

Nucleic acid polymers: Biochemistry, molecular mechanism and application in the achievement of functional cure

**Andrew Vaillant, Ph.D.
Chief Scientific Officer
Replicor Inc.**

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**SCIENCE OF
HBV CURE**

Presented by

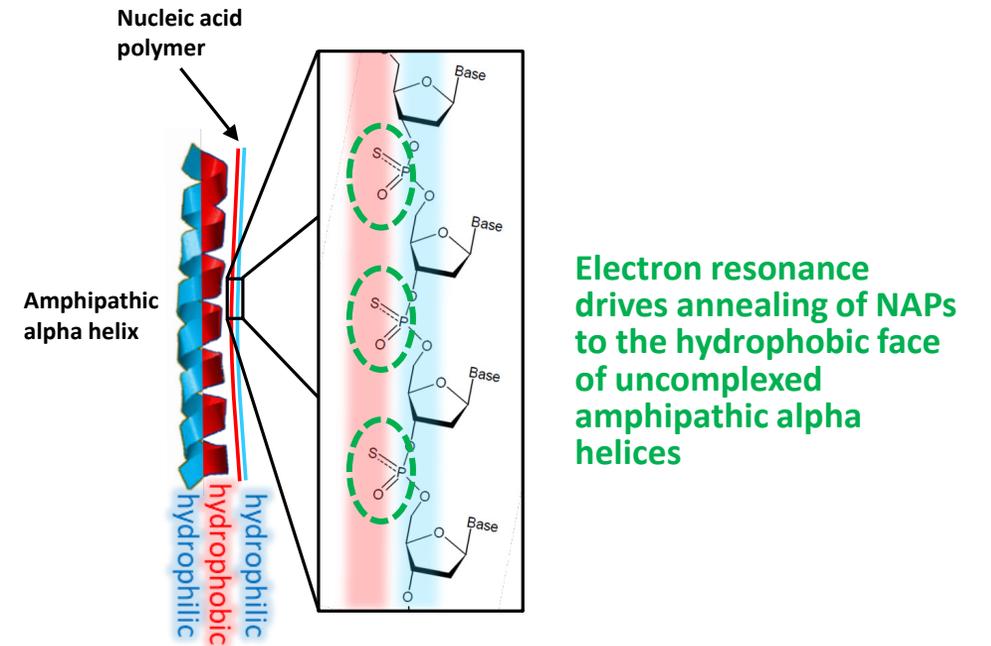
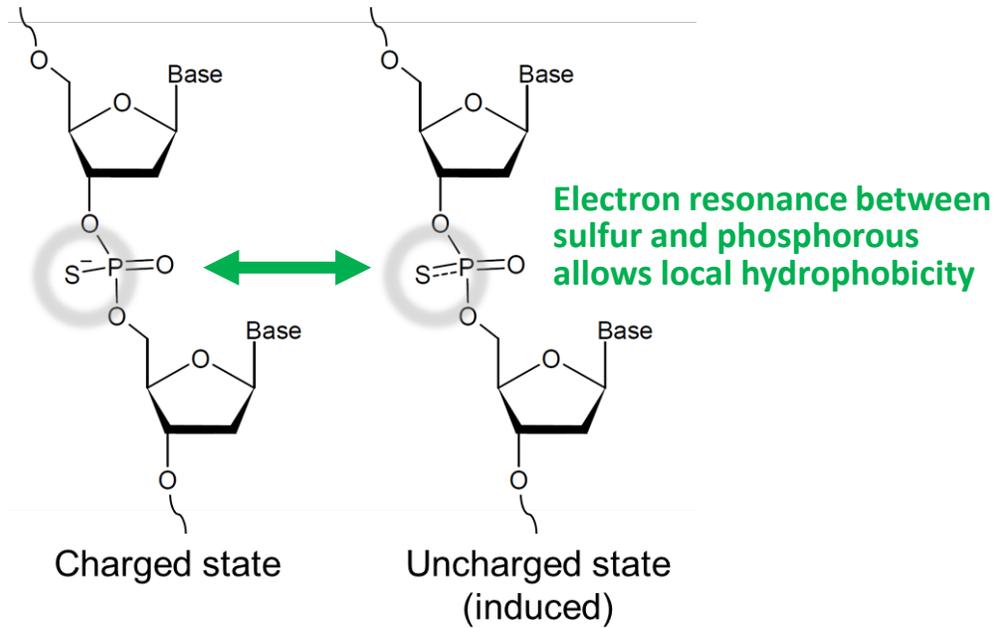


Disclosures

Shareholder and employee, Replicor Inc.

