291-5298
9. Blanchet et al., Antiviral Res 2019; 164: 97-105
10. Noordeen et al., AAC 2013; 57: 5299-5306
11. Kanehara et al et al., Biochemistry 1992; 118: 1305-1320
12. Leroy et al., NAR 1994; 22: 1600-1606
13. Geinguenaud et al., 2000 Biochemistry 39: 12650-12658

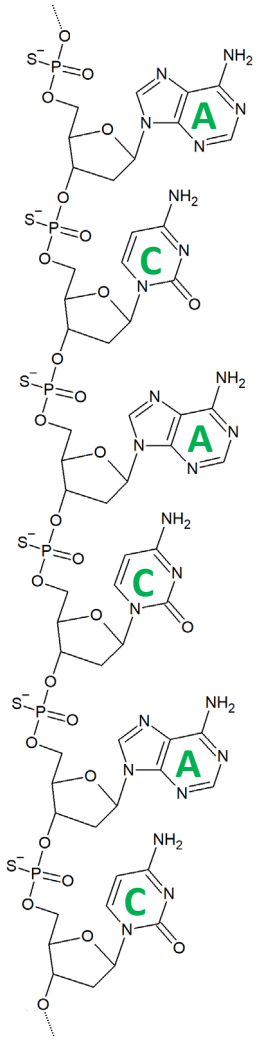
Identifying the subcellular compartment for NAP activity



1. Huovila et al J Cell Biol 1992; 118:1305-1320
2. Zeyen et al., Cell Microbiol Epub Feb 4 2020
3. Patient et al., J Virol. 2007; 81: 3842-3851
4. Patzer et al., J Virol. 1984: 51: 346-353
5. Simon et al., Virology 1988; 166: 76-81
6. Prange et al., Biol Chem 1999; 380: 305-314
7. Lambert and Prange PNAS 2003; 100: 5199-5204
8. Wang et al., AAC 2010; 54: 2070-2177
9. Prange et al., Biol Chem. 1999; 380: 305-314
10. Hartman-Stuhler and Prange J Virol. 2001; 75: 5343-5351
11. Lambert et al., J Virol. 2007; 81: 9050-9060
12. Jurgens et al., Nat Chem Biol. 2013; 9: 540-547
13. Awe et al., FEBS Lett 2008; 582: 3179-3184
14. Ostapchuk et al., EMBO 1994; 13: 1048-1057
15. Bruss et al., EMBO 1994; 13: 2273-2279
16. Watanabe et al., PNAS 2007; 104: 10205-10210
17. Bruss, Virus Res. 2004; 106: 199-209
18. Pastor et al., Sci Rep. 2019; 9: 16178
19. Jiang et al., J Virol. 2016; 90: 3330-3341

Freedom to remove toxic properties of oligonucleotides

Transitioning from REP 2055 to REP 2139



REP 2055
1st gen clinical candidate

Repetitive adenosine / cytidine sequence (already in REP 2055)[1]

- ***Does not affect antiviral activity (recall activity is sequence independent)***
- Eliminates secondary structure formation and off target interaction
- Blocks recognition by TLR 9 (no CpG motifs present) – no stimulation of TLR 3, 7 or 8 detected[2]
- Efficiently quenches all immunoreactivity[2]

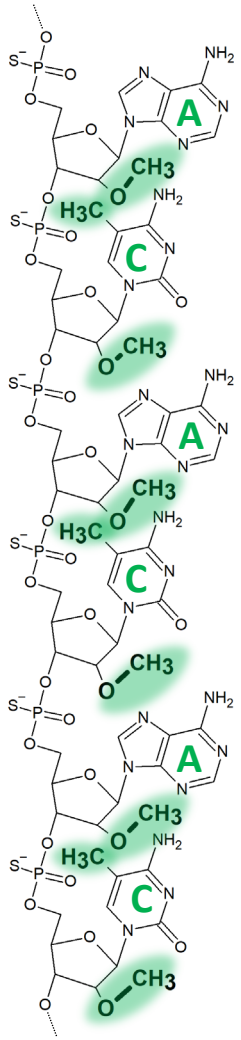
2'O-methylation of all ribose sugars in backbone

- ***Does not affect antiviral activity (verified in vivo and in human studies)[1]***
- Increases oligonucleotide hydration along long axis of the NAP[3]
  - improves solubility, reduces off target protein interactions
  - **essential for chelate complex formulation**
- Enhanced nuclease stability
- Further blocks recognition by TLR 3, 7 and 8

5-methylation of cytosine

- ***Does not affect antiviral activity***
- Further blocks recognition by TLRs and RIG-I / NOD in cytoplasm[2]
- Identifies NAP as a "self" nucleic acid in mammals

All modifications are naturally occurring (no mitochondrial toxicity)



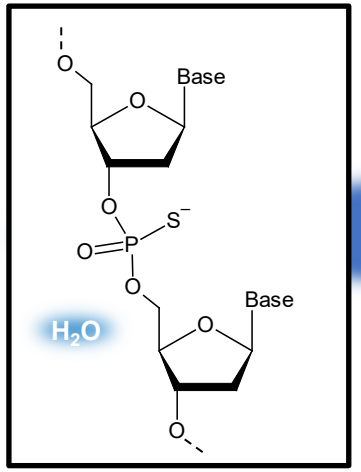
REP 2139
Lead clinical candidate

1. Vaillant. ACS Inf Dis 2019; 10: 675-687
2. Real et al., Sci Reports 2017; 7: 43838

Sugar modification drives hydration in NAPs

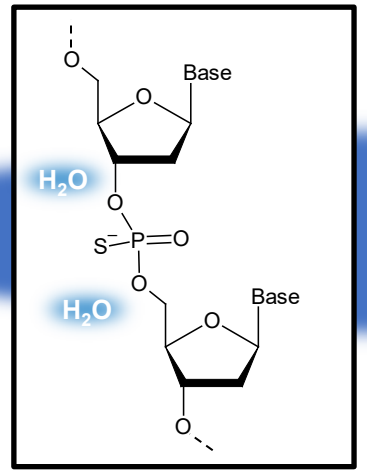
REP 2055
(poorly hydrated)

REP 2139
(well hydrated)



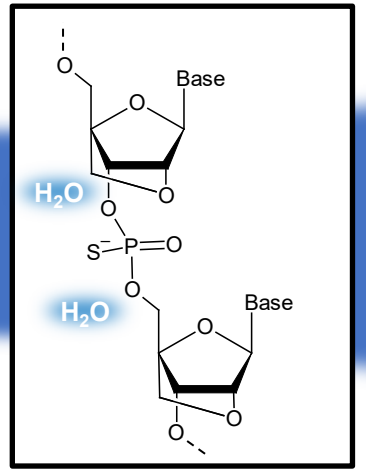
Phosphorothioate DNA
(R-diastereomer)

- destabilizes oligonucleotides[1-3]
- shifts sulfur into the major groove with weaker electron resonance[4]
- **Weaker hydration** [5]



Phosphorothioate DNA
(S-diastereomer)

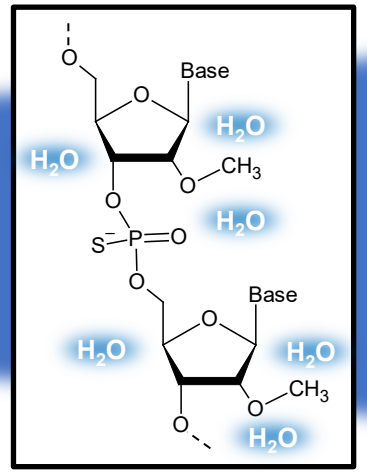
- stabilizes oligonucleotides[1-3]
- shifts sulfur into aqueous space with stronger electron resonance[4]
- **Improved hydration vs Rs**[5]



Phosphorothioate LNA RNA

Constrained 2' methylene bridge prevents RNA hydration
Comparable hydration to DNA[6,7]

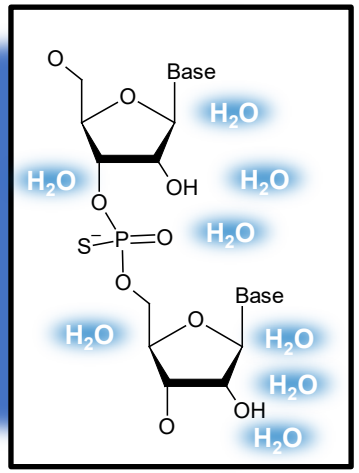
Hepatoxicity[8-10]
(increased off target hybridization)



Phosphorothioate 2' O-methyl RNA

Unconstrained 2' oxygen permits hydration

Increases water solubility[11]
Blocks off target protein interactions[12,13]
Increased nuclease stability[14]
Naturally occurring[15,16]
(no mitochondrial toxicity)



Phosphorothioate RNA

Most water soluble

Rapidly degraded by efficient endonuclease attack[17]

