HBsAg, Subviral Particles and Their Clearance in Establishing Functional Cure of Chronic HBV Infection

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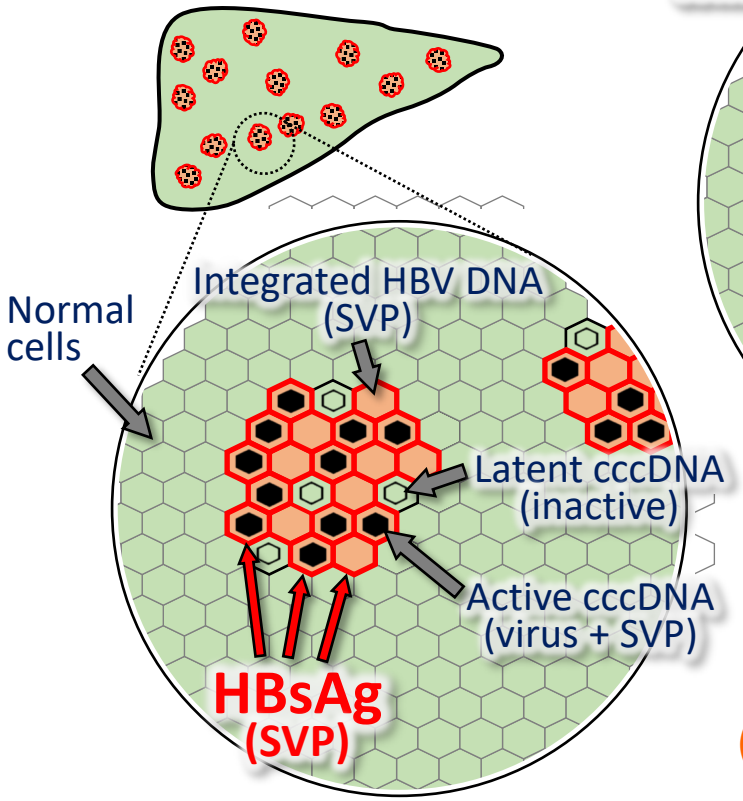
Getting rid of therapy in chronic HBV infection

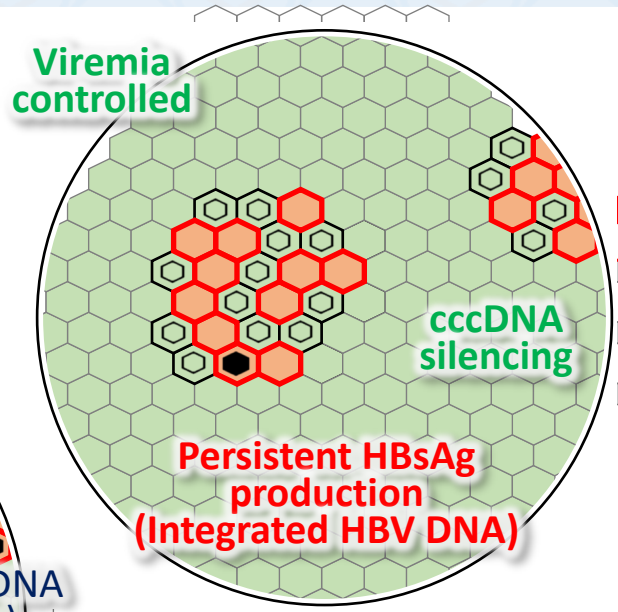
Chronic HBV



HBsAg blocks immune control

Progression of liver disease

Risk of HCC



Current Therapy

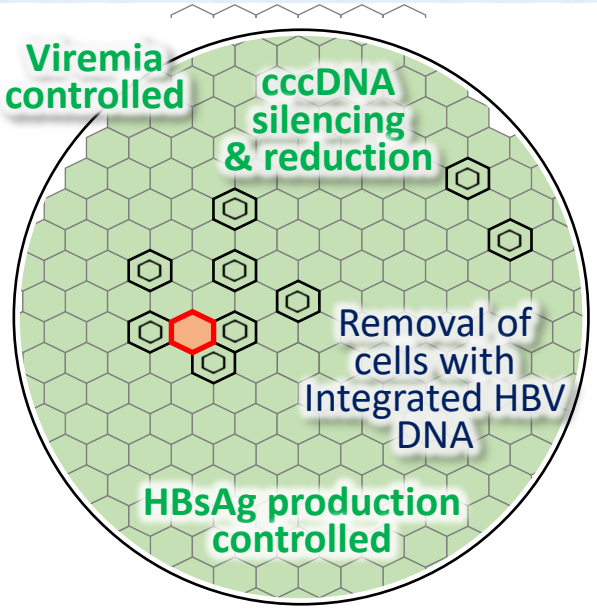
(NUCs)

No immune control

Life long therapy (viral rebound if removed)

Controlled progression of liver disease

Risk of HCC (late initiation of therapy)



Functional Cure

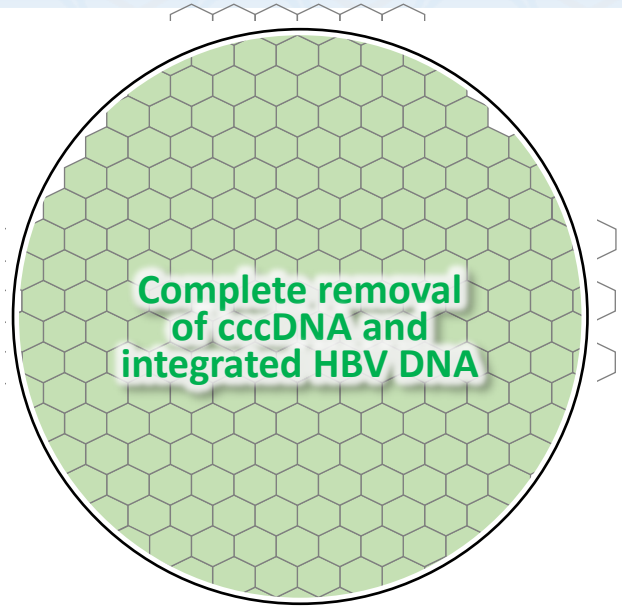
Restoration of immune control

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

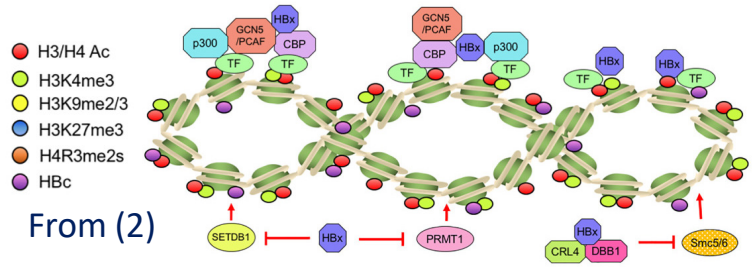
Reversal of liver disease

Reduced risk of HCC

Persistence of latent cccDNA is a high barrier

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]

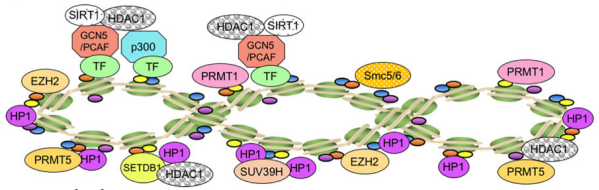
Rapid turnover (1-4 weeks)[3,4] → **rapid fixation of escape mutants to NUCs, CAMs and RNAi/antisense**

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

Targeting cccDNA inactivation / clearance

Capsid assembly modulators:

- Efficient HBV DNA and RNA declines are a result of their antiviral mechanism[1]
- negligible effects on cccDNA to date[1-5] (HBeAg, HBcrAg) in monotherapy
- **96% rebound rate following 12-18 months of vebicorvir (ABI-H0731) + NUC therapy[6]**

Can we do better with novel CAMs?

NUCs already have good activity against cccDNA!

1. Lahlali et al., AAC. 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. Tu et al., JHEP Reports 2020 3: 100195
6. https://investor.assemblybio.com/node/10876/pdf

NUCs target cccDNA inactivation / clearance

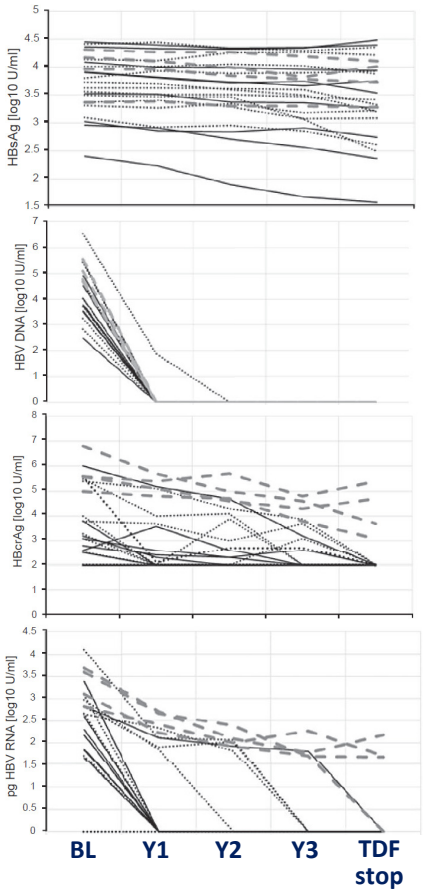
HBsAg
Production is independent from viral replication and cccDNA activity

HBV DNA
RT inhibition
+
cccDNA inhibition / removal

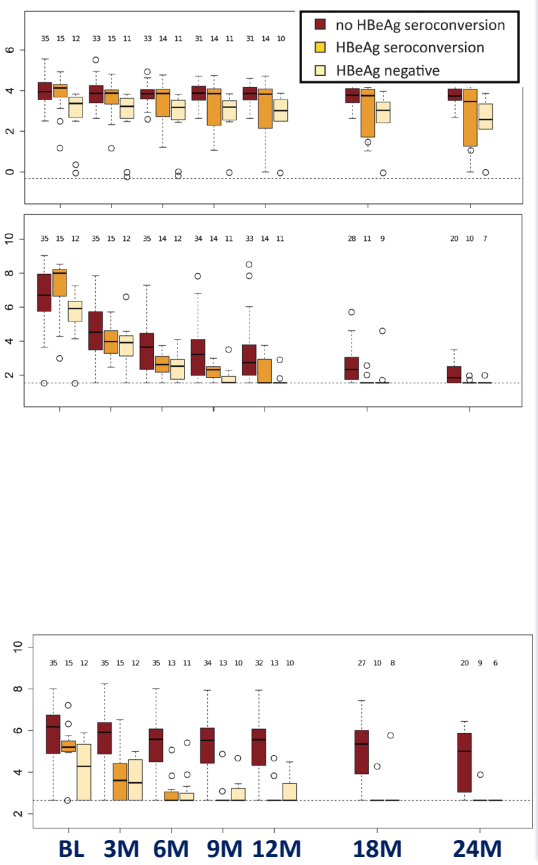
HBcrAg
cccDNA inhibition / removal

HBV RNA

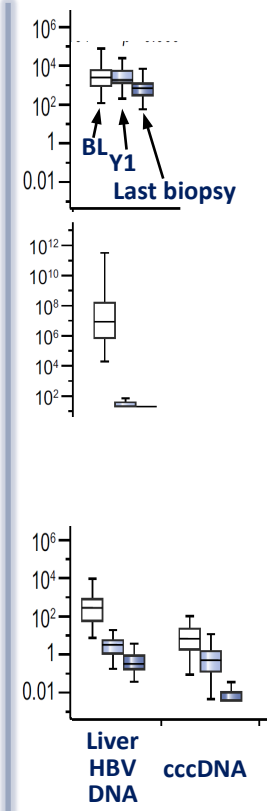
TDF[1]



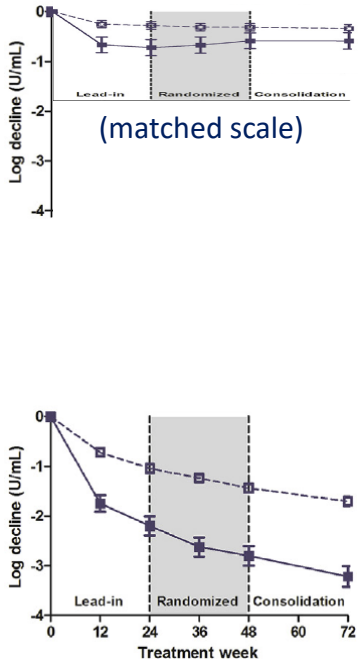
3TC or TDF[2]



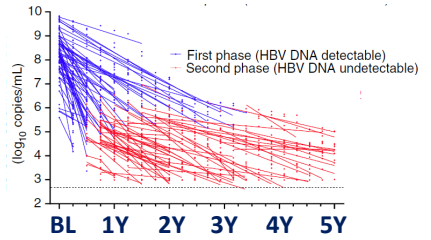
3TC, LdT, L-FMAU, ADV, ETV, or TDF[3]



ETV[4]



LdT, LdT + ADV[5]



Silencing of cccDNA within the first 12 weeks of therapy[2,5]

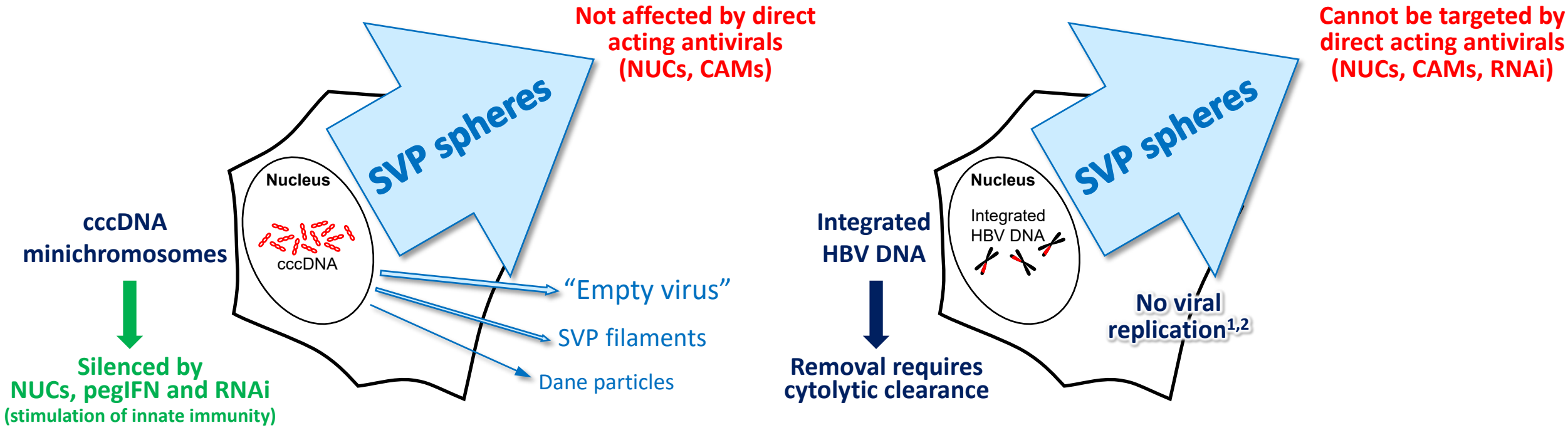
Reduction of cccDNA burden within 1st year of therapy[3] or with HBeAg seroconversion[6]

HBsAg persists during NUC therapy despite cccDNA inactivation/clearance

1. Carey et al., Hepatol. 2020 72: 42-57
2. Bommel et al., Hepatology 2015; 61: 66-76
3. Lai et al., J Hepatology 2017; 66: 275-281
4. Van Campenhout et al., Clin Microbiol Inf 2016; 22: 571.e6
5. Liu et al., Alimentary Pharmacol Therap 2020; 52: 692-700
6. Suslov et al. J Heptaol 2021; 74: 794-800

The challenge of controlling HBsAg production in the liver

Subviral particles (SVP) constitute 99.99% of circulating HBsAg



Present in HBeAg positive and negative infection[3]

Transaminase flares signal removal of integrated HBV DNA from the liver

1. Tu et al., Viruses 2017; 9: 75
2. Yang et al., J Cancer 2018; 9: 3225-3235
3. Budinska et al., Emerg Microb Inf. 2018; 7: 142