Nucleic acid polymers (NAPs)

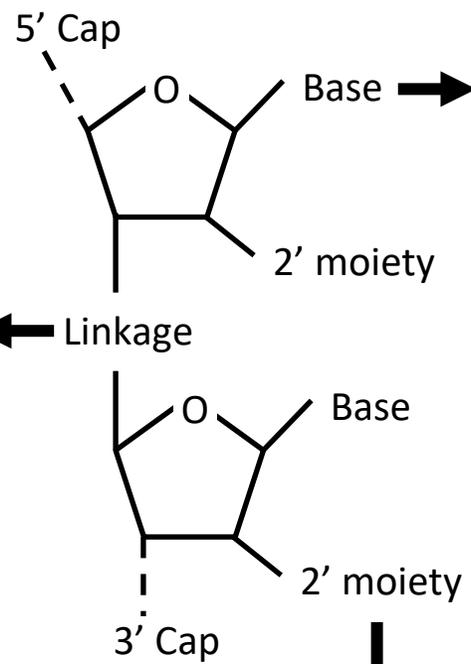
Oligonucleotides with sequence independent antiviral activity



Previously described NAP modifications

US patent 8,008,269 (2003)

- Phosphodiester**
 - Phosphorothioate (mixed chirality)
 - Phosphorothioate (R-diastereomer)
 - Phosphorothioate (S-diastereomer)
 - Phosphorodithioate
- Phosphotriester**
 - Aminoalkyl phosphotriester
 - Methyl phosphonate
 - 3' amino phosphoroamidate
 - Aminoalkyl phosphoramidate
 - Thiophosphoroamidate
 - Thioalkylphosphonate
- Thioalkylphosphotriester**
 - Boranophosphate
 - Selenophosphate
 - Carboranyl phosphate



- Guanine (G)**
- Adenosine (A)**
- Cytidine (C)**
- Thymine (T)**
- Uracil (U)**
- 5-methyl C**
- 5-hydroxymethyl C
- Xanthine
- Hypoxanthine
- 2-amino A
- 6-methyl A/G
- 2-propyl G
- 2-thiol U
- 4-thiol U
- 2-thiol T/C
- 5-halo U/C
- 5-propynyl U/C

- 6-azo U/C/T
- 8-halo A/G
- 8-amino A/G
- 8-thiol A/G
- 8-hydroxyl A/G
- 8-thioalkyl A/G
- 8-aza A/G
- 7-deaza A/G
- 3-deaza A/G
- 5-halo U/C
- 7-methyl A/G**
- 2-halo A
- Carbazol C
- 2-pyridine
- 2-aminopyridine

STOPs™ are LNA-modified NAPs

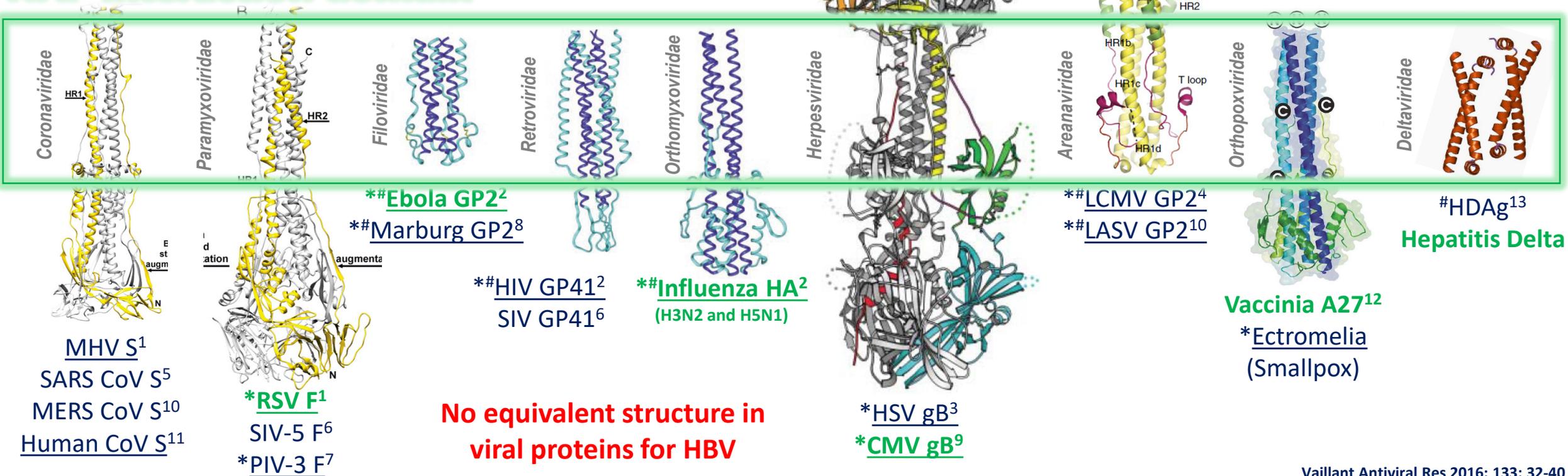
- Naturally occurring
- Non-naturally occurring
- Increased nuclease degradation
- Safe in humans **REP 2139**
- Inhibits NAP-target interaction
- Known toxicity in humans