1. Stec et al., J Am Chem Soc. 1998; 120: 7156-7167
2. Iwamoto et al., Nat Biotech. 2017; 35: 845-851
3. Østergaard et al., Nuc Acid Res. 2020; 48: 1691-1700
4. Lan et al., Sci Rep. 2016; 6: 25737
5. Karwowski et al., Bioorgan Med Chem Lett. 2001; 1001-1003
6. Kaur et al., Biochem. 2006; 45: 7347-7355
7. Pande and Nilsson, Nuc Acids Res. 2008; 36: 1508-1516
8. Swayze et al., Nuc Acids Res. 2007; 35: 687-700
9. Burel et al., Nuc Acid Res. 2016; 44: 2093-2109
10. Takeshi et al., Sci Rep. 2016; 6: 30377
11. Egli and Pallan. An Rev Biophy Biom Stru. 2007;36:281-305
12. Yu et al., Bioorganic Med Chem. 1996; 4: 1685-1692
13. Yoo et al., Nuc Acid Res. 2004; 32: 2008-2016
14. Monia et al., J Biol Chem. 1996; 271: 14533-14540
15. Darzacq, et al., EMBO 2002: 21: 2746–2756.
16. Rebane et al., BMC Mol Biol. 2002; 3: 1
17. Roehl et al., Mol Ther Nuc Acids 2017; 8: 1-12

Hydration status does not affect NAP activity

NAP	REP 2055	REP 2139
Hydration	poor	good
Activity in vivo[1-3]	equivalent	
UNC7938 mediated endosomal release (HepG2.2.15)[4]	IC_{50} ~35nM *(influenced by in vitro tox)*	IC_{50} ~125nM
Minimum effective dose in humans (unpublished data)	equivalent	
Activity in humans[5]	equivalent	

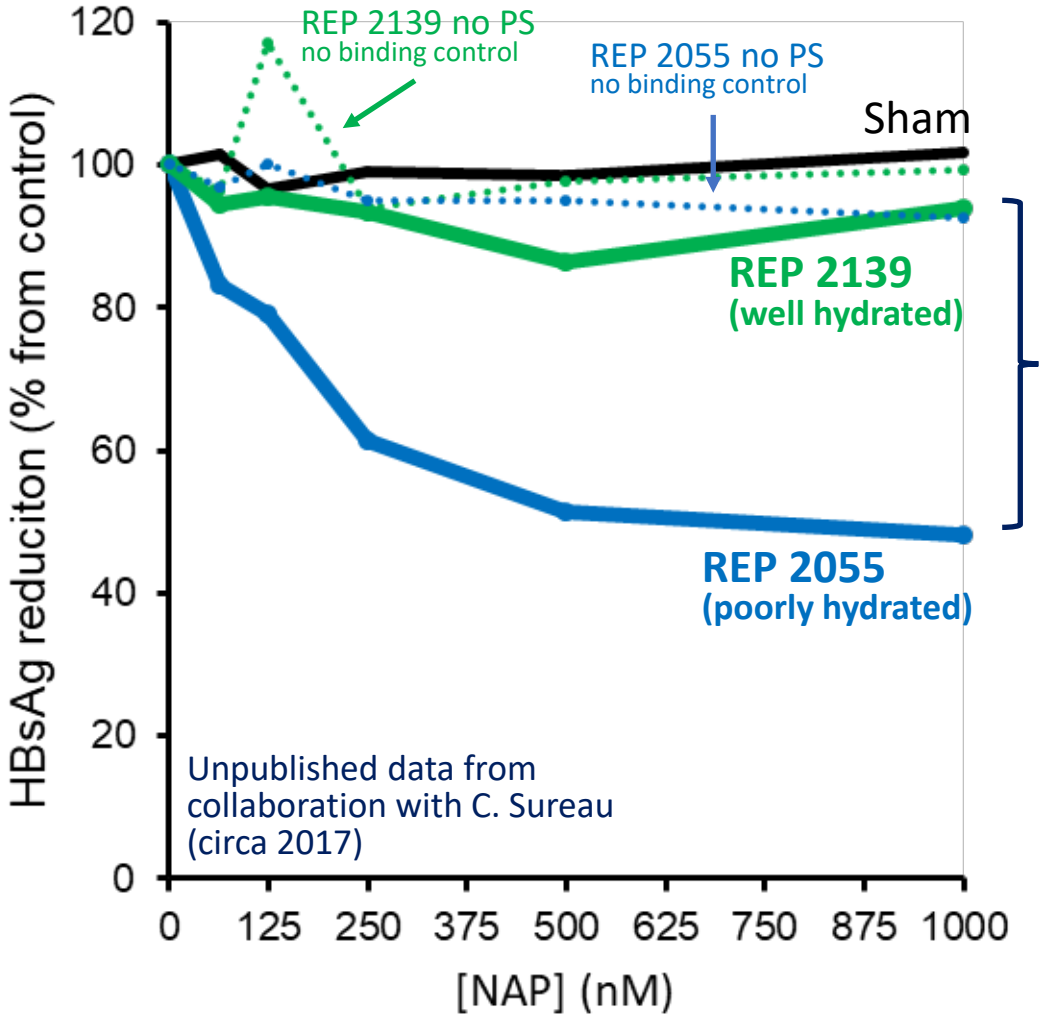
All poly AC phosphorothioate NAPs are equivalently active with best *in vitro* estimate of IC_{50} ~100-125nM

REP 2139 hydration optimizes safety and maximizes solubility

1. Noordeen et al., Plos One 2015; 11: e0140909
2. Roehl et al., Mol Ther Nuc Acids 2017; 8: 1-12
3. Quinet et al., Hepatol. 2018; 67: 2127-2140
4. Blanchet et al., Antiviral Res. 2019; 164: 97-105
5. Al-Mahtab et al., PLoS One 2016; 11: e01566667

Why not transfection?

Oligofectamine transfection of NAPs in HepG2.2.15 cells



Equivalent activity *in vitro*[1,2], *in vivo*[3-6] and in humans[7]

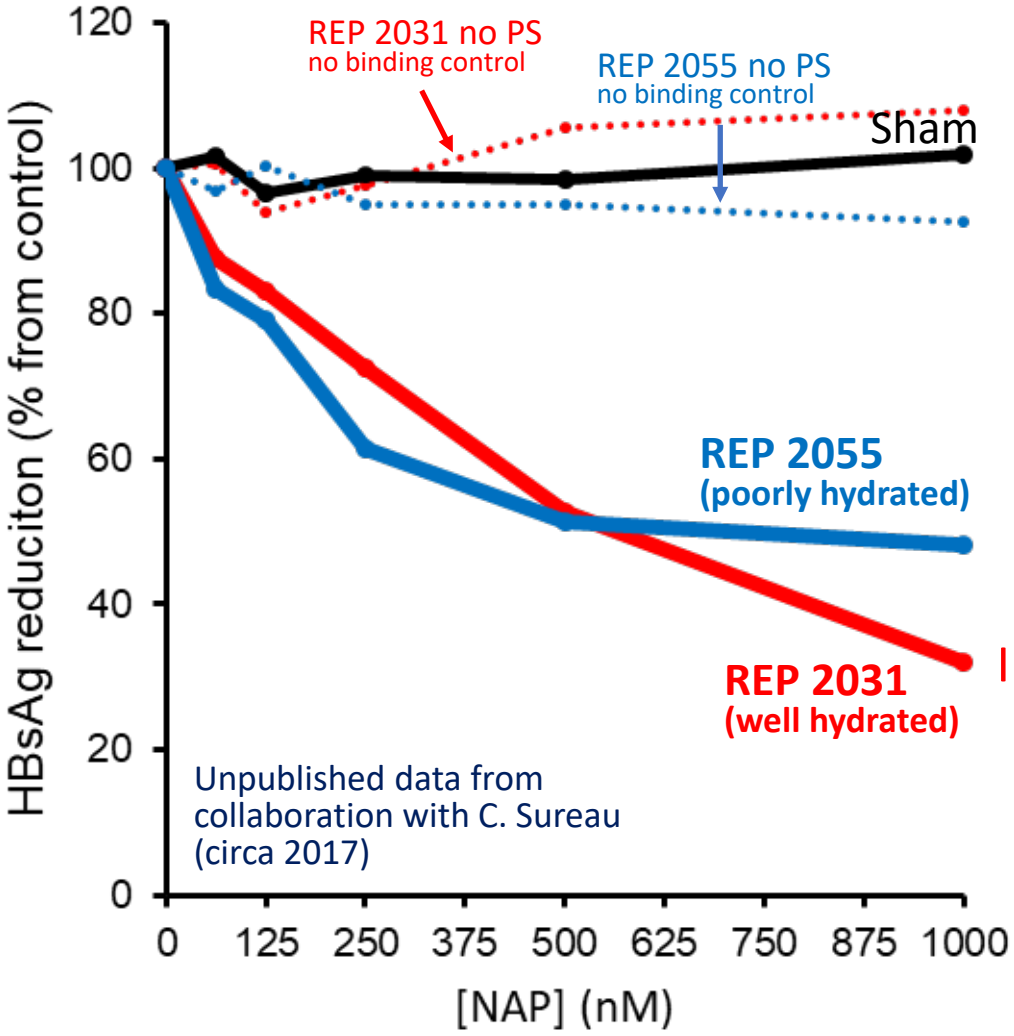
Transfection (liposome formation) is inhibited by oligonucleotide hydration[8-12]