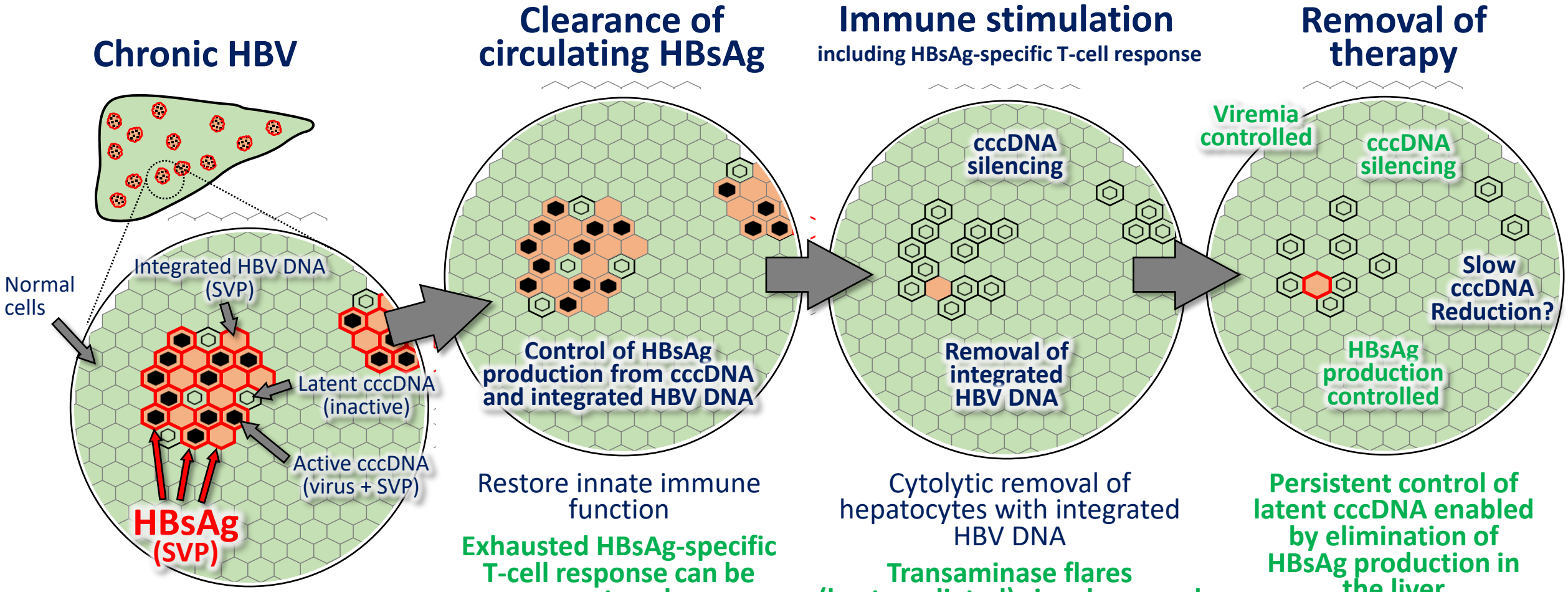
Production of excess SVP drives chronicity of HBV infection

Immunoinhibitory properties of SVP

Immune function	Target of inhibition	Effect observed
Innate	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	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

The path to functional cure



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 HBsAg still present
- Predicts clinical futility for achieving functional cure[1-3]

> 4 log_{10} IU/mL reduction from baseline → **Potent HBsAg loss 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 (dsRNA) 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

Approaches to targeting SVP

All oligonucleotide-based
Specific activity requires delivery to hepatocytes

GalNAc RNAi

Inhibition of HBsAg synthesis
Designed to engage RISC-mediated cleavage of HBV RNA
dsRNA stimulates innate immunity via TLR3 – *sequence independent!*[2-4]
Cannot be fully quenched without blocking RISC-loading![5]

GalNAc antisense

Inhibition of HBsAg synthesis
Designed to engage RNAse H mediated cleavage of HBV RNA
ssDNA (CpG) stimulates innate immunity via TLR9[2]
ssRNA (U-rich) stimulates innate immunity via TLR7[2]

Nucleic acid polymers

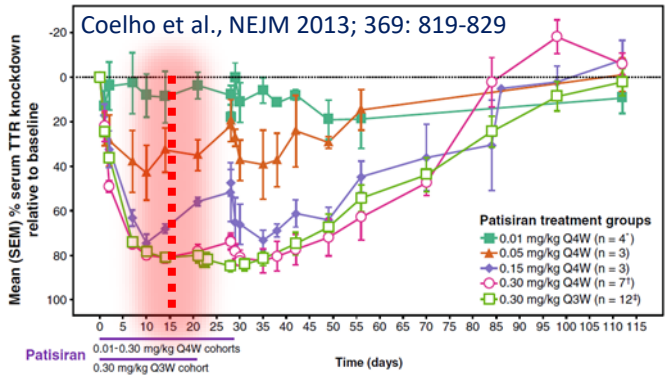
Single point mutation abolishes hydridization-dependent mRNA cleavage[1]

Similar pharmacodynamic response with optimal dosing

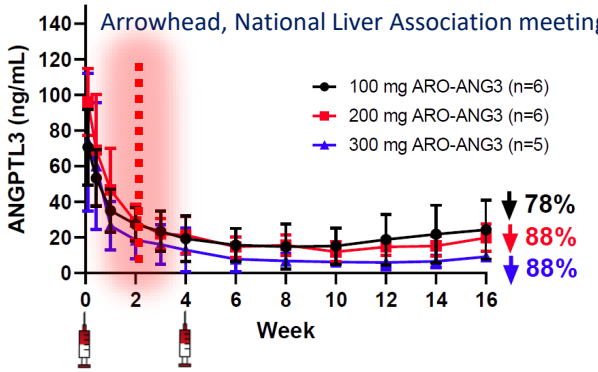
Well described ability to stimulate innate immunity

1. Vickers et al., J. Biol. Chem. 2003; 278: 7108-7118
2. Kawai and Akira Int Immunol 2009; 21: 317-337.
3. Robbins et al., Oligonucleotides 2009; 19: 89-101
4. Robbins et al., Hum Gene Ther 2008; 19: 991-999
5. Leuschner et al., EMBO Reports 2006; 7: 314-320

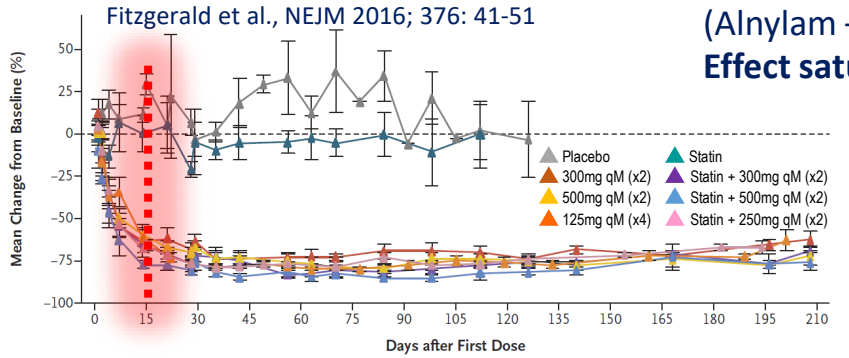
TLR-independent RNAi pharmacodynamic signatures (true RNAi effect)



LNP-RNAi
(Alnylam – PCKS9)
Effect saturated at 0.3 mg/kg



GalNAc-RNAi
(Arrowhead - ANGPTL3)
Effect saturated at 100mg



GalNAc-RNAi
(Alnylam – PCKS9)
Effect saturated at < 125mg

Very low interpatient variability in response

Saturated effect (~1 log_{10} decline) with multiple dosing 2 weeks after first dose

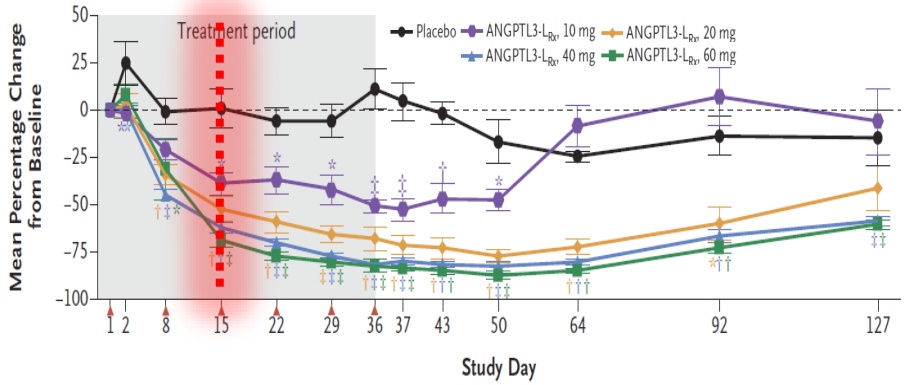
Residual intact target mRNA remains!

Seo et al., Cell Host & Microbe 2013; 14: 435-445

Cytoplasmic RISC competition with endogenous miRNA
Deactivation of RISC by TLR3 activation

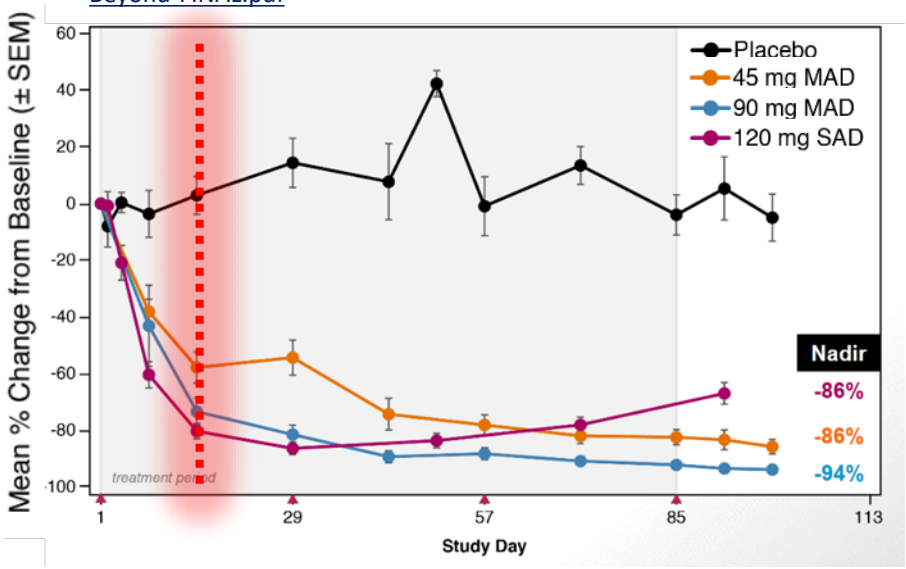
TLR-independent GalNAc-antisense pharmacodynamic signatures (true antisense effect)

Graham et al., NEJM 2017; 377: 222-232



GalNAc-antisense
(Ionis / Akcea = ANGPTL3)
Effect saturated at 40mg

https://akceatx.com/wp-content/uploads/Tsimikas_EuATTR_Tegsedi-Beyond-FINAL.pdf



GalNAc-antisene
(Ionis / Akcea = TTR)
Effect saturated at 90mg

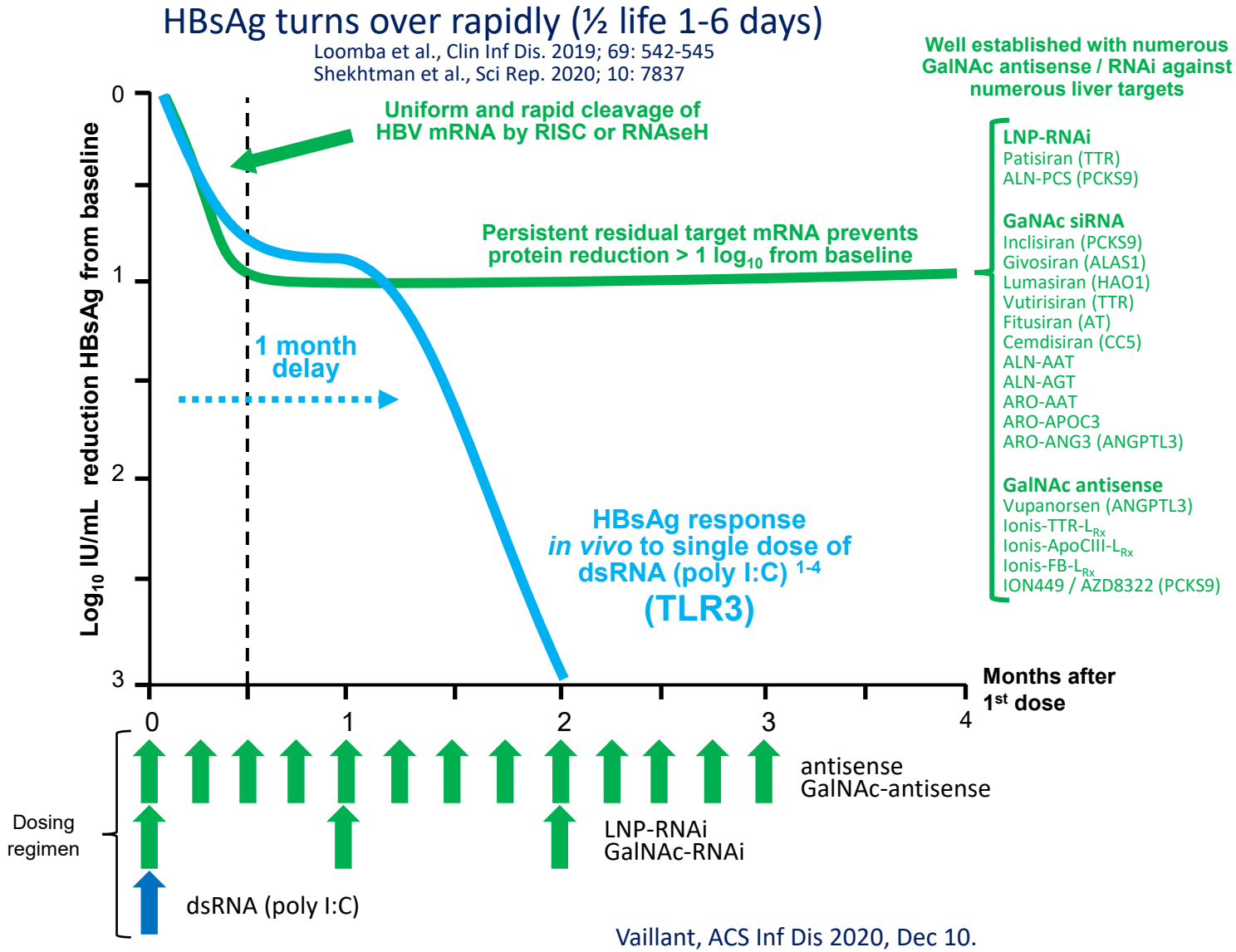
Very low interpatient variability in response

Saturated effect (~1 log_{10} decline) with multiple dosing 2 weeks after first dose

Intact residual target mRNA remains!
Antisense is consumed in RNAseH mediated target mRNA cleavage

Equivalent pharmacodynamic response as RNAi

Expected HBsAg response with efficient target engagement by antisense / RNAi



Vaillant, ACS Inf Dis 2020, Dec 10.