- H (DNA)**
- OH (RNA)**
- O-methyl** → Enhances hydration – reduces off target protein interactions
- O-methoxyethyl → Unpredictable acute thrombocytopenia
- O-alkyl
- O-alkenyl
- O-alkynyl
- S-methyl
- SH
- N-methyl
- Locked nucleic acids (LNA)**
 - Induction of rigid A-form structure – poor target engagement
 - Increased endonuclease degradation poorly suited for clinical application
 - Hepatotoxic due to LNA-driven promiscuous pre-mRNA degradation

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



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

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 Struct Biol 2016; 23: 513-521
5. Lamb and Jardetzky Curr Op Struct 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

*SAR consistent with hydrophobic α -helical interaction with NAPs

#NAP/glycoprotein interaction validated

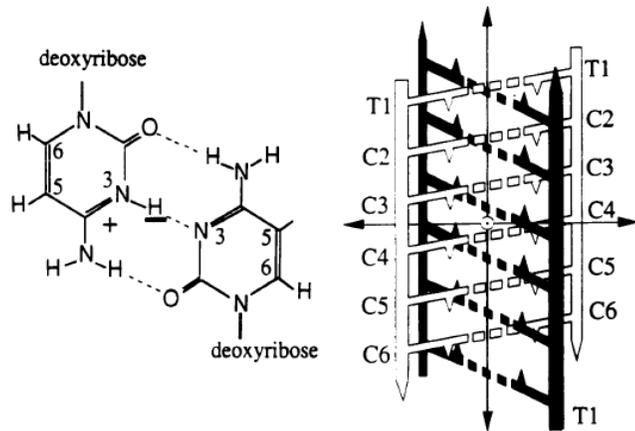
In vitro activity

In vivo activity – active against liver / lung / spleen viral infections

(consistent with demonstrated accumulation of NAPs in liver, lungs and spleen)

REP 2031: identifying the subcellular location of 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	potent and equivalent	
Post entry activity in HBV <i>in vitro</i> ^{8,9}	potent	absent / weak
Antiviral activity against HBV <i>in vivo</i> ¹⁰	potent	absent



Polypyrimidines (i.e poly cytidine) undergo tetramerization at acidic pH (< 6.8)^{11,12}

Loss of ability to target exposed amphipathic alpha helices

Tetramerization inhibited by doping poly C with purine nucleotides (adenosine)¹³

Genesis of the poly AC sequence

NAP activity in HBV infection occurs in acidified compartments of the secretory pathway (i.e. ERGIC)

- Vaillant et al., AAC 2006; 50: 1393-1401
- Guzman et al., Antiviral Ther 2007; 12: 1147-1156
- Bernstein et al., AAC 2008; 52: 2727-2733
- Cardin et al., Virol J 2009; 6: 214
- Lee et al., Virology 2008; 372: 107-117

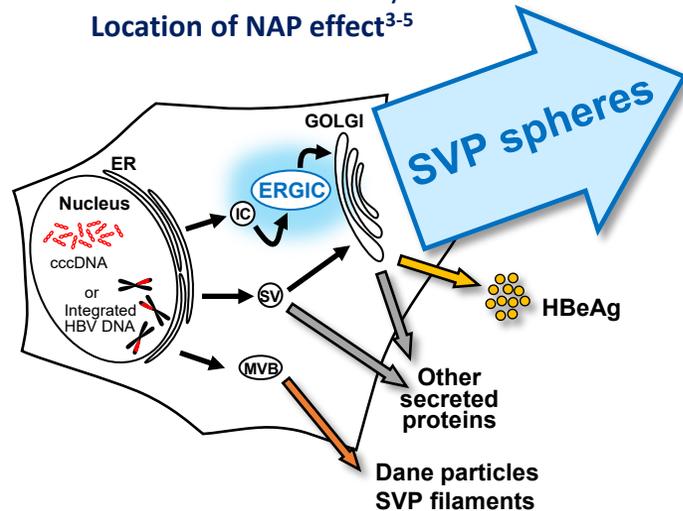
- Matsumura et al., Gastroenterol 2009; 137: 673-681
- Kocisko et al., AAC 2006; 50: 1034-1044
- Noordeen et al., AAC 2013; 57: 5291-5298
- Blanchet et al., Antiviral Res 2019; 164: 97-105
- Noordeen et al., AAC 2013; 57: 5299-5306