Also observed with other PS-ONs *in vitro*[13-15]

1. Noordeen et al., AAC 2013; 57: 5291-5298
2. Blanchet et al., Antiviral Res 2019; 164: 97-105
3. Noordeen et al., AAC 2013; 57: 5299-5306
4. Noordeen et al., Plos One 2015; 11: e0140909
5. Roehl et al., Mol Ther Nuc Acids 2017; 8: 1-12
6. Quinet et al., Hepatol. 2018; 67: 2127-2140
7. Al-Mahtab et al., PLoS One 2016; 11: e01566667
8. Akhtar et al., NAR 1991;19:5551-5559
9. Jääskeläinen et al., Biochem Biophys Acta 1994; 1195: 115-123
10. Teixeira et al., Biophys Chem 2001;92:169-81
11. Campbell et al., Biochim Biophys Acta 2001; 1512: 27-39
12. Wang et al., Molecules 2018; 23: 2564
13. Amarzguioui et al., Nuc Acid Res. 2003;31:589-95.
14. Fabani and Gait, RNA 2008; 14: 336-346
15. Wan and Seth. J Med Chem 2016;59:9645-9667

Why not transfection?

Oligofectamine transfection of NAPs in HepG2.2.15 cells



Unpublished data from collaboration with C. Sureau (circa 2017)

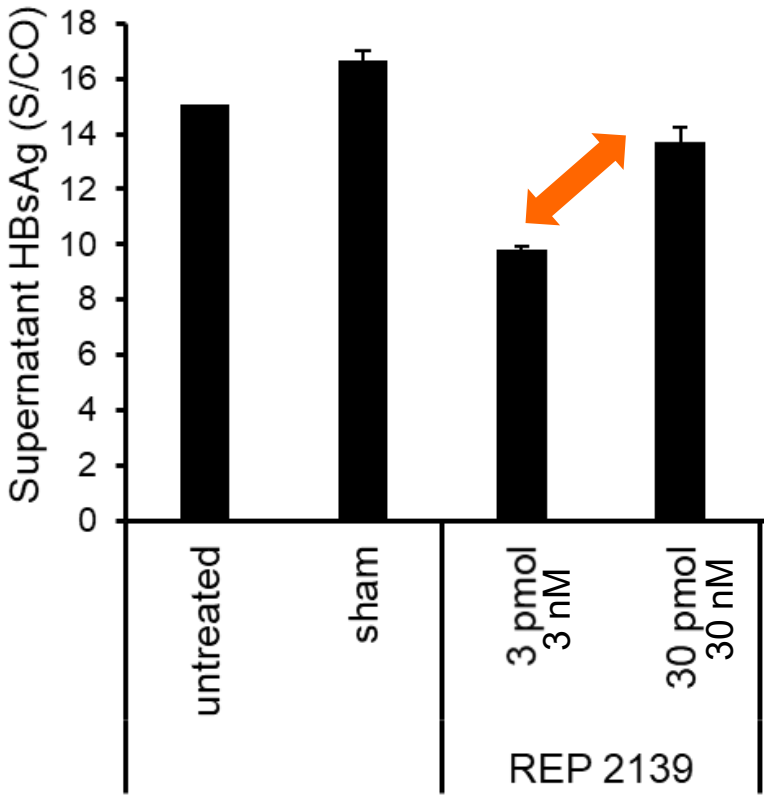
Oligofectamine interferes with HBsAg assembly / transit through the ERGIC

Inactive *in vitro*[1,2] and *in vivo*[3]

1. Noordeen et al., AAC 2013; 57: 5291-5298
2. Blanchet et al., Antiviral Res 2019; 164: 97-105
3. Noordeen et al., AAC 2013; 57: 5299-5306

Why not transfection?

RNAiMAX transfection of NAPs in HepG2.2.15 cells



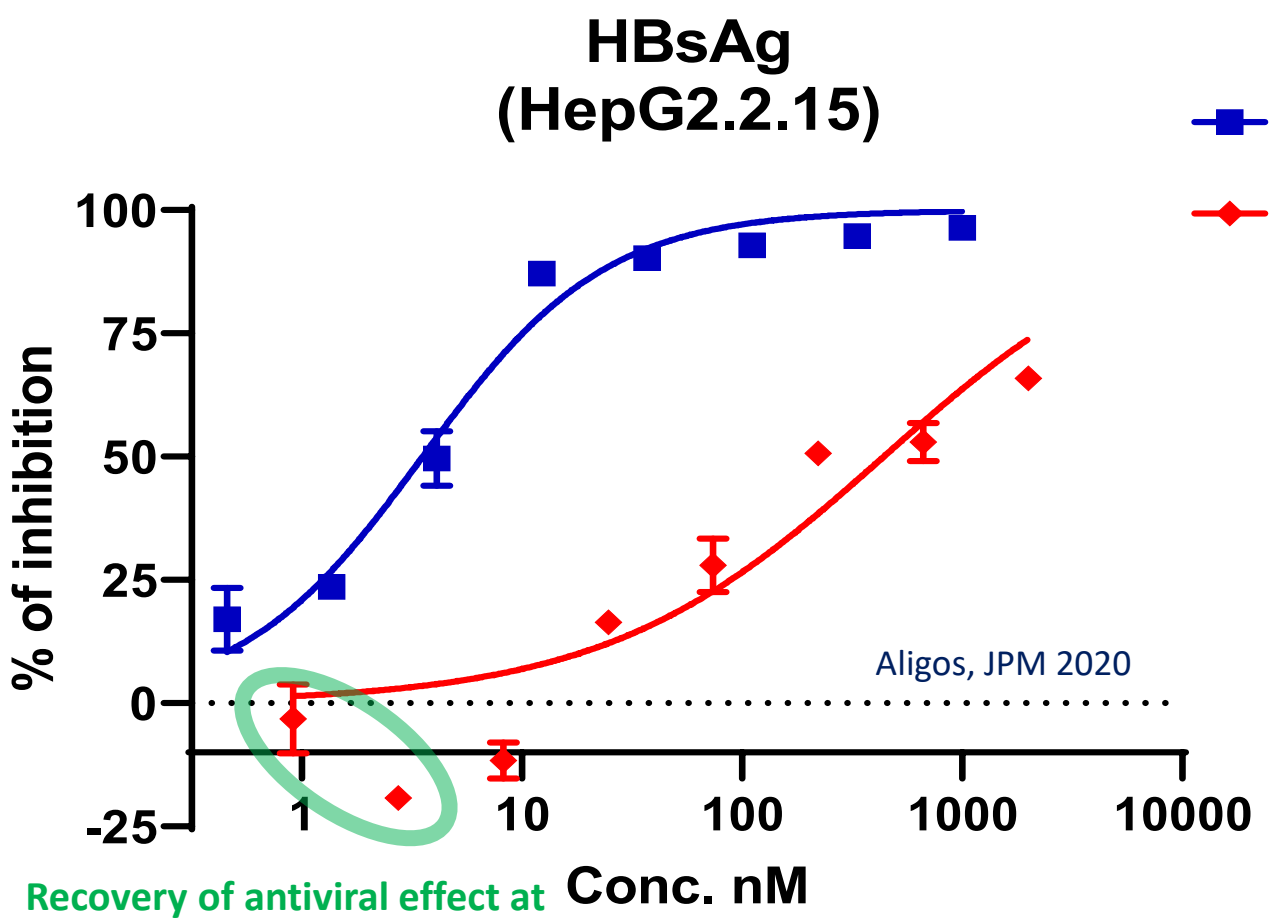
REP 2139 inhibits HBsAg secretion following transfection with RNAiMAX but only at low concentrations!

REP 2139 hydration blocks liposome formation

Unpublished data from collaboration with M. Roggendorf (circa 2014)

Why not transfection?