< 1 log_{10} HBsAg decline in 2 weeks:
No hybridization with HBV mRNA
RNAi escape mutations present

> 1 log_{10} decline in HBsAg:
additional off-target effects

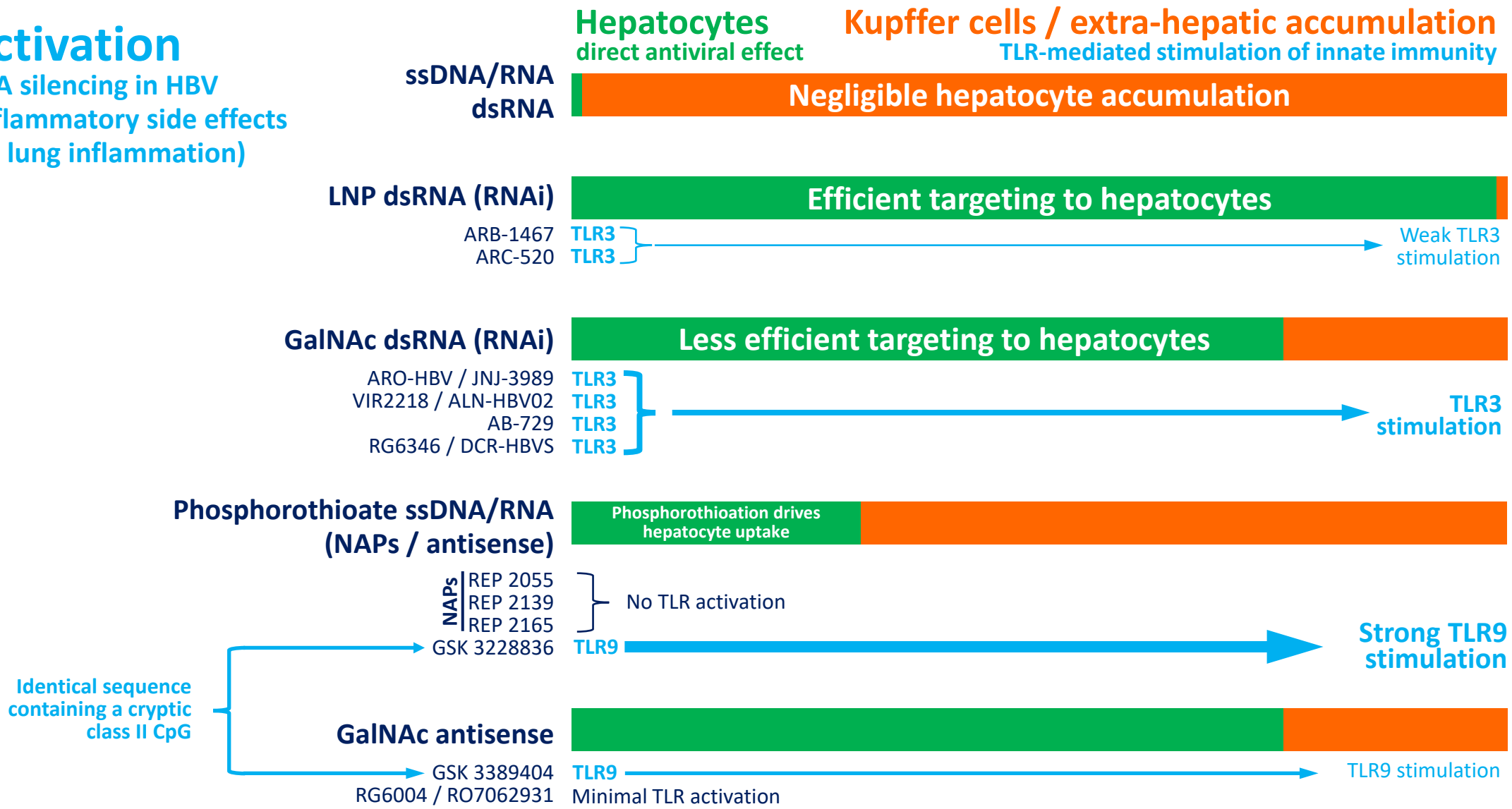
Delayed > 1 log_{10} HBsAg response :
dsRNA stimulation of TLR3
innate inactivation of cccDNA

1. Du et al., Acta Biomater 2021 1: S1742-706(21)00073
2. Wu et al., J Virol 2014; 10421-10431
3. Chen et al., Oncol Rep 2012; 28: 200-206
4. Ijichi et al., J Med Vriol 1994; 43: 161-165

Oligonucleotide partitioning influences TLR activation

TLR activation

- cccDNA silencing in HBV
- pro-inflammatory side effects (TLR3 - lung inflammation)



GSK 3228836 and 3389404

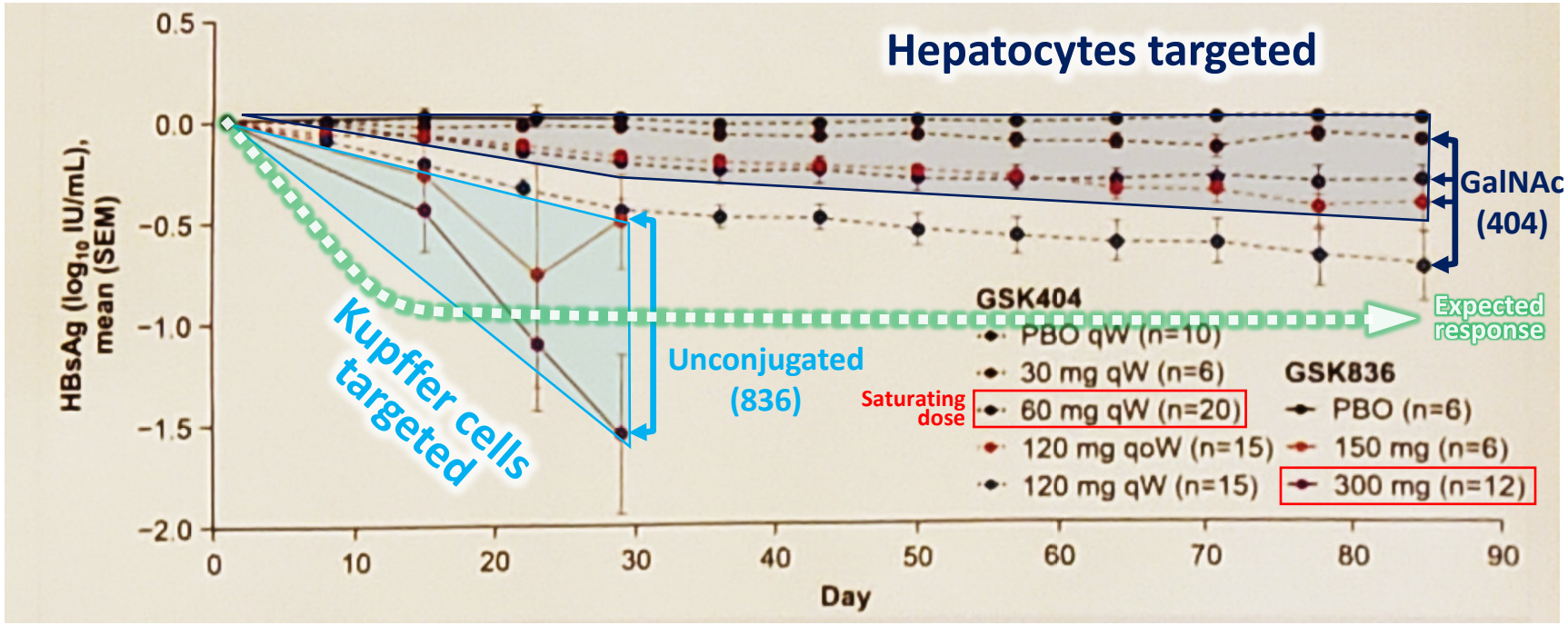
antisense targeting HBx → targets mRNA from cccDNA and integrated HBV DNA

Cryptic class B CpG
(TLR9 stimulation)

Enriched in hepatocytes → GalNAc-GCAGAGGTGAAGCGAAGTCG **GSK 3389404 / IONIS HBV$_{Rx}$ (GSK 404)**

Enriched in Kupffer cells (more TLR reactive) → GCAGAGGTGAAGCGAAGTCG **GSK 3228836 / IONIS HBV-L$_{Rx}$ (GSK 836)**

2'MOE 2'MOE



GalNAc drives hepatocyte targeting
Weak HBsAg response indicates HBV mRNA is not efficiently cleaved

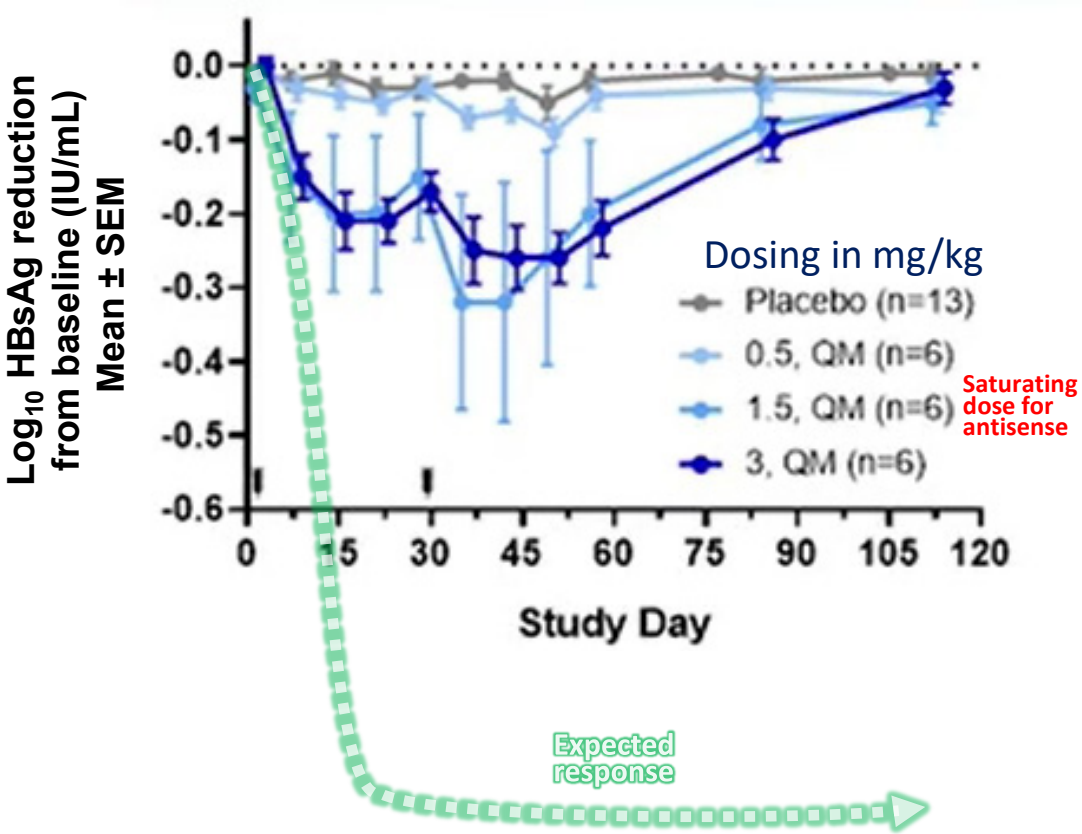
Development halted!

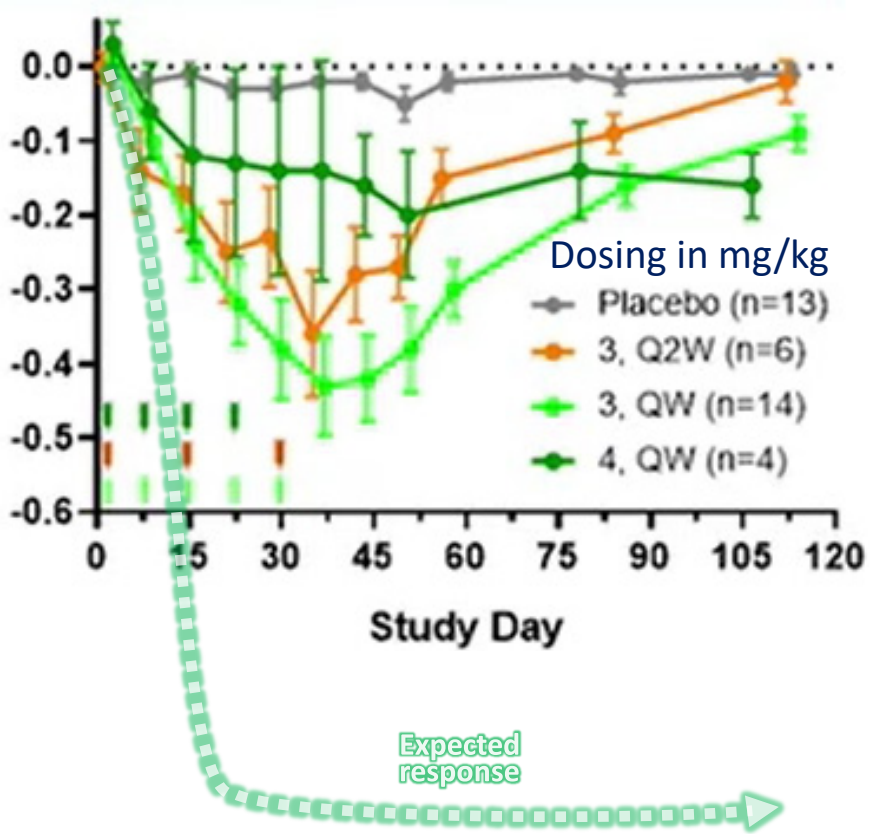
Kupffer cells targeted without GalNAc
Strong HBsAg response limited to 3 patients with low HBsAg at baseline
Consistent with TLR9 stimulation of innate immunity (cccDNA silencing)

Theodore et al., HEPDART 2019 O15

Roche RG6004 (RO7062931)

GalNAc LNA antisense gapmer targeting HBx→ targets mRNA from cccDNA and integrated HBV DNA





Highly variable and weak HBsAg response indicates minimal cleavage of HBV mRNA

Escape mutants present at baseline or rapidly fixed via cccDNA turnover

Development of RG6004 / RO7062931 halted

Yuen et al., J Hepatology 2020 73: S51

ARB-1467 (TKM-HBV)

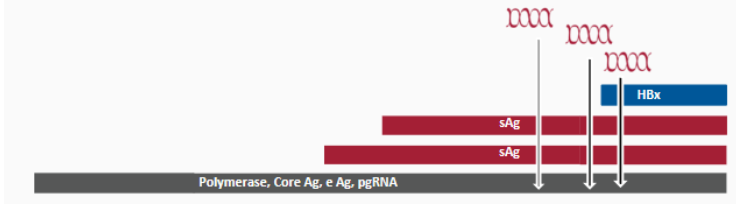
LNP: one RNAi targeting HBx and two RNAi targeting HBsAg (+ETV or TDF)

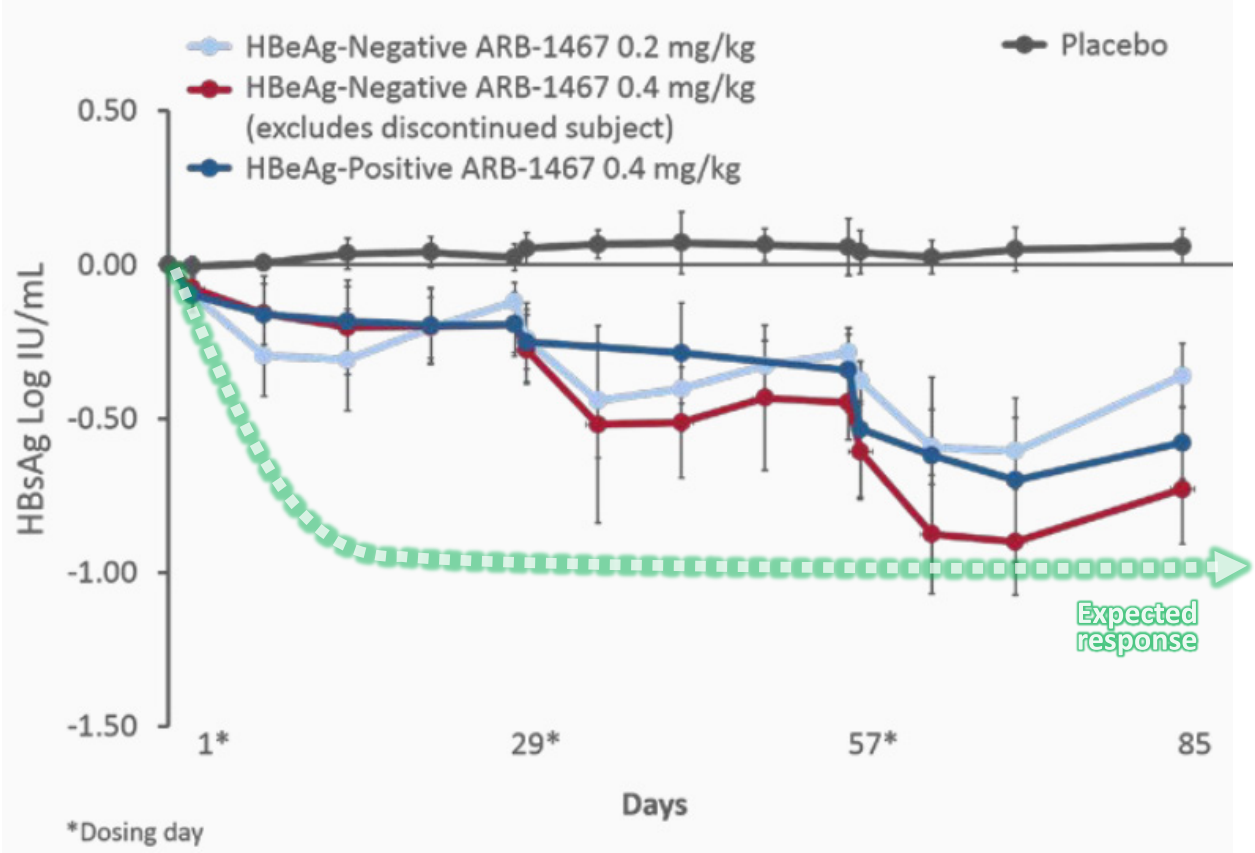
Optimum design for efficacy:

High efficiency targeting to hepatocytes (LNP)