- Kanehara et al et al., Biochemistry 1997; 118: 1305-1320
- Leroy et al., NAR 1994; 22: 1600-1606
- Geinguenaud et al., 2000 Biochemistry 39: 12650-12658

The challenge of mechanistic evaluation of NAPs in vitro

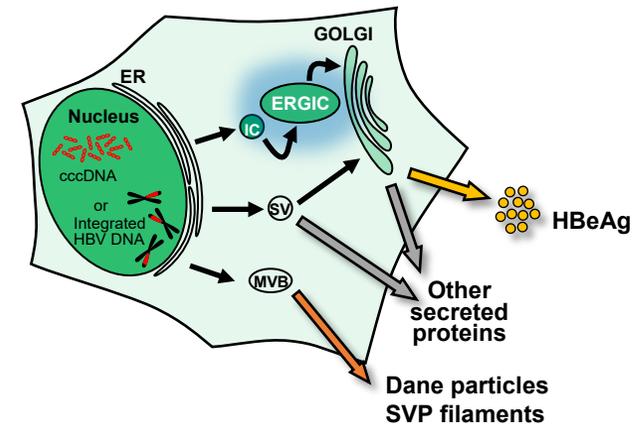
Subviral particle production *in vivo* > 99.99% of HBsAg

ER – Golgi intermediate compartment (ERGIC)
Location of SVP assembly^{1,2}
Location of NAP effect³⁻⁵



NAP trafficking and antiviral effect *in vivo* and humans

NAPs transit via endosomes
through ER / Golgi



NAPs do not affect:

1. secretory functions⁵⁻⁹
2. cccDNA transcription^{5,9}
3. HBV RNA translation⁹
4. immune function¹⁰

1. Huivola et al., J Cell Biol. 1992; 118: 1305-1320
2. Patient et al., J Virol. 2007; 81: 3842-3851
3. Noordeen et al., AAC 2013; 57: 5291-5298

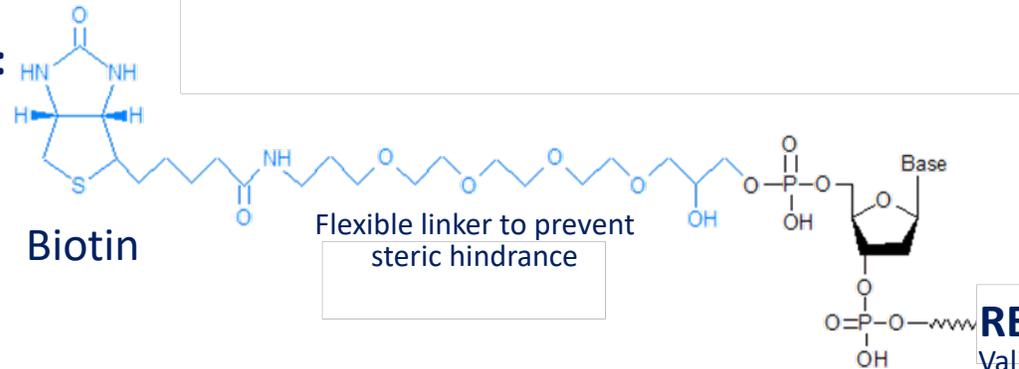
4. Noordeen et al., AAC 2013; 57: 5299-5306
5. Blanchet et al., Antiviral Res 2019; 164: 97-105
6. Noordeen et al., PLoS ONE 2015; 10: e0140909

7. Al-Mahtab et al., PLoS ONE 2016; 11: e0156667
8. Quinet et al., Hepatology 2018; 67: 2127-2140
9. Boulon et al., Antiviral Res. 2020; 183: 104853

10. Real et al., Sci Reports 2017; 7: 43838

NAP target identification in HBV