RNAiMAX transfection of NAPs in HepG2.2.15 cells



Recovery of antiviral effect at lower REP 2139 doses

Poly A,5-MeC PS 40mer
LNA / 2'OMe RNA altimer
(poorly hydrated)

ALG-010133

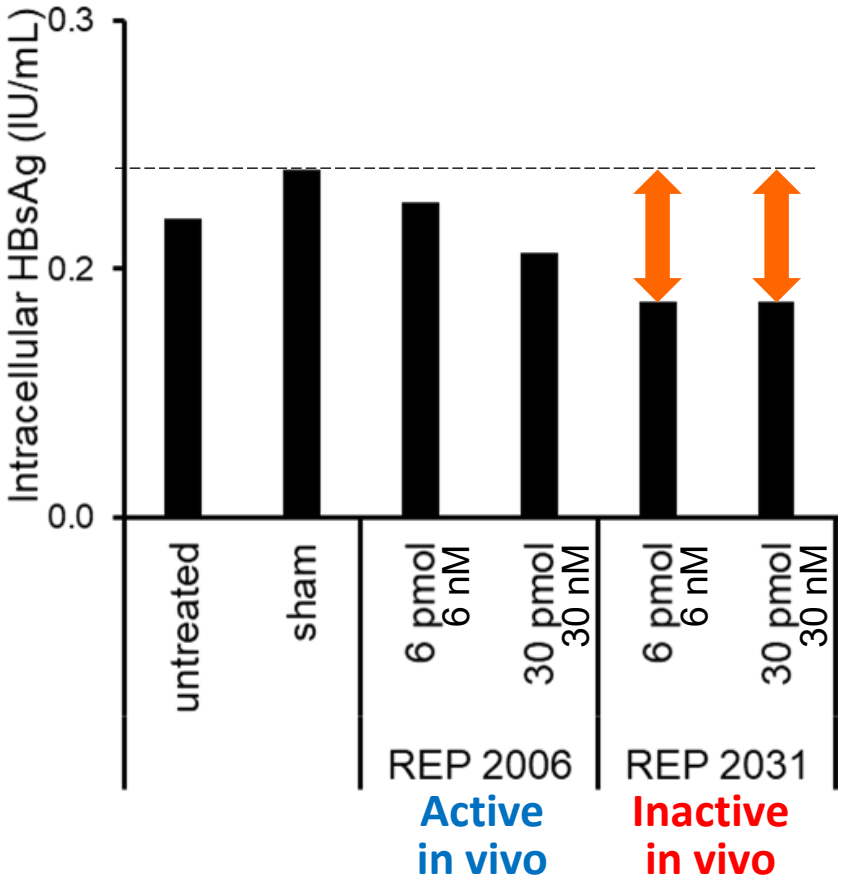
ALG-010004 (REP 2139)

Poly A, 5-MeC PS 40mer
Full 2'OMe RNA
(well hydrated)

Better apparent activity of ALG-010133 is driven by more efficient transfection, not better specific activity

Why not transfection?

RNAiMAX transfection of NAPs in HepG2.2.15 cells



REP 2031 demonstrates inhibition of HBsAg secretion

RNAiMAX interferes with HBsAg assembly / transit through the ERGIC

Unpublished data from collaboration with M. Roggendorf (circa 2014)

Making sense of transfection data with NAPs

RNAiMAX transfection of NAPs into HepG2.2.15 cells

All described in US 7,358,068 (Replicor)

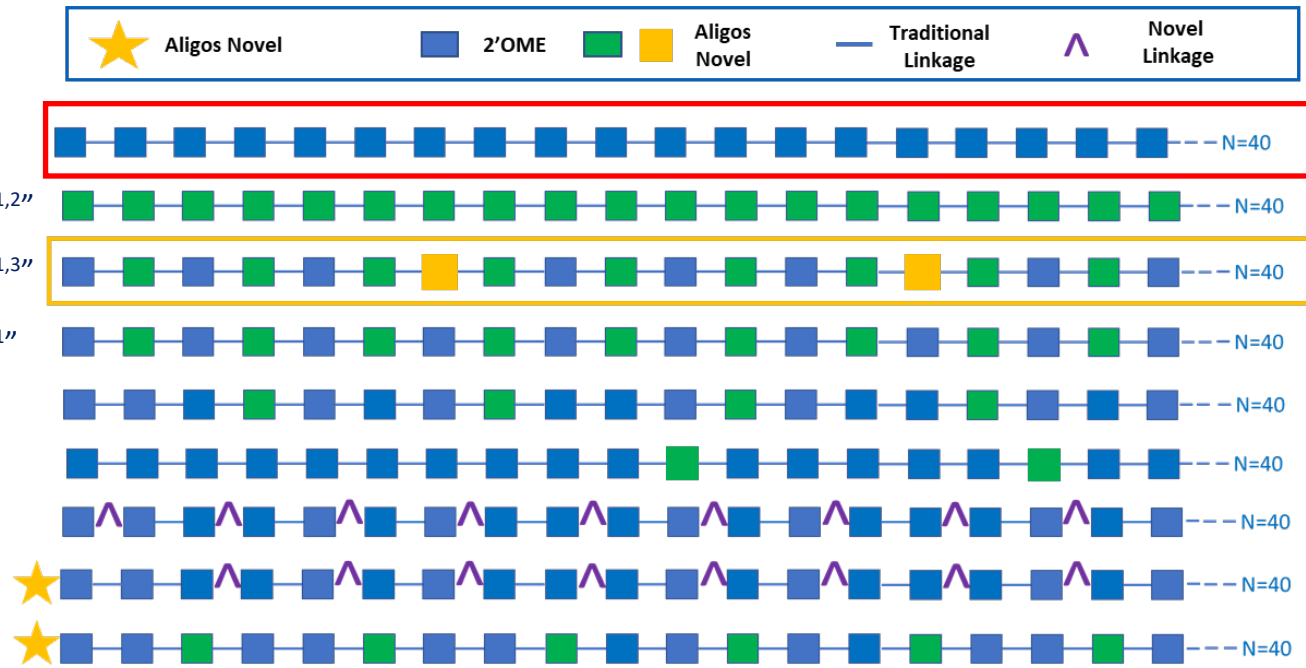
2'OH or similar

Phosphorothioate mixed stereoisomer

5' conjugate

LNA

Phosphorothioate R stereoisomer



"REP 2055[1,2]"

"REP 2165[1,3]"

"REP 2163[1]"

ALG #	HepG2.2.15	
	EC_{50} nM	CC_{50} nM
REP-2139	~500	>10000
ALG-10000	3.9	200
ALG-10133	3.2	>2000
ALG-10093	2.5	>2000
ALG-10128	21	>2000
ALG-10129	32	>2000
ALG-10048	23	>2000
ALG-10122	12	>2000
ALG-10125	79	>2000

All are 40mer poly A,5-MeC PS NAPs

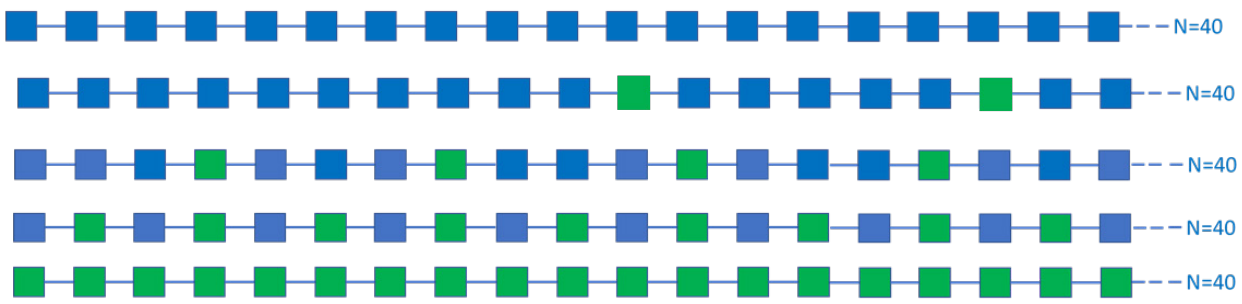
All have the same specific activity

Aligos, JPM 2020
Aligos provisional patent US 62/757,632 filed Nov 8, 2018 "Nucleic Acid Polymers"

1. Roehl et al., Mol Ther Nuc Acids 2017; 8: 1-12
2. Al-Mahtab et al., PLoS One 2016; 11: e01566667
3. Bazinet et al., Gastroenterol. 2020; 158: 2180-2194

Making sense of transfection data

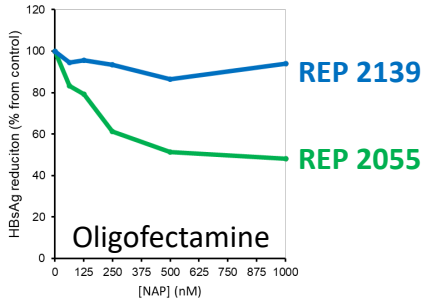
2'Ome RNA LNA



REP-2139	~500	>10000
ALG-10129	32	>2000
ALG-10128	21	>2000
ALG-10093	2.5	>2000
ALG-10000	3.9	200

LNA doping of REP 2139 reduces hydration and increases transfection efficiency **(Beware hepatoxicity)**