Targeting of HBsAg (two loci) and HBx: covers cccDNA and integrated HBV DNA

best effort against mutational escape





Highly variable and weak HBsAg response indicates minimal cleavage of HBV mRNA

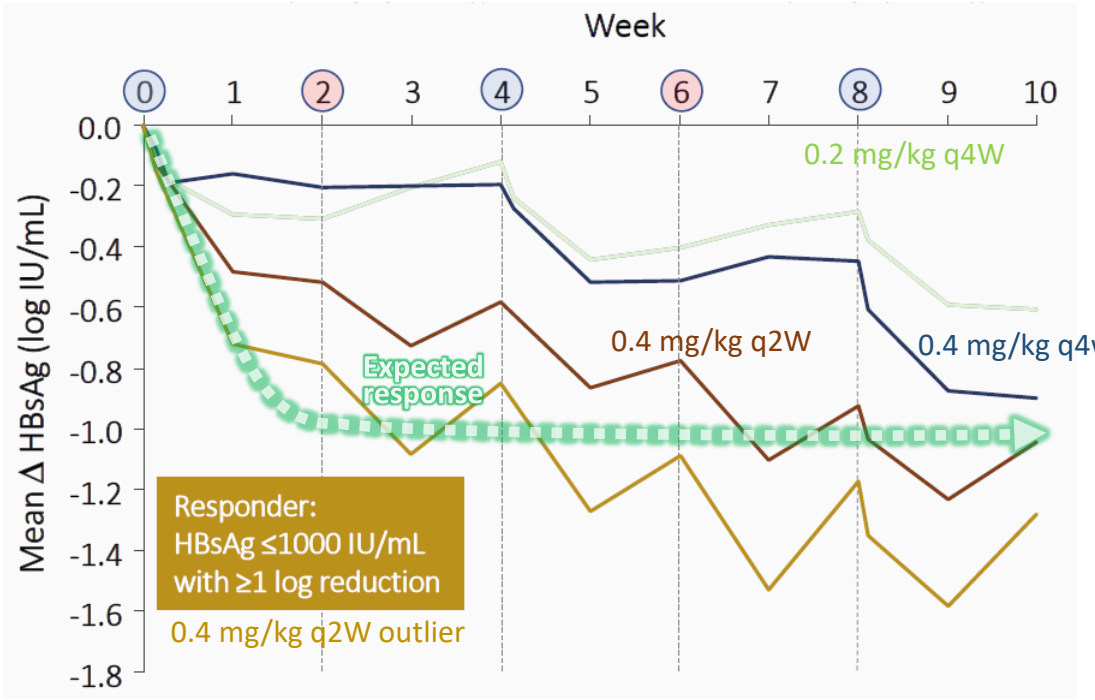
Escape mutants present at baseline or rapidly fixed via cccDNA turnover

Eley et al., Hepatology 2017; 66: 23A

ARB-1467 (TKM-HBV)

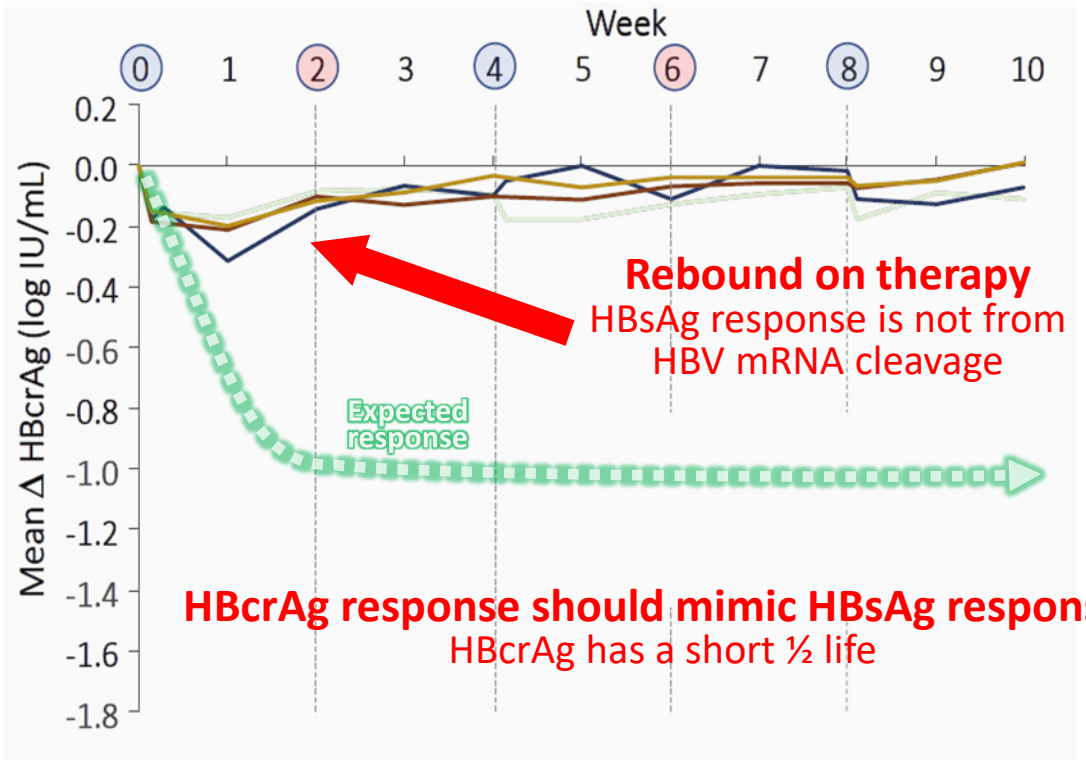
LNP: one RNAi targeting HBx and two RNAI targeting HBsAg (+ETV or TDF)

HBsAg



Weak HBsAg response indicates HBV mRNA not efficiently cleaved

HBcrAg



Agarwal et al., Hepatology 2017; 66: 22A

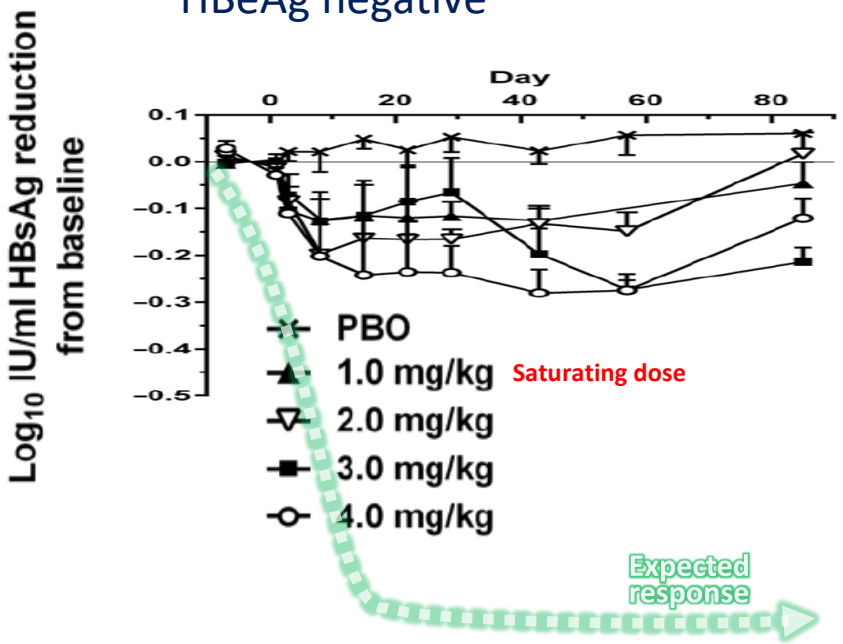
Development of ARB-1467 halted

ARC-520 (single dose)

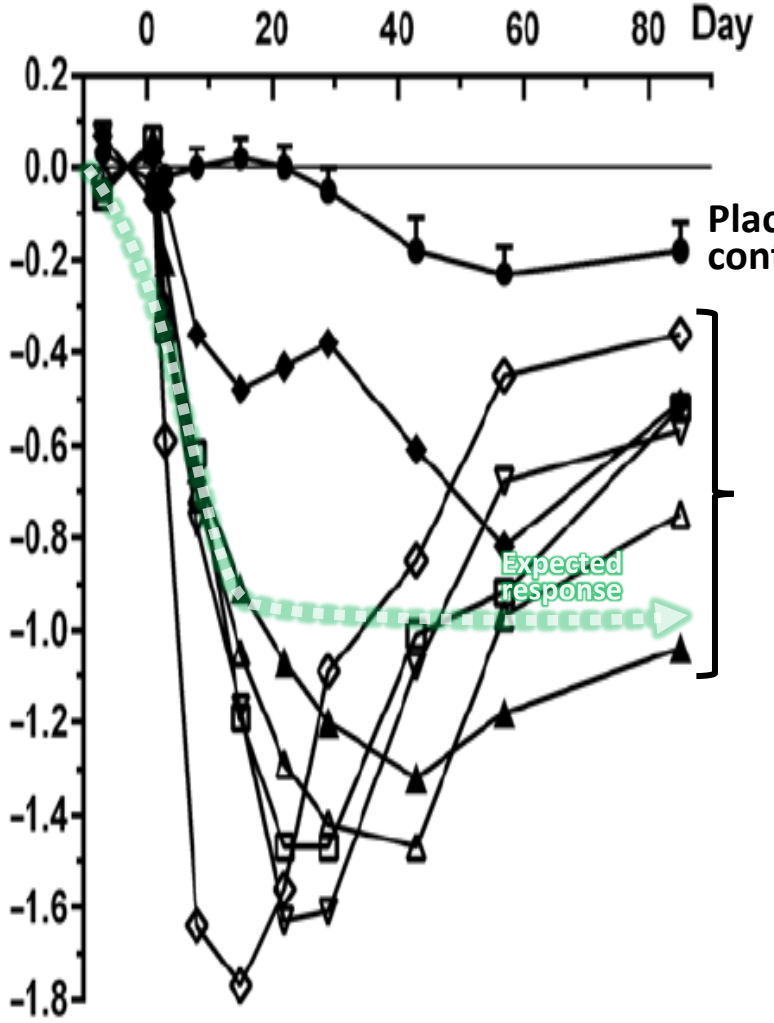
LNP: two RNAi targeting HBsAg (+ETV or TDF)

High efficiency targeting to hepatocytes

HBeAg negative



Variable and weak response
HBV mRNA is not being cleaved



HBeAg positive (scale matched)
4.0 mg/kg!

Pure RNAi effect should be equivalent in HBeAg negative and positive

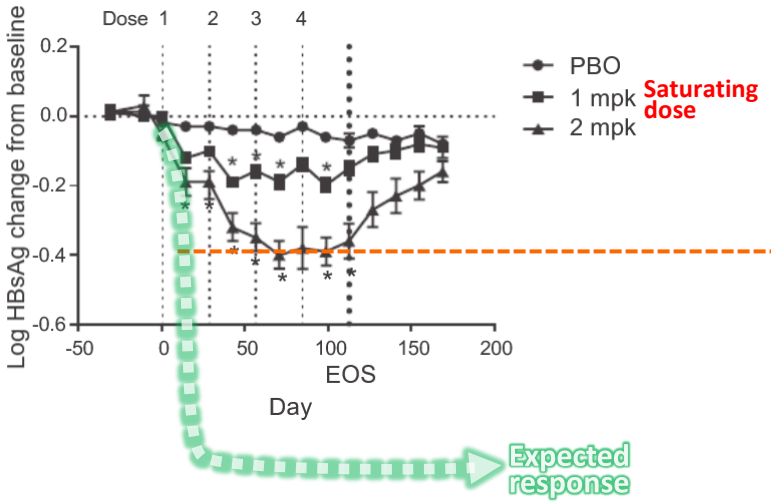
HBeAg positive patients respond better to immunotherapy

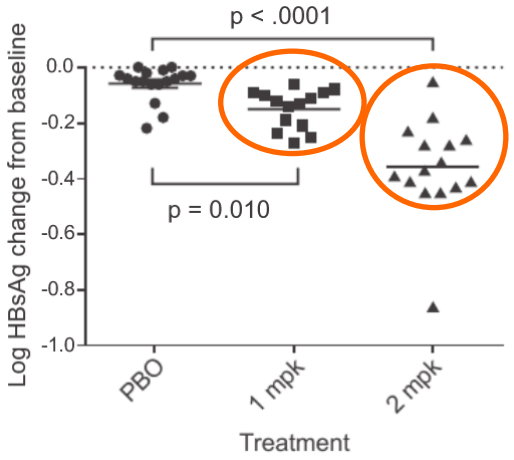
Wooddell et al., Sci Trans Med 2017 9: eaan0241

ARC-520 (multiple dose)

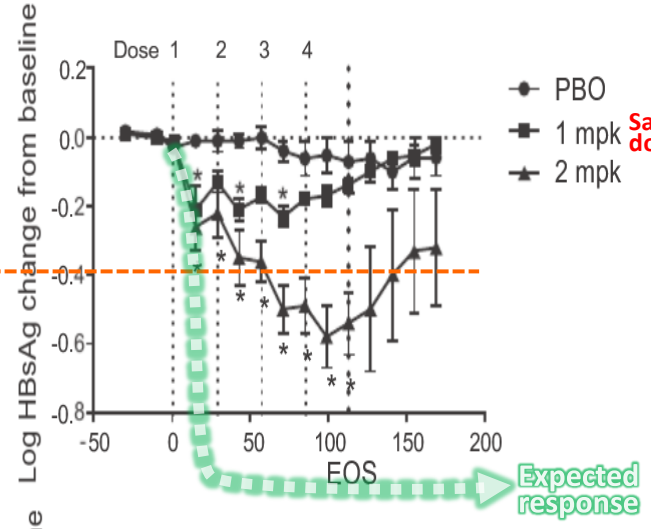
LNP: two RNAi targeting HBsAg (+ETV or TDF)

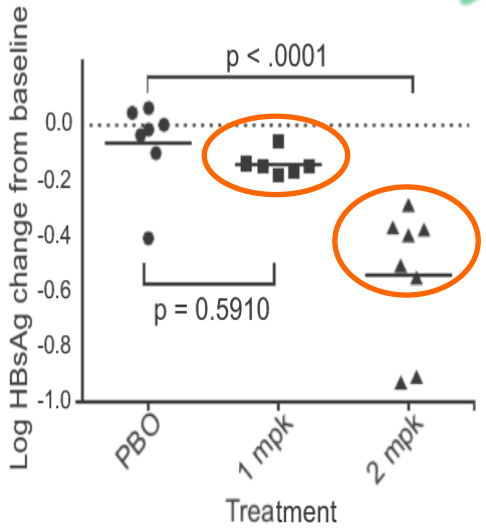
HBeAg negative





HBeAg positive (scale matched)





Effect is still stronger in HBeAg positive participants

Variable and weak response indicates mRNA is not being targeted

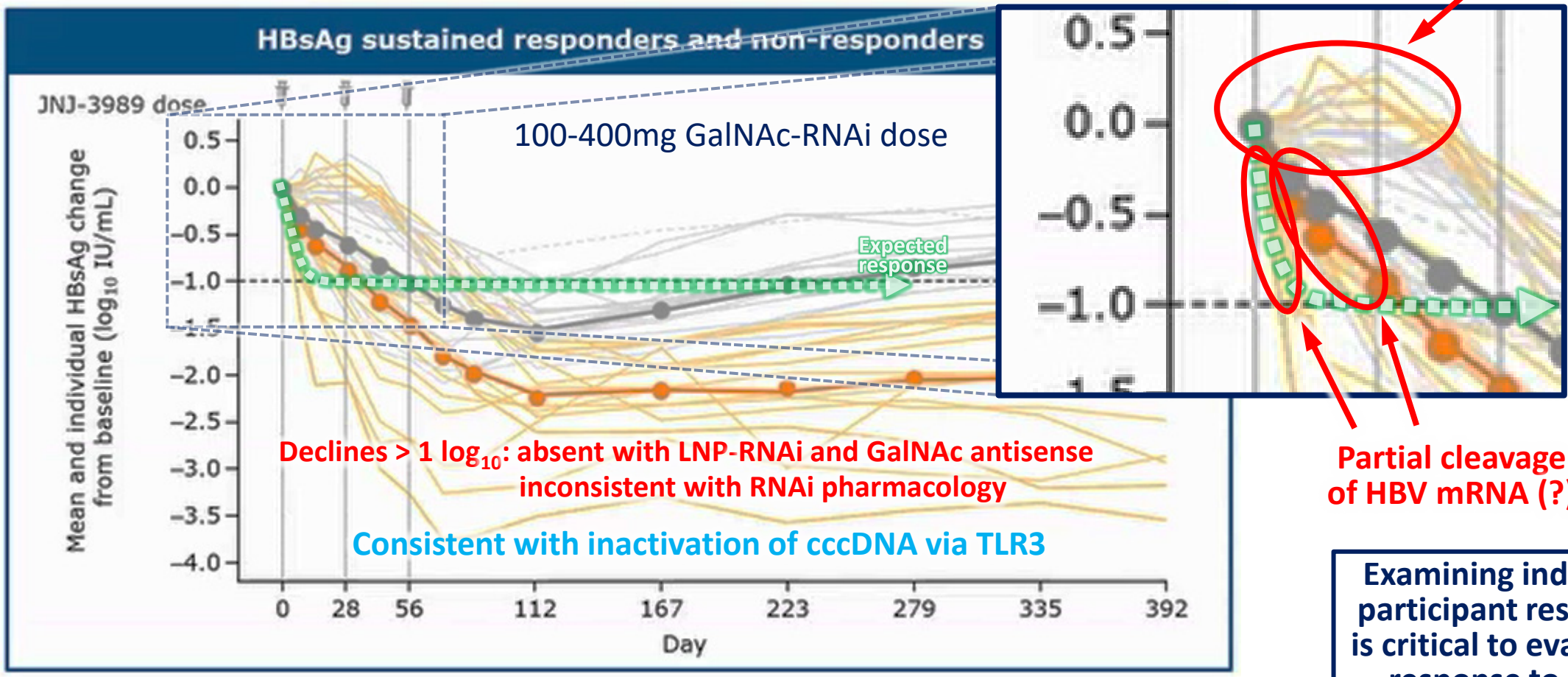
ARC-520 / ARC-521 abandoned
(LNP toxicity issues)

Yuen et al., Hepatology 2020 72: 19-31

JNJ-3989 (ARO-HBV)

Two GalNAc-RNAi targeting HBx and HBsAg (+ETV)

GalNAc vs LNP RNAi: Weaker hepatocyte targeting / **greater TLR activation**



No HBsAg decline
No cleavage of HBV mRNA

Partial cleavage of HBV mRNA (?)