Similar hydration status as REP 2055



Mixed R/S stereoisomer R stereoisomer

REP-2139	~500	>10000
ALG-10048	23	>2000

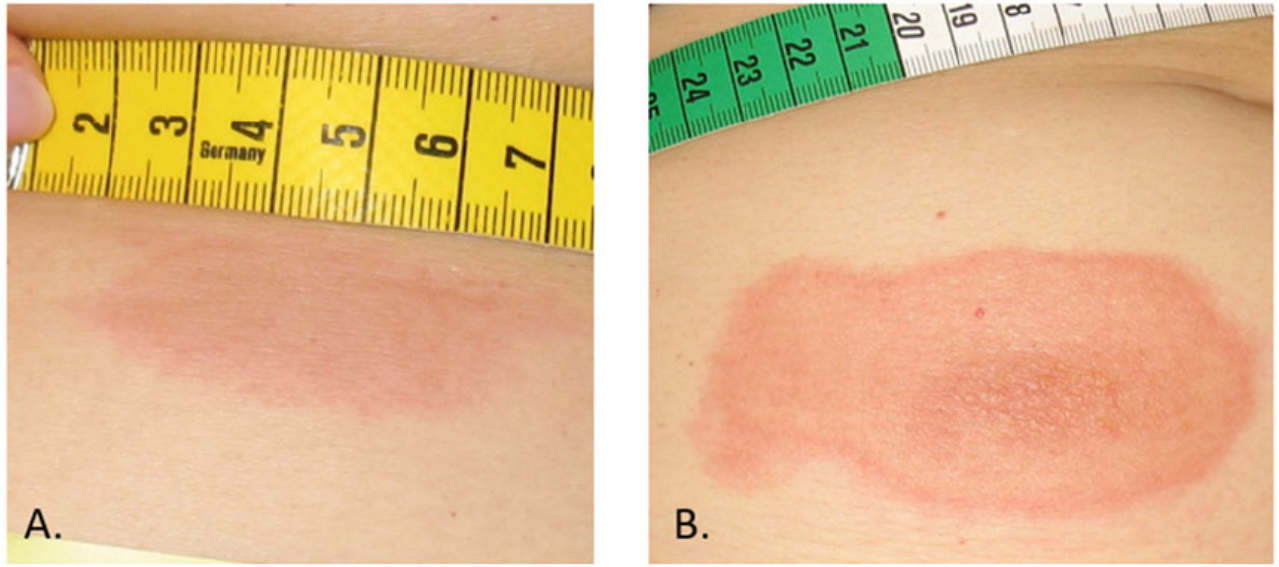
R-stereoisomer enrichment of REP 2139 reduces hydration and increases transfection efficiency

Aligos, JPM 2020

Administration tolerability with phosphorothioate oligonucleotides

All PS-ONs are poorly tolerated by IV infusion and SC injection

IV: fever, chills, headache, rigor, asthenia
SC: pain, itching, induration, swelling, scarring



SC injection site reactions (ISRs) occur with doses as low as 5mg
Occur with 5-MeC, 2'OMe, 2'MOE, and LNA modifications

ISRs are highly prevalent with current approved PS-ON therapies:
Kynamro: 85% (off market)
Tegsedi: 49%
Waylivra: 82%

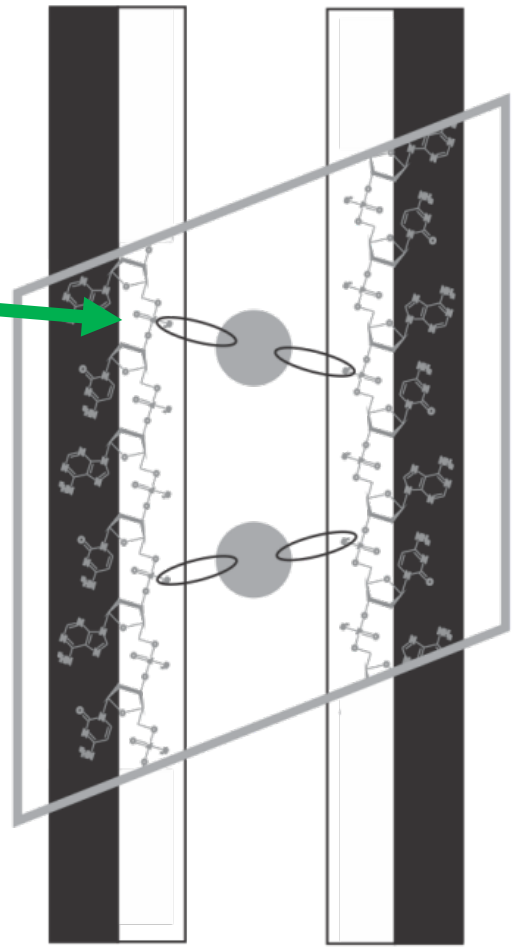
Longer length of NAPs enhances their administration reactivity

Van Meer et al., Br J Clin Pharmacol 2016; 82: 340-351

Solving NAP administration tolerability with chelate complexes

IV and SC reactivity is driven by chelation of divalent metals by oligonucleotides during administration

- Chelation occurs by formation of intermolecular metal bridges between non-bridging oxygen or sulfur in linkages
- A universal property of DNA and RNA oligonucleotides

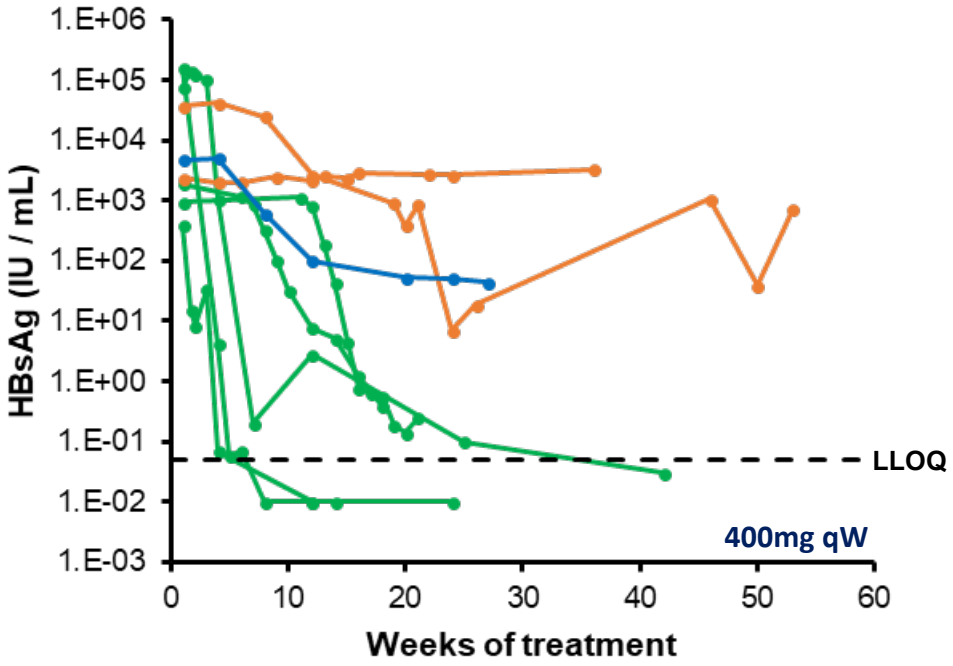


Formulation of oligonucleotides in solution as chelate complexes reduces or eliminates IV infusion tolerability and ISR reactivity with SC injection

Replicor patents US 8531211 and 8716259
(also allowed in all major jurisdictions WOW)

Integrating NAPs into existing therapies

REP 2055 monotherapy
HBeAg positive chronic HBV infection



Good HBsAg response

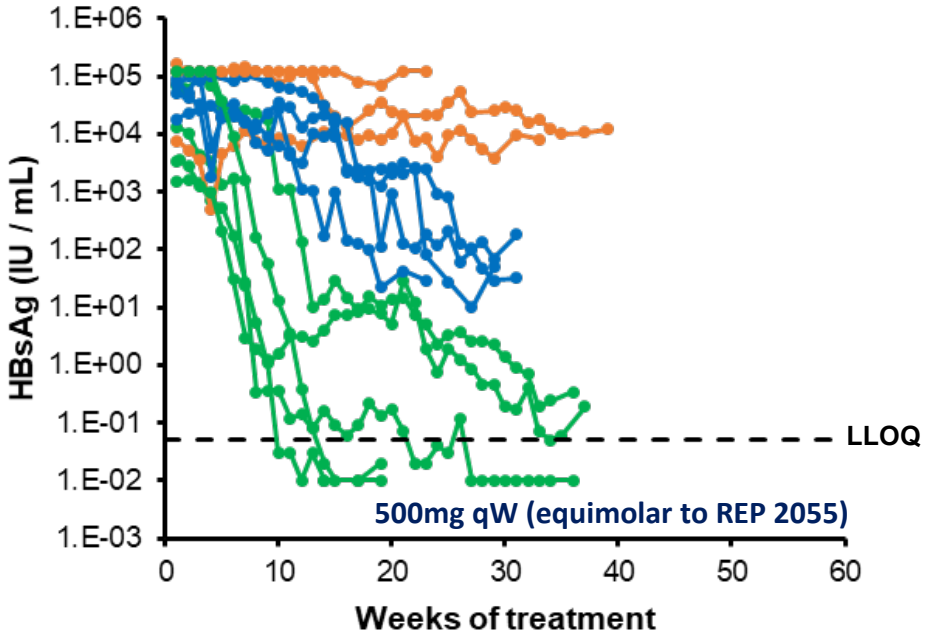
Poor administration tolerability (IV and SC)