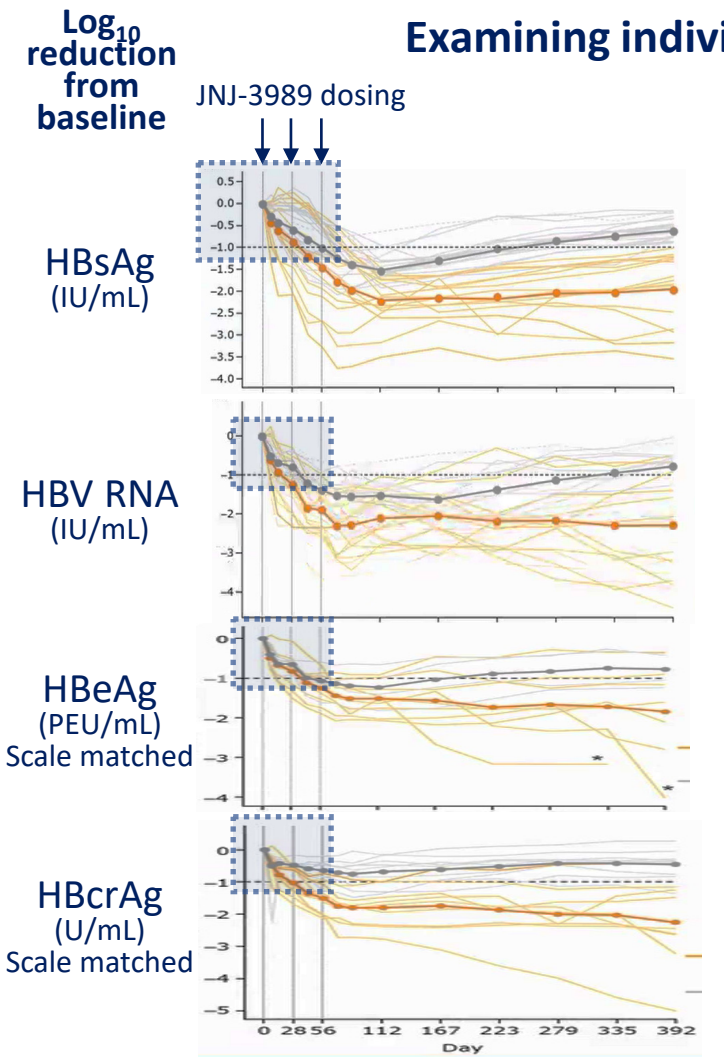
Examining individual participant responses is critical to evaluating response to RNAi

Gane et al., J Hepatology 2020;73: S20

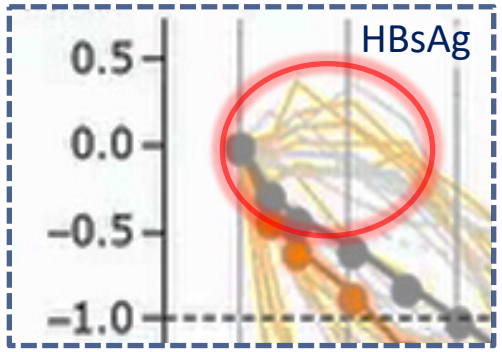
JNJ-3989 (ARO-HBV)

Two GalNAc-RNAi targeting HBx and HBsAg (+ETV)

Examining individual participant responses is critical to evaluating response to RNAi

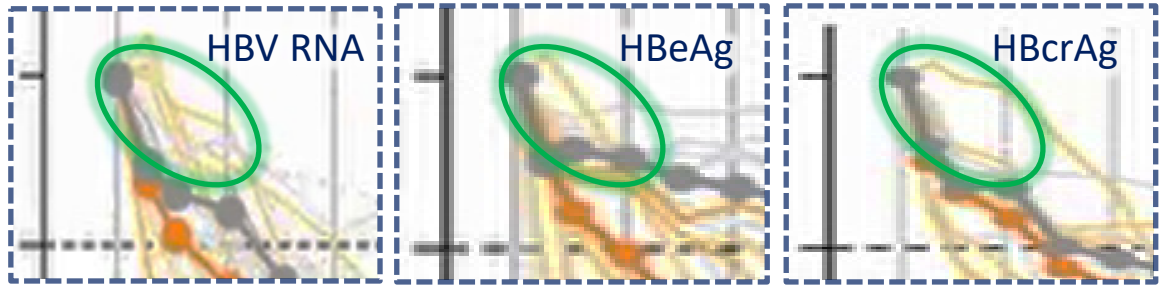


No HBsAg response
No cleavage of HBV mRNA



cccDNA
+
integrated HBV DNA

cccDNA silencing



cccDNA

Initial biomarker response similar to NUCs!

Consistent with inactivation of cccDNA without affecting integrated HBV DNA

Gane et al., J Hepatology 2020;73: S20

JNJ-3989 (ARO-HBV)
GalNAc-RNAi + ETV + CAM (JNJ-6379)

LP4 – AASLD 2019

400mg GalNAc RNAi dosing!

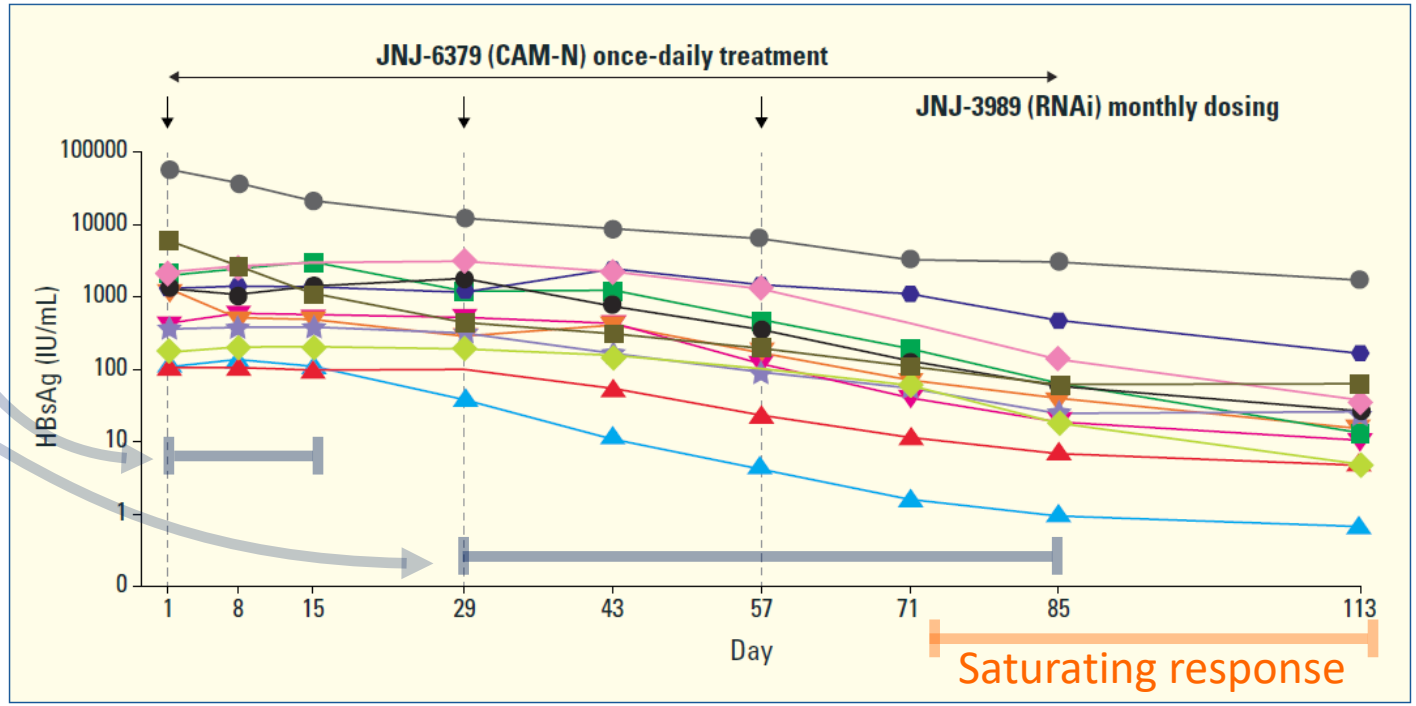
HBsAg response during first 2 weeks
- absent (7/12 participants)
- << 1 log_{10} from baseline (12/12 participants)

Delayed HBsAg response > 4 weeks
- 12/12 participants

HBV mRNA cleavage negligible or absent

HBsAg response consistent with TLR3 stimulation / cccDNA silencing

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-mediated lung inflammation[1-3]

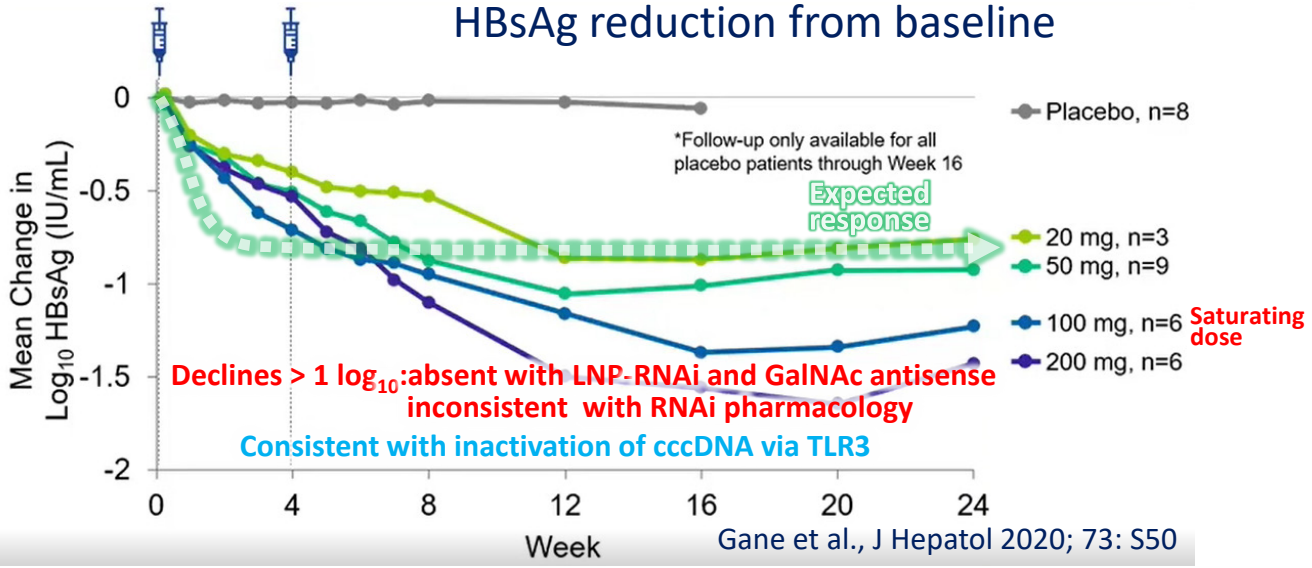
Also observed for ARC-520 (LNP), ARO-AAT (GalNAc) and ALN-AAT (GalNAc)

Increased cytokine responses in humans consistent with TLR3 activation observed with ARC-520[4-7]

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

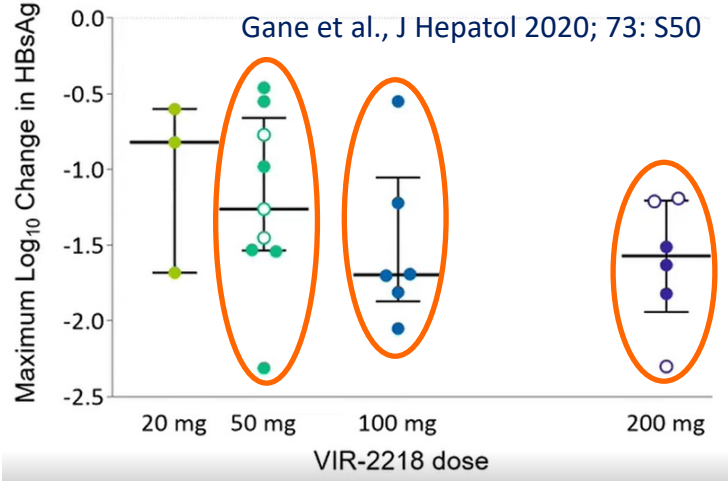
VIR-2218 (ALN-HBV02)
GalNAc-RNAi targeting HBx (+ETV)

HBsAg reduction from baseline



Gane et al., J Hepatol 2020; 73: S50

Hypervariable response in HBsAg

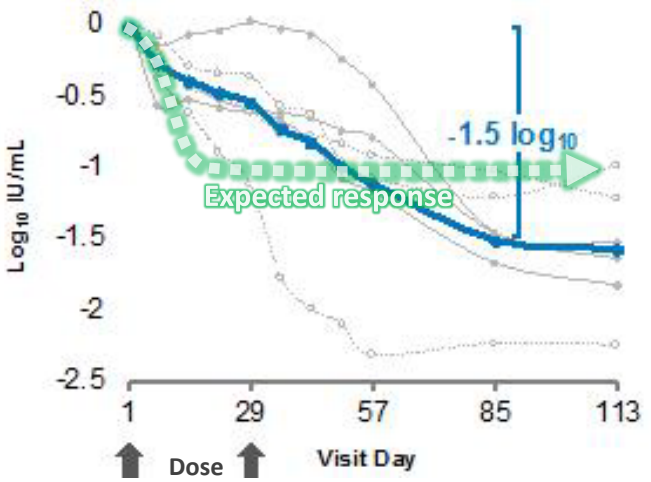


Gane et al., J Hepatol 2020; 73: S50

HBsAg response during first 4 weeks
- Absent (1/6 participants)
- << 1 log_{10} from baseline (4/6 participants)

Delayed HBsAg response > 4 weeks
- 6/6 participants
- Hypervariable responses

200mg Individual participant responses
Vir SEC filing March 2020



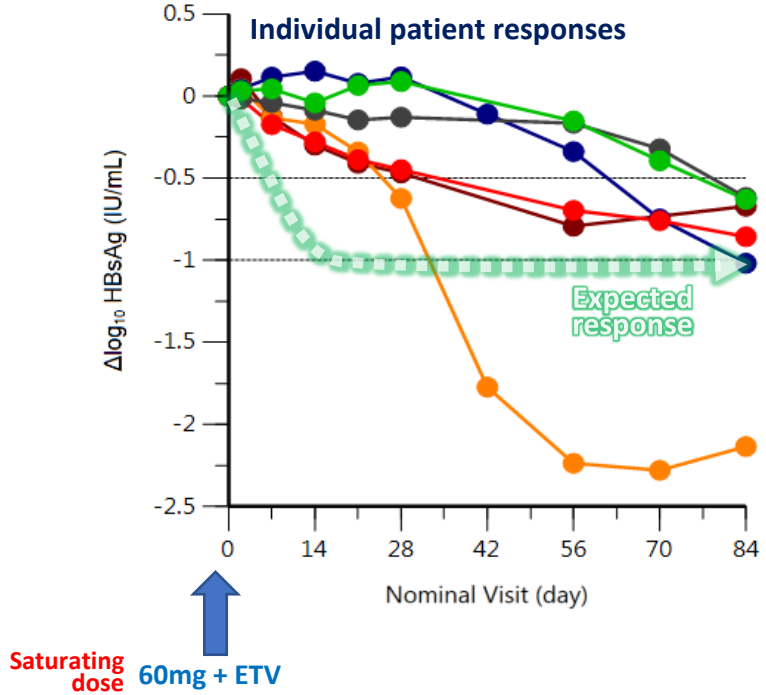
HBV mRNA cleavage minimal or absent in most patients

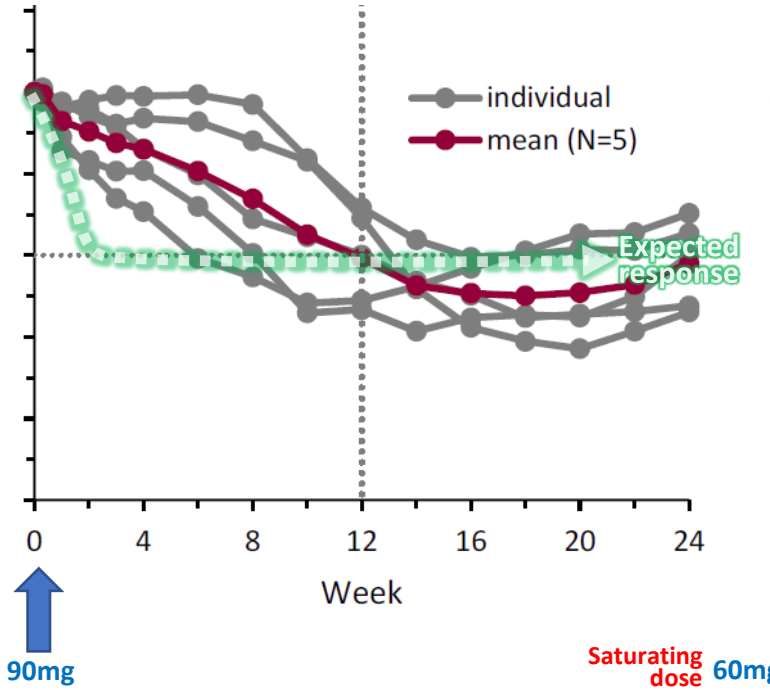
Delayed HBsAg response consistent with TLR 3 simulation

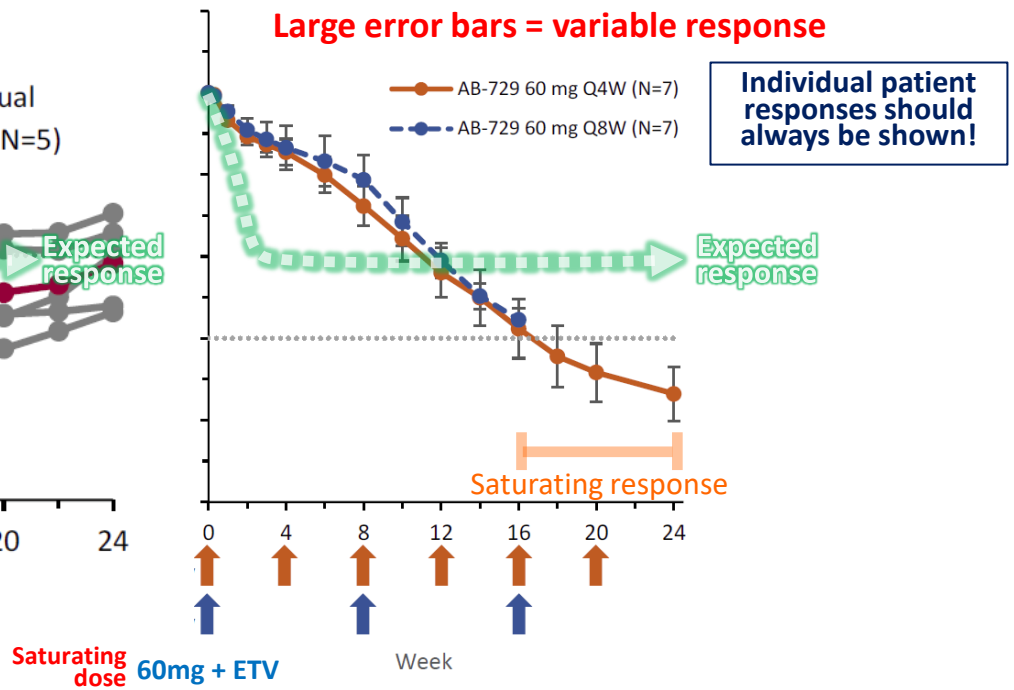
Grade 3 upper respiratory tract infection reported!
(likely TLR3-mediated)

AB-729
GalNAc-RNAi targeting HBx

HBsAg reduction from baseline







HBsAg response during first 2 weeks

- Absent (3/6 @60mg, 2/5 @ 90mg)
- << 1 $\log_{10}$ from baseline (all participants)

Delayed HBsAg response > 4 weeks

- All participants

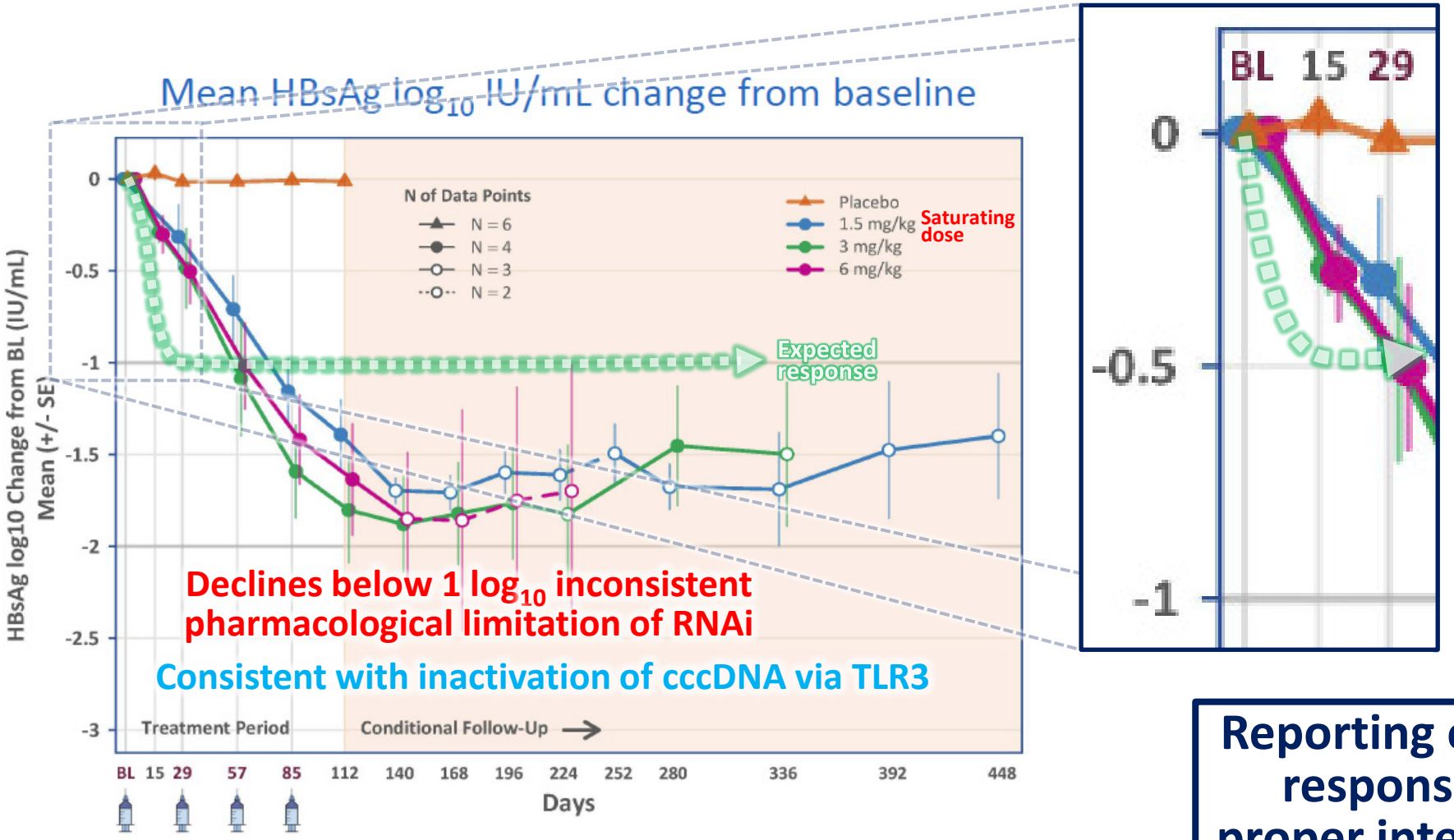
HBV mRNA cleavage minimal or absent in most patients

Delayed HBsAg response > 1 $\log_{10}$ from baseline consistent with TLR 3 simulation

http://www.arbutusbio.com/portfolio/ab-729-galnac-rnai.php
Gane et al., APASL 2021

RG6346 (DCR-HBVS)

GalNAc-RNAi targeting HBsAg (+ NUCs)



Large error bars indicate highly variable HBsAg response

Overall weak HBsAg response indicates mRNA cleavage is minimal or absent

Reporting of individual patient responses is CRITICAL for proper interpretation of HBsAg response to RNAi / dsRNA

Yuen et al., AASLD 2020, LO-9

RNAi / antisense for HBV (???)
The clinical story so far….

HBsAg response is much stronger with targeting of RNAi to Kupffer cells / periphery (GalNAc)

- **Responses should be much stronger with targeting to hepatocytes with RNAi effect**
- Indicates an immunostimulatory mechanism is involved in HBsAg response to RNAi

HBsAg response to antisense is much weaker than RNAi with optimal dosing

- **Response should be equivalent! Weak HBsAg response to antisense confirms lack of RNAi effect**

HBsAg response is weak or absent 2 weeks following start of antisense / RNAi in most patients

- **No cleavage of HBV mRNA is occurring – no targeting of integrated HBV DNA**
- **Escape mutants are present at baseline or rapidly evolve during treatment**
- Consistent with prevalence of numerous HBV quasispecies and rapid turnover of cccDNA

HBsAg response > 1 $\log_{10}$ from baseline (after 1 month) in all patients with GalNAc-RNAi

- **Inconsistent with well characterized pharmacological limitation of RNAi in humans**
- **Absent with LNP-RNAi (more efficient hepatocyte targeting and minimal TLR exposure)**
- **Consistent with TLR3 mediated HBsAg response to dsRNA *in vivo***
- **Consistent with TLR3-mediated side effects and cytokine signalling observed with RNAi in humans**
- **Indicates HBsAg responses to RNAi are driven by TLR3-mediated cccDNA silencing**

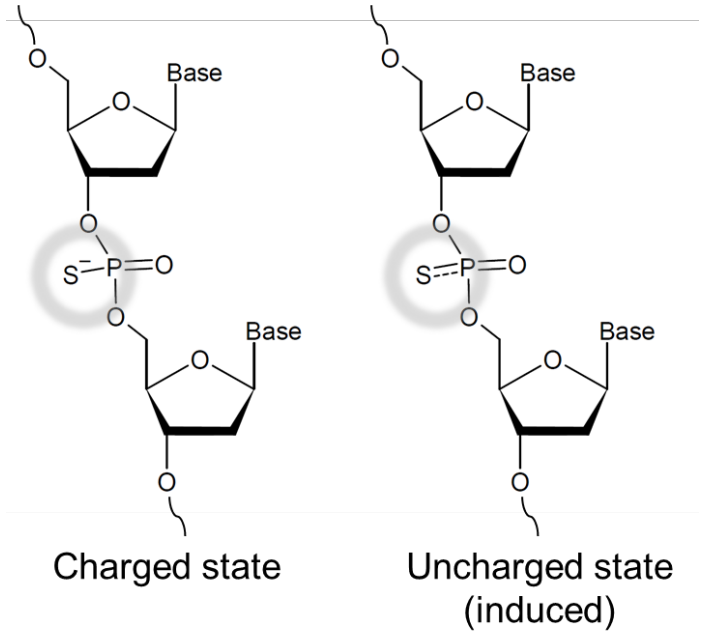
Moving forward to correctly interpret RNAi / dsRNA clinical data in HBV

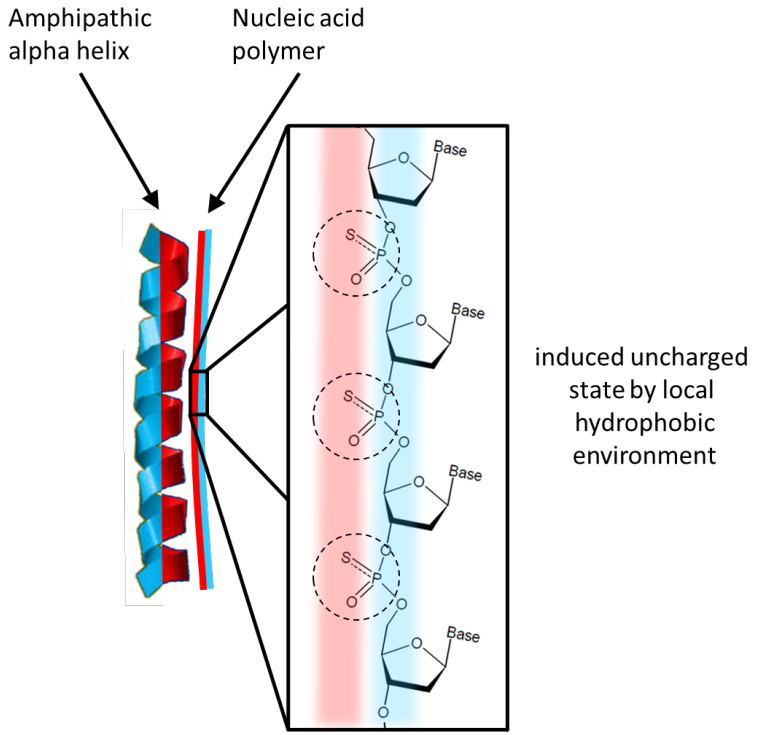
- **RNAi = dsRNA = TLR3 stimulation (sequence independent, cannot be blocked without losing RNAi activity)**
- Disclose individual patient responses to HBsAg, HBV RNA, HBcrAg, and HBeAg, separate according to HBeAg status
- **Exclude patients with HBsAg < 1000 IU/mL (these will respond better to TLR3 stimulation)**
- Disclose correlation between baseline HBsAg and HBsAg response on therapy

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

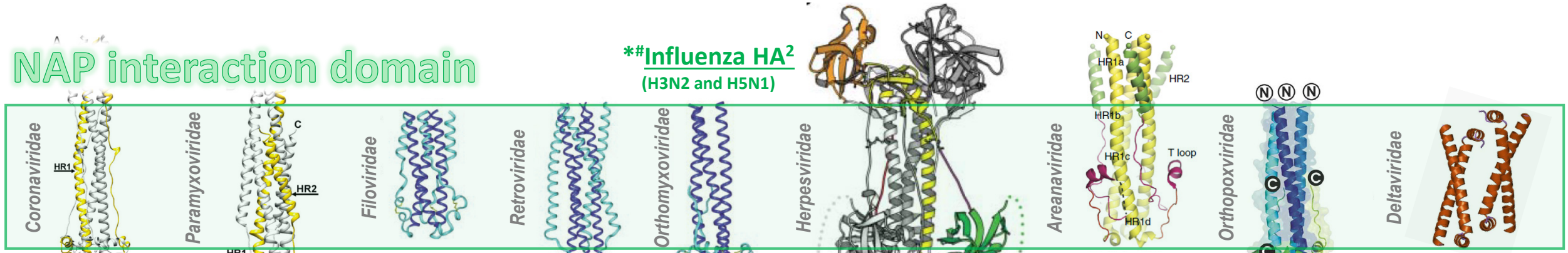
HBV, HDV, HCV, HSV, CMV, RSV, PI-3, LCMV, HIV, Influenza, Ebola, Marburg, Lassa Fever, Coronaviruses, prion disease + others

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

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)

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

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

Vaccinia A27[12]
*Ectromelia
(Smallpox)