(Grade 2 SC ISR with doses as low as 25mg)

1/8 participants with sustained virologic control (5 years)

1/8 participants with sustained functional cure (5 years)

REP 2139-Ca monotherapy
(calcium chelate complex)
HBeAg positive chronic HBV infection



Similar HBsAg response

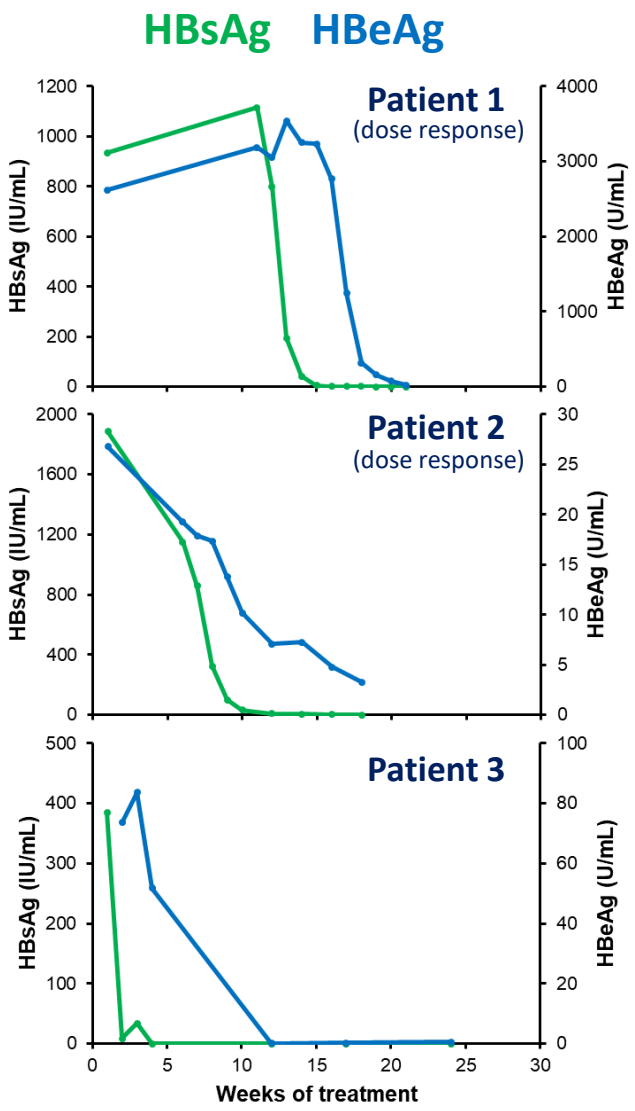
Improved administration tolerability (IV and SC)

Due to chelate complex formulation

HBsAg response alone is insufficient for high rates of control

Al-Mahtab et al., 2016 PLoS One 11: e0156667

HBeAg response is disconnected from HBsAg response



Quantitative declines in HBsAg and HBeAg during REP 2055 monotherapy in the REP 101 study (unpublished data)

- Measured using the Roche Impact platform
- HBeAg declines are delayed by ~4-5 weeks from HBsAg declines.
- HBeAg decline and seroconversion likely driven by immunological reconstitution following reduction in hepatic and circulating HBsAg

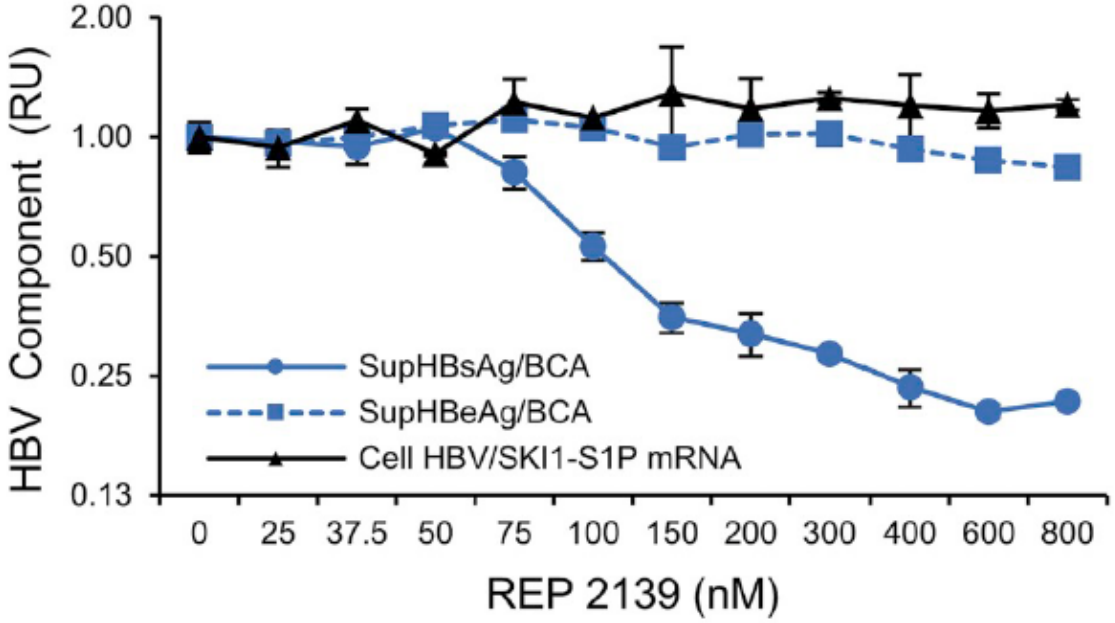
NAPs do not directly inhibit production or secretion of HBeAg
Consistent with published *in vitro* data[1,2]

1. Blanchet et al., Antiviral Res. 2019; 165: 97-105
2. Boulon et al., Antiviral Res. 2020 epub June 22

Non-specific effects on cellular secretion with RNAiMAX

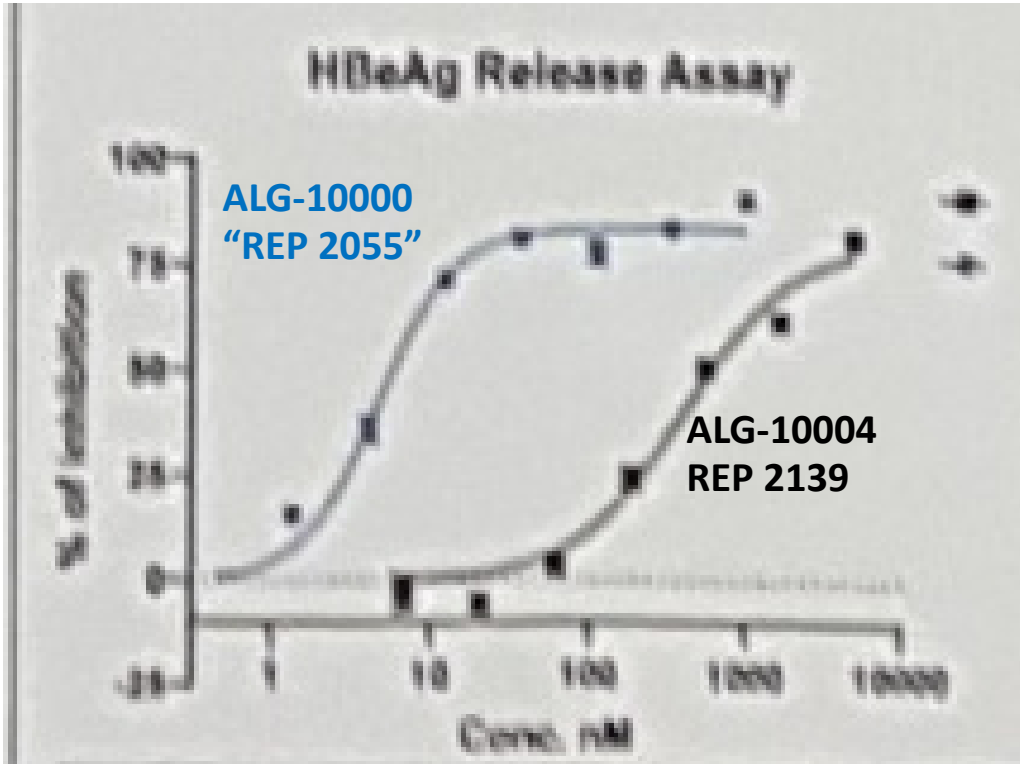
REP 2139 has no effect on HBeAg secretion in HepG2.2.15 cells with UNC7938 endosomal release

Blanchet et al., Antiviral Res. 2019; 165: 97-105



Consistent with clinical data

In vitro data, Aligos Poster # 689, AASLD 2019



Inhibition of HBeAg secretion with RNAiMAX:

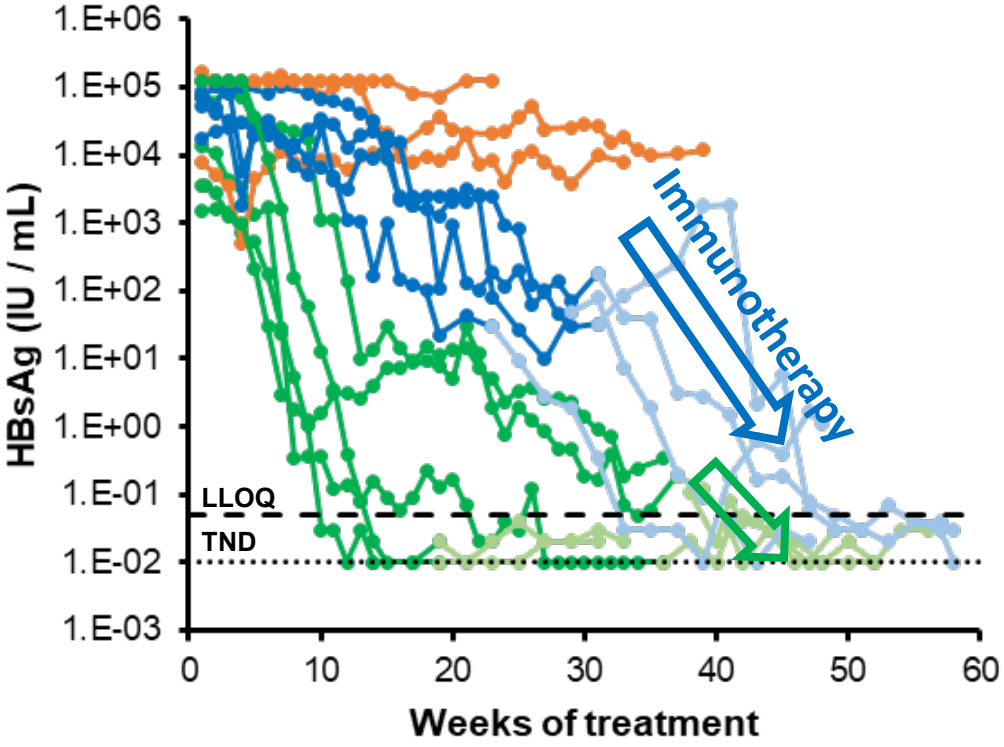
Directly interferes with HBeAg secretion
or
Directs NAPs to non-physiological compartments, blocking HBeAg secretion

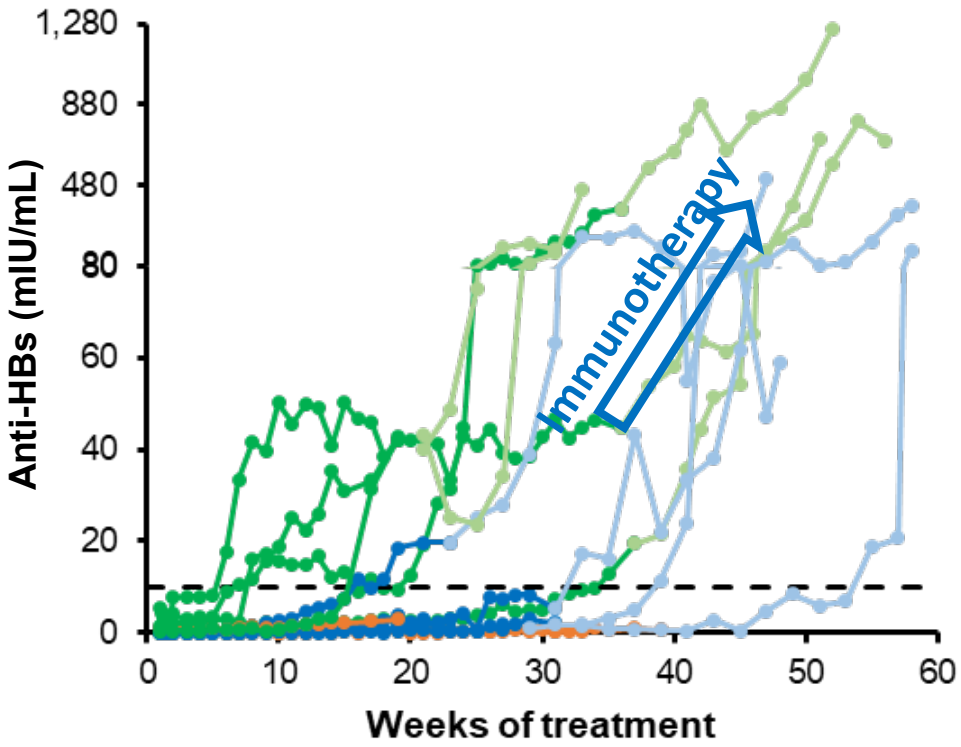
Antiviral response with REP 2139 is improved with addition of immunotherapy

HBsAg decline < 1 log_{10} did not receive immunotherapy
HBsAg decline > 1 log_{10} but > 1 IU/mL
HBsAg decline to < 1 IU/mL

Shaded portions of lines indicate introduction of immunotherapy

pegIFN or thymosin α1





Improved HBsAg and anti-HBs response

All participants on combination therapy achieve < 0.05 IU/mL HBsAg on therapy

4/9 achieve virologic control > 2 years after removal of all therapy

Al-Mahtab et al., 2016 PLoS One 11: e0156667

REP 401: Putting the pieces of the puzzle together

Steps to achieve functional cure

Remove SVP → **NAP chelate complex (REP 2139-Mg)**
Administration now asymptomatic

↓

Remove immune inhibition

↓

Restore innate control of cccDNA → **TDF**, **pegIFN**
Prevent reactivation of latent cccDNA

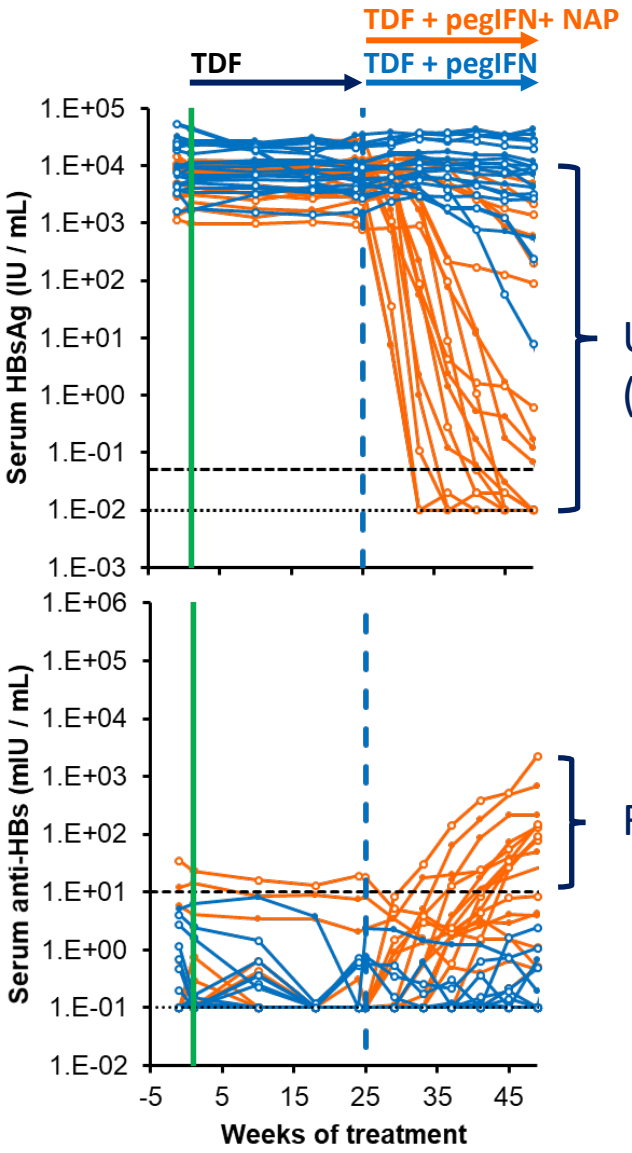
Restore HBsAg specific T-cell response → **pegIFN[1,2] (or thymosin α1[3])**
Removal of hepatocytes with integrated HBV DNA to clear SVP producing capacity in the liver

↓

Functional cure

1. Micco et al., J Hepatol. 2013; 58: 225-33
2. de Niet et al., J Hepatol 2016; 64: 539-46
3. Naylor and Mutchnick, J Viral Hep. 2018; 25: 4-9