#HDAg[13]
Hepatitis Delta

No equivalent structure in viral proteins for HBV

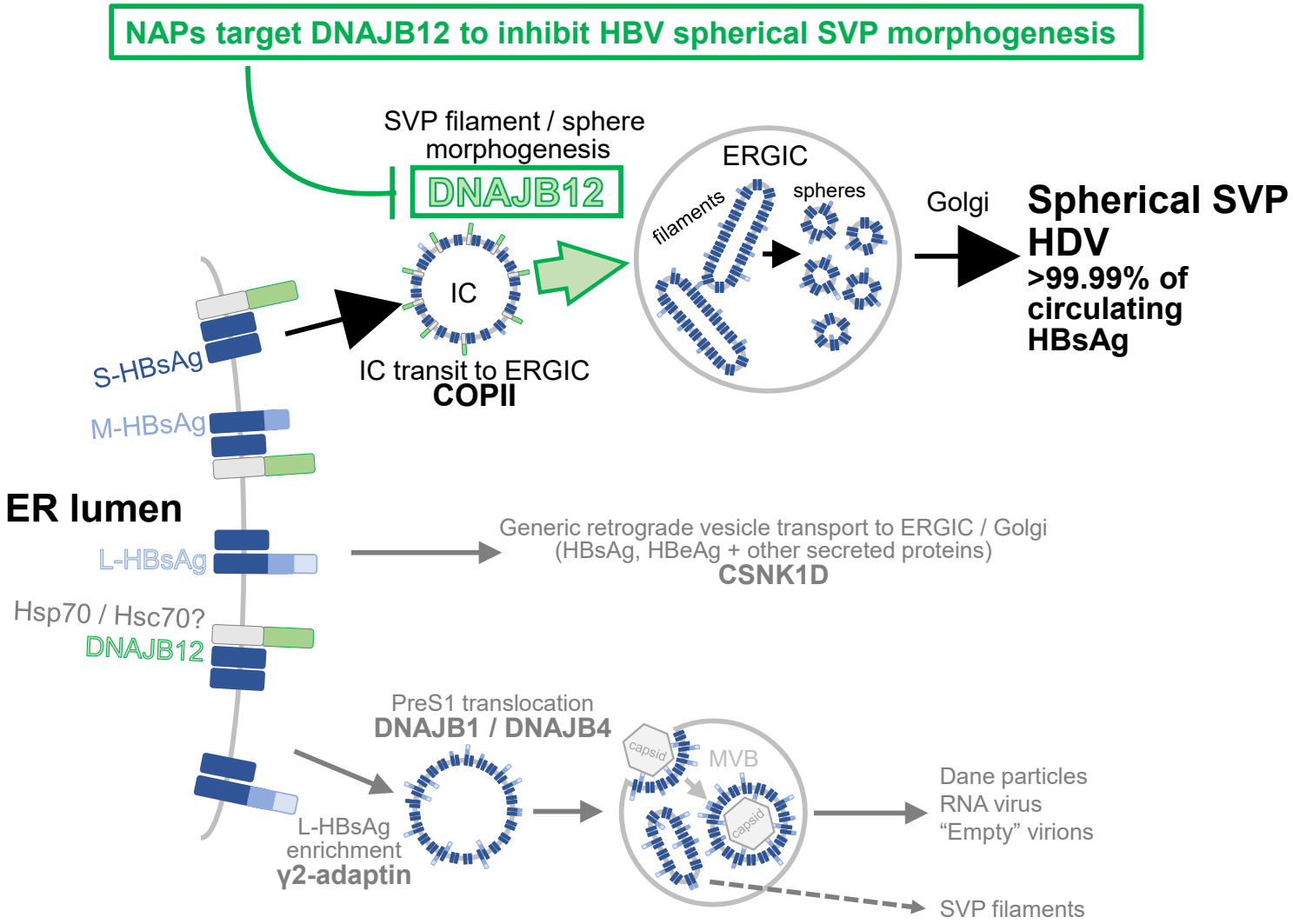
NAPs do not interact with any HBV proteins

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

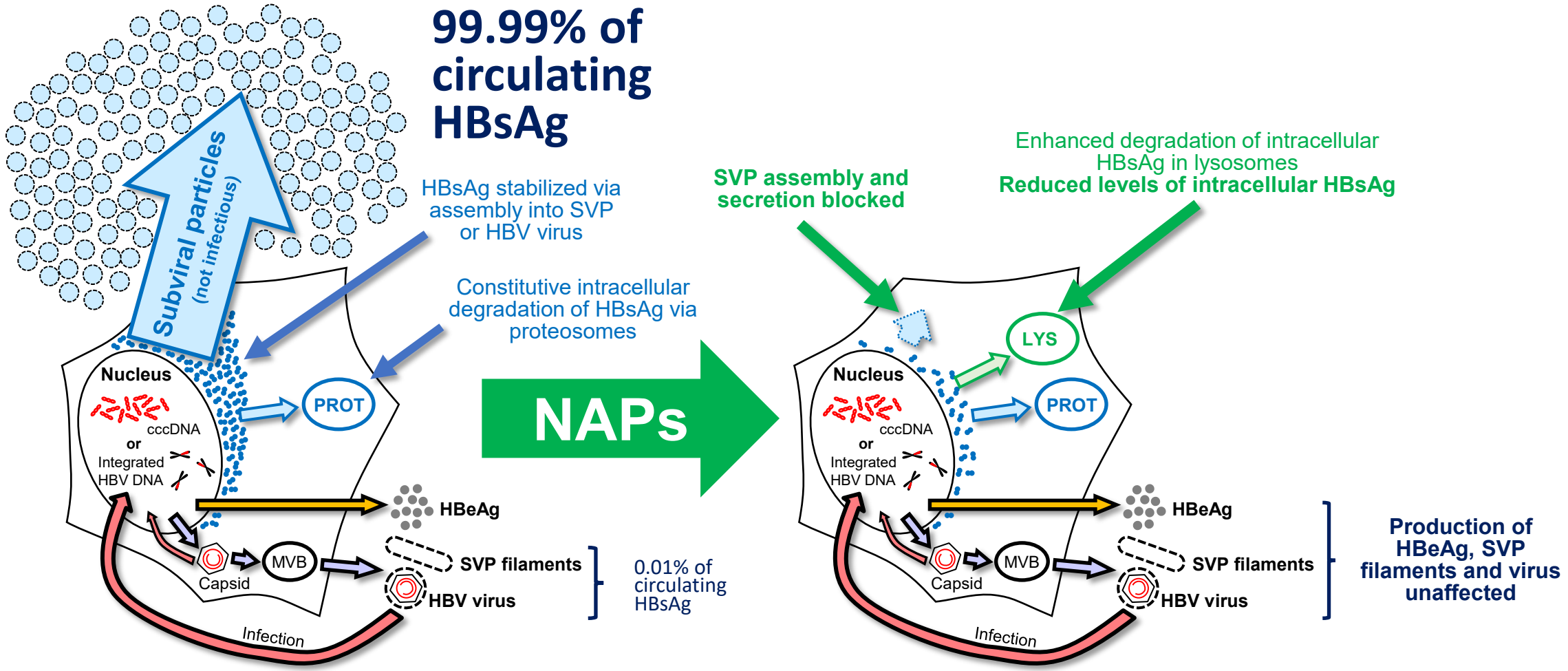
Mechanism of action of NAPs in HBV



Amphipathic α-helical interactions in the J-domain of DNAJB12 consistent with conserved target interface

Boulon et al., AASLD 2020 LP-42

Antiviral effects of NAPs



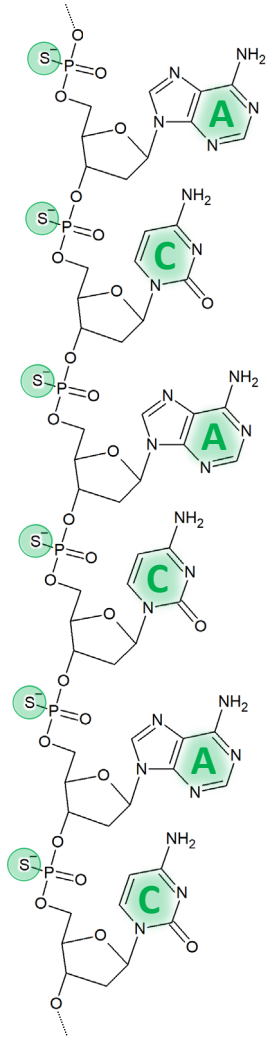
Boulon et al., Antiviral Res. 2020; 183: 104853

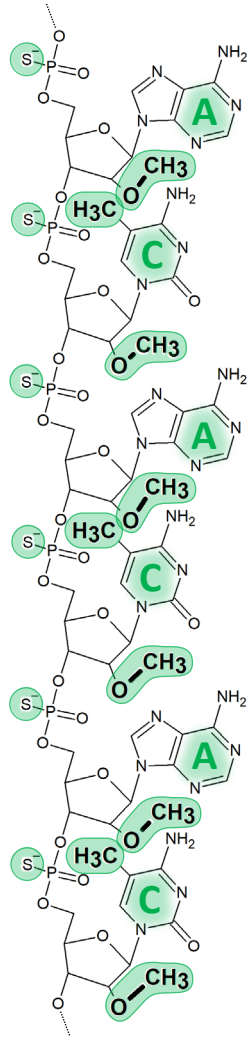
Clinical optimization of NAPs

REP 2055
1st gen clinical candidate

Equivalent activity *in vitro*, *in vivo* and in humans

REP 2139
Lead clinical candidate





Activity is length and phosphorothioation dependent
40mer phosphorothioate[1]
High affinity and selectivity for J-domain of DNAJB12

Activity is sequence independent
Repetitive adenosine / cytidine sequence[1] (safety)
Eliminates secondary structure, quenches immunoreactivity in ssDNA or ssRNA[2]

REP 2139 improvements

All naturally occurring with no impact on antiviral activity

2'O-methylation of all ribose sugars in backbone
- Improves solubility, reduces off target protein interactions
- Enhanced nuclease stability
- Quenches immunoreactivity

Optimizes chelate complex formation (required for well tolerated administration)

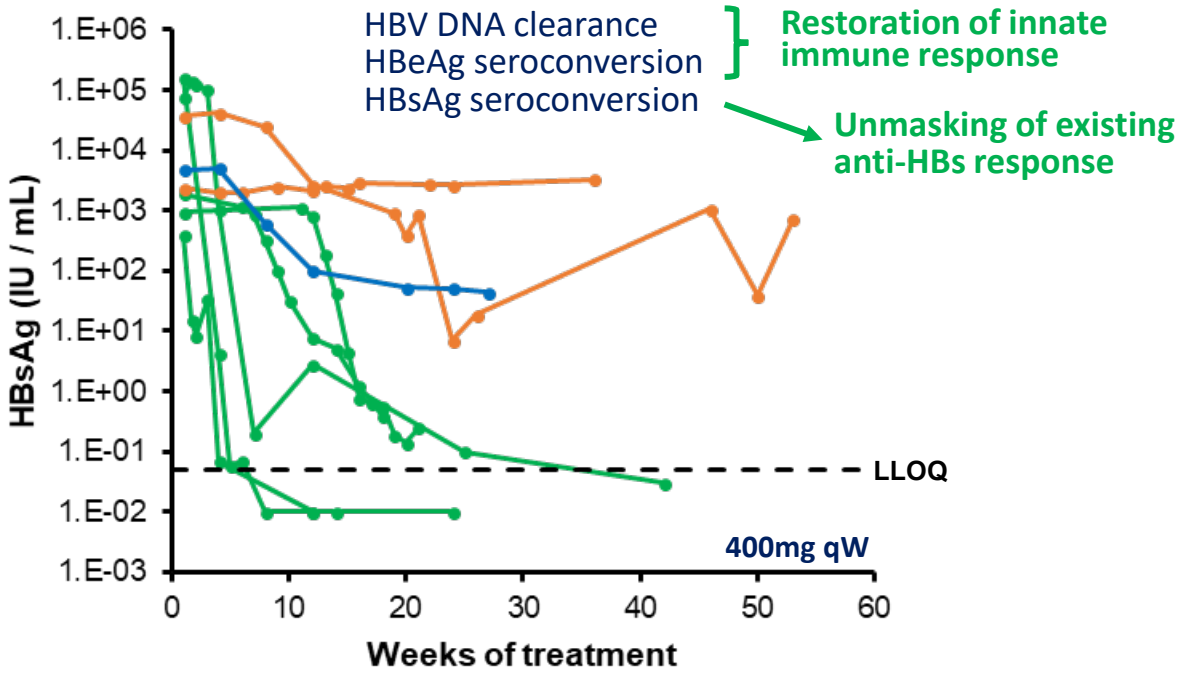
5-methylation of cytosine
- Quenches immunoreactivity

Exclusion of locked nucleic acids early in development (e.g. ALG-10133)
- Not naturally occurring, hepatotoxic
- Induces strong 2° structure and substantially reduces oligonucleotide flexibility
  reduces binding to J-domains
- Unsuitable for clinical use with NAPs (additional undisclosed property)

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

Integrating NAPs into existing therapies

REP 2055 monotherapy
HBeAg positive chronic HBV infection



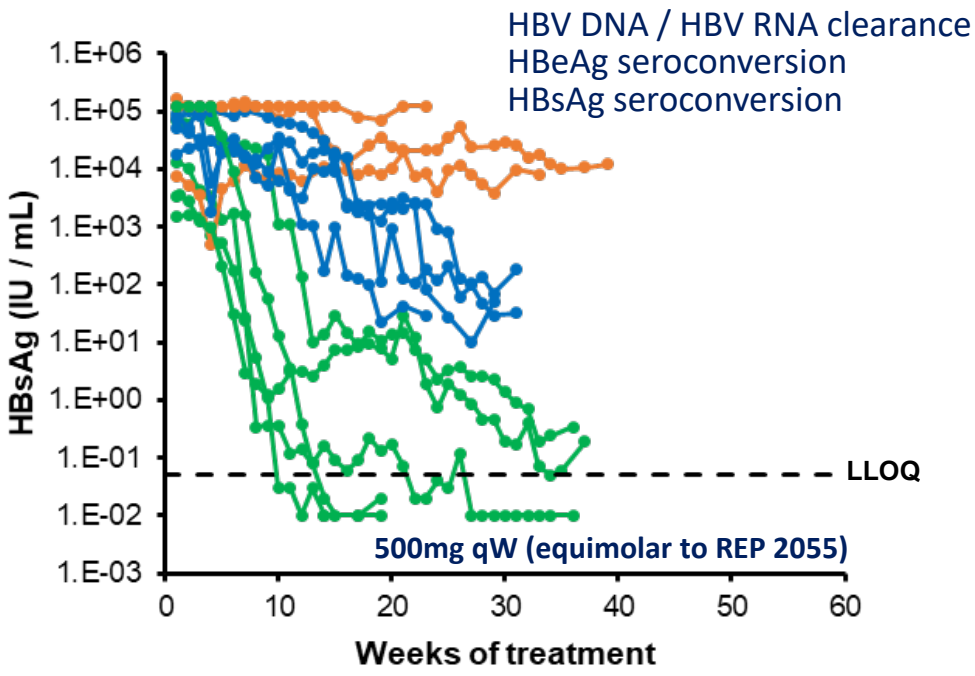
Good HBsAg response

Poor administration tolerability (IV and SC)

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

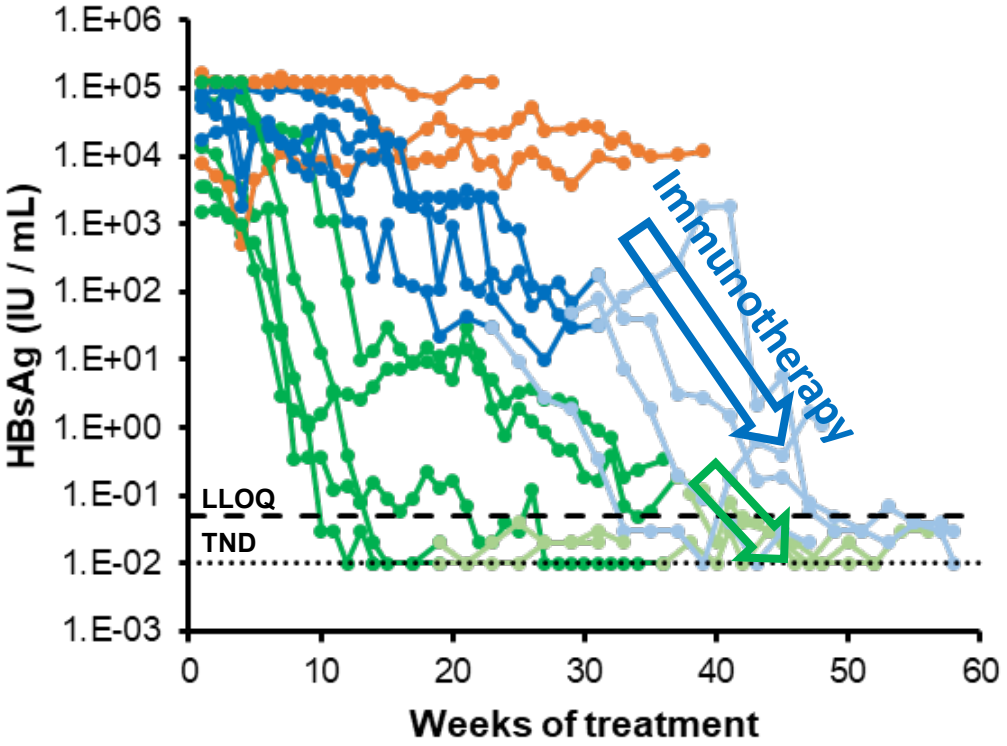
Jansen et al., J Hepatology 2015: 62: S250

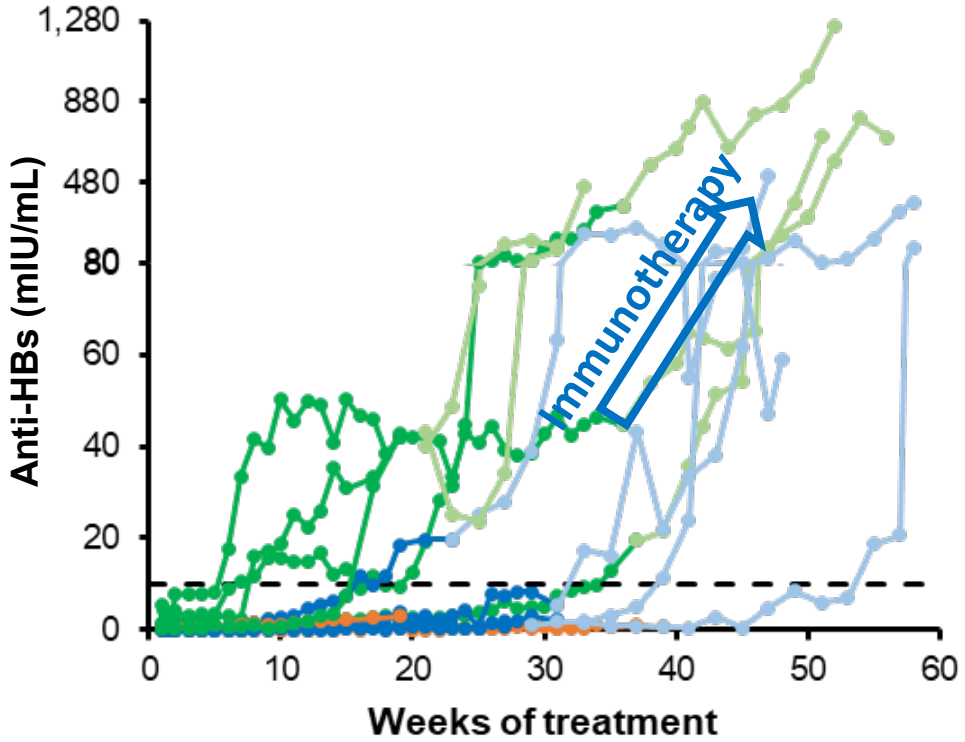
Al-Mahtab et al., 2016 PLoS One 11: e0156667

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

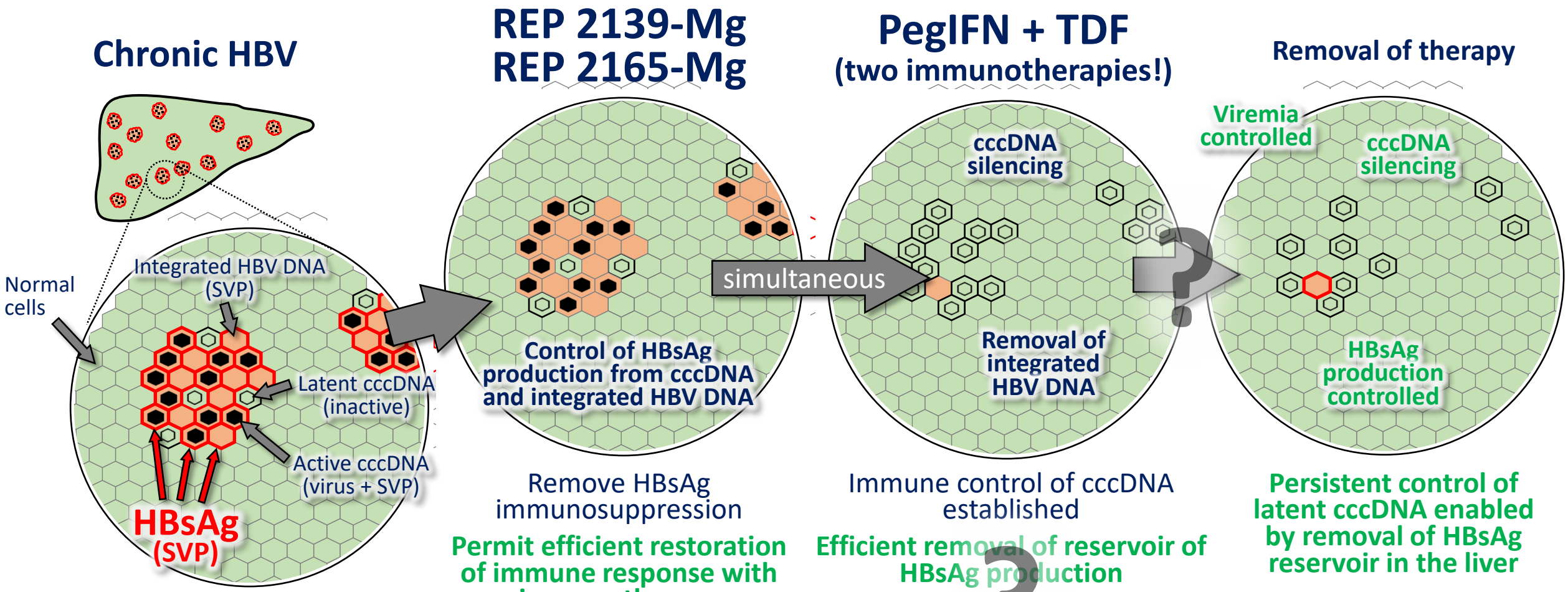
2 year follow-up post therapy:

3/9 with virologic control

1/9 with functional cure

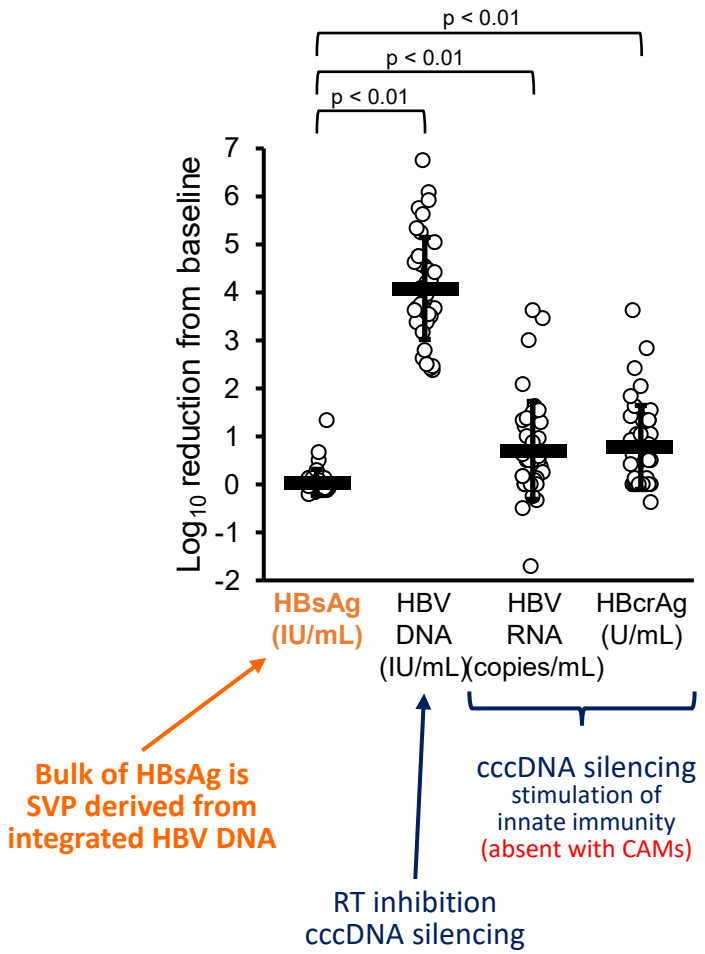
Al-Mahtab et al., 2016 PLoS One 11: e0156667

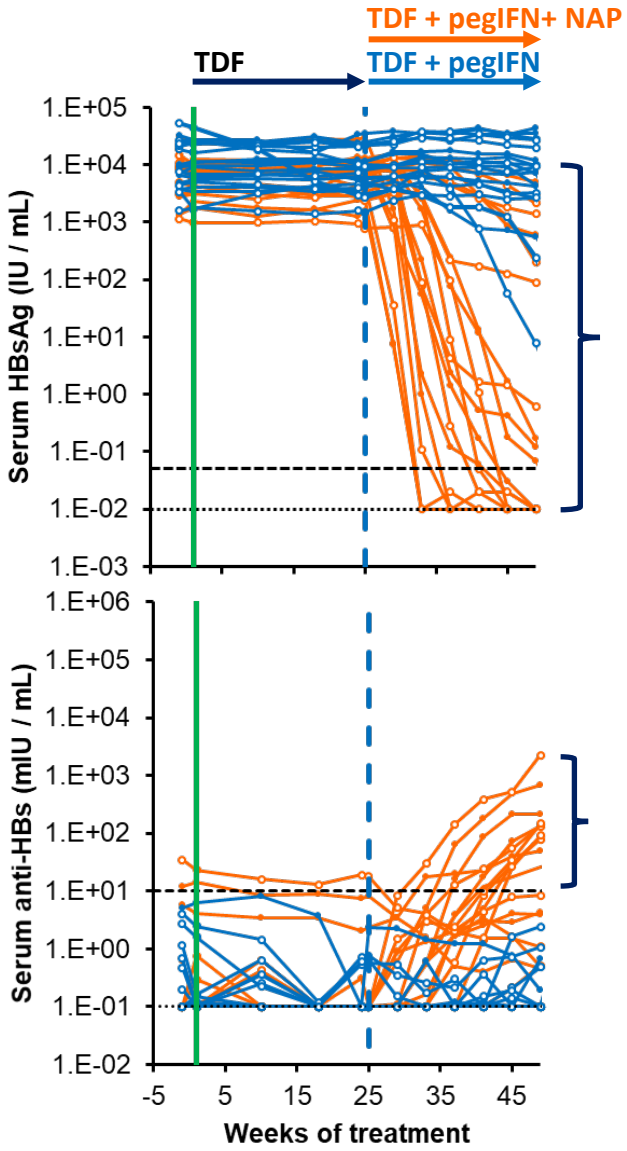
REP 401: Putting the pieces of the puzzle together



REP 401: NAPs dramatically improve responses with TDF + pegIFN

Bifunctional nature of 24 weeks TDF in the REP 401 study



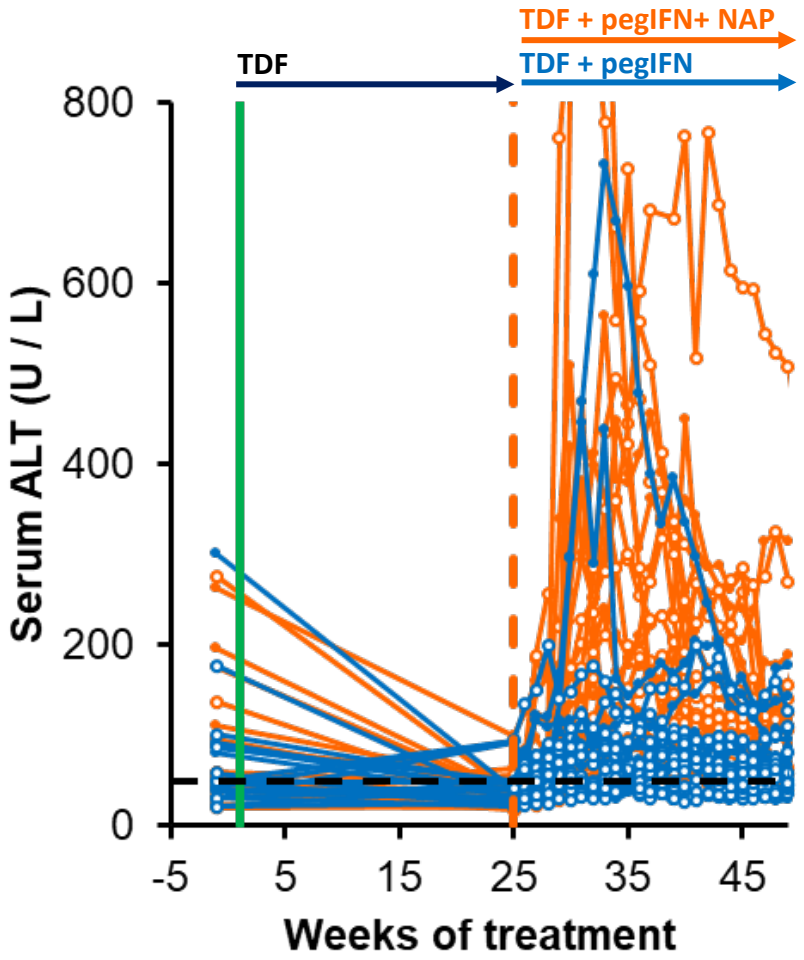


Up to 7 log_{10} decline from baseline (to < 0.005 IU/mL)
Potential HBsAg decline

Rapid HBsAg seroconversion

Bazinet et al., Gastroenterol. 2020; 158: 2180-2194
Bazinet et al., EASL 2020, LBP-33

REP 401: NAPs dramatically improve response over TDF + pegIFN



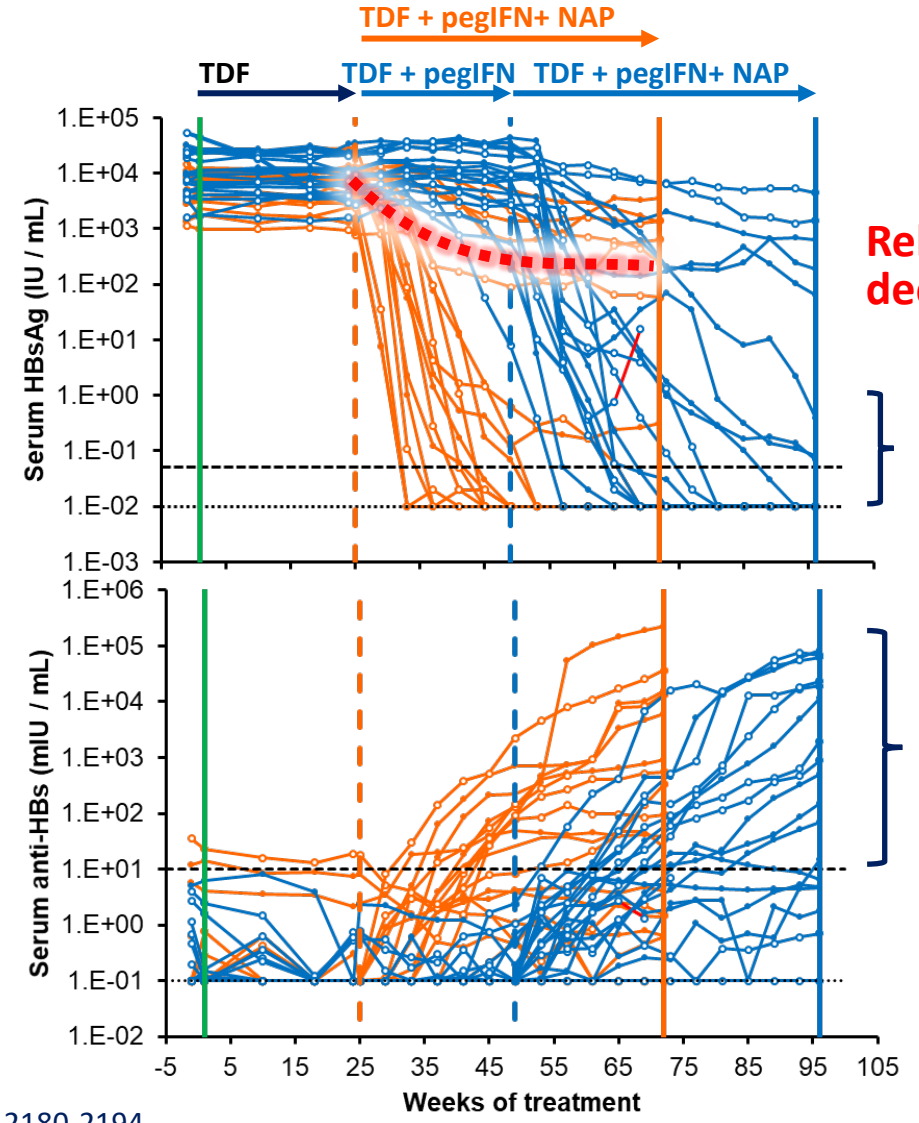
Dramatic increase in host mediated transaminase flares[1]

- 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

Consistent with the safe and beneficial nature of host mediated transaminase flares during therapy with approved agents, even in cirhottics[3]

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

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



Relatively weak HBsAg decline with GalNAc-RNAi

70% HBsAg < 1 IU/mL

52% HBsAg < 0.005 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 24-48 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	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**

Efficient silencing of cccDNA

Removal of integrated HBV DNA

Bazinet et al., Gastroenterol. 2020; 158: 2180-2194
Bazinet et al., Hepatol Comm 2021, in press

Summary

Subviral particles (SVP):
The Key to Functional Cure

> 99.99% of circulating HBsAg
Prevent immune control and function of immunotherapy
Removal during therapy is essential for functional cure
Cannot be targeted by direct acting antivirals (NUCs / CAMs)

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

RNAi acts like dsRNA in human HBV infection:

Negligible targeting of HBV mRNA (and integrated HBV DNA) in most subjects
HBsAg response likely reflects TLR3-mediated inactivation of cccDNA
Can TLR3 activation with dsRNA play a role in functional cure?

NAPs: Target SVP assembly / secretion from cccDNA and integrated HBV DNA
Allow efficient, host-mediated clearance of HBsAg

Immunotherapy used in combination is associated with:
high rates of asymptomatic host-mediated transaminase flares
high rates of functional cure, silencing of cccDNA and removal of integrated HBV DNA

Transition to SC administration in cirrhotics in REP 501 study (HBV / HDV) will improve convenience for all patients.