REP 401: NAPs dramatically improve responses with TDF + pegIFN

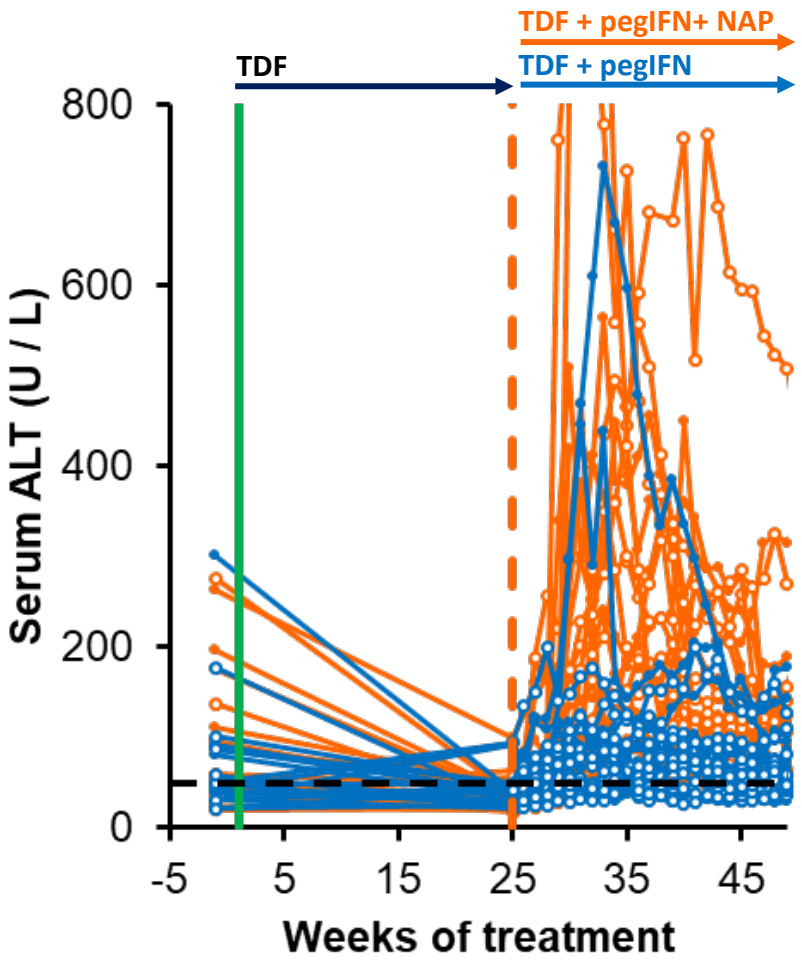


Up to 6 $\log_{10}$ decline from baseline (to < 0.05 IU/mL)

Rapid HBsAg seroconversion

Bazinet et al., Gastroenterol. 2020; 158: 2180-2194

REP 401: NAPs dramatically improve response over TDF + pegIFN



Bazinet et al., Gastroenterol. 2020; 158: 2180-2194

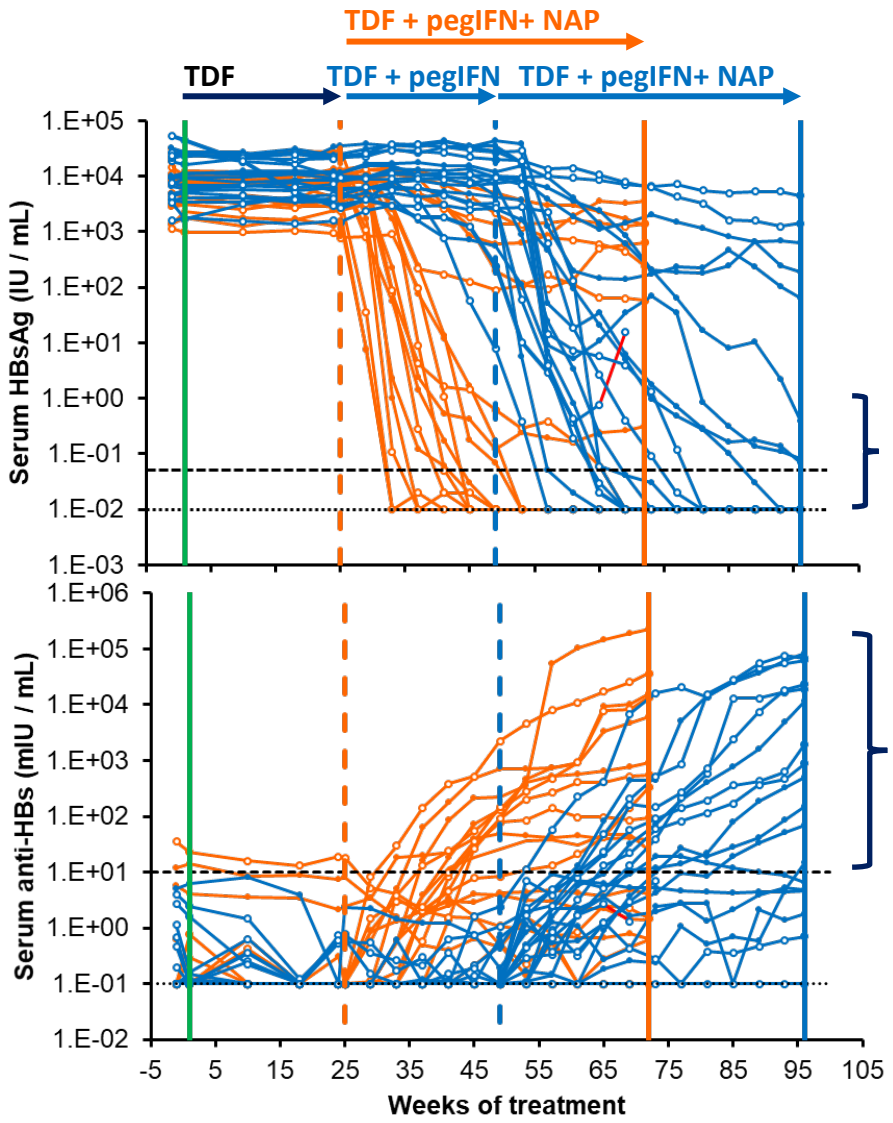
Dramatic increase in host mediated transaminase flares

- occur in 95% of participants
- no alteration in liver function
- asymptomatic
- correlated with functional cure
- Involved in clearance of cccDNA and integrated HBV DNA (?)

Consistent with beneficial nature of host mediated transaminase flares during therapy with approved agents[1-10]

1. Nair and Perrillo, Hepatology 2001; 34: 1021-1026
2. ter Borg et al., J Clin Virol. 2008; 42: 160-1634
3. Sonneveld et al., Clin Inf Dis. 2013; 6: 100-105
4. Nagaoka et al., Hepatol Res. 2016; 46: E89-E99
5. Chi et al., J Gastrol Hepatol. 2016; 31: 1882-1887
6. Seo et al., Clin Moml Mepatol. 2017; 23: 154-159
7. Yano et al., Biomed Rep. 2017; 7: 257-262
8. Jeng et al., J Viral Hep. 2018; 25: 421-428
9. Wong et al., Liv Int. 2018; 38: 1760-1769
10. Brahmania et al., Clin Gastrol Hepatol 2019; 17: 2541-2551

REP 401: Transition to NAP addon from TDF + pegIFN elicits similar responses



70% < 1 IU/mL

52% < 0.05 IU/mL

70% HBsAg seroconversion

Anti-HBs up to 223,055 mIU/mL

Bazinet et al., Gastroenterol. 2020; 158: 2180-2194

REP 401: Outcomes

Completed treatment and ≥ 24 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	**Virologic control (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%**

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

Collaboration with Abbott Diagnostics
Late breaking poster to be presented at EASL 2020

HBsAg isoform analysis planned for presentation at AASLD 2020

Bazinet et al., Gastroenterol. 2020; 158: 2180-2194

REP 2139-Mg: next steps

1. Target identification underway
   Data suggests an ERGIC resident chaperone involved in SVP assembly
2. Verify efficacy of REP 2139-Mg with SC administration
3. Verify safety of REP 2139-Mg in advanced fibrosis / cirrhosis
4. Assess efficacy / safety of pegIFN vs thymosin α1

To be addressed in upcoming phase II REP 501 trial in HBV / HDV co-infection

5. Expand genotype assessment and include other patient cohorts (e.g. HIV / HBV co-infection)

To be addressed in upcoming multi-country phase II trial (s) in NUC-suppressed HBV mono-infection or HBV/HIV co-infection

Summary

Subviral particles:

- an evolved mechanism for immunosuppression
- not targeted by direct acting antivirals or innate immunity
- clearance during therapy is essential to achieve high rates of functional cure

Host mediated transaminase flares are correlated with functional cure

- HBsAg reactive T-cell response may be required to remove intrahepatic reservoir of SVP production

RNAi / antisense

- clinical HBsAg response is inconsistent with RNAi / antisense response, is TLR3 agonism involved?
- is integrated HBV DNA being targeted in humans? how to integrate with existing therapies?

NAPs selectively target SVP assembly and the bulk of HBsAg secretion

- transfection cannot be used to assess NAPs *in vitro*
- accompanied by intracellular HBsAg decline / hepatic HBsAg clearance
- HBsAg clearance from the blood requires host immune functions
- chelate complex formulation and immunotherapy required: well tolerated therapy achieving high rates of functional cure
- host-mediated transaminase flares associated with high rates of functional cure
- **profound control demonstrated by experimental assays suggests removal of integrated HBV DNA**
- **transition to SC administration will improve convenience**
- **demonstration of safety in cirrhotic patients will expand utility in advanced disease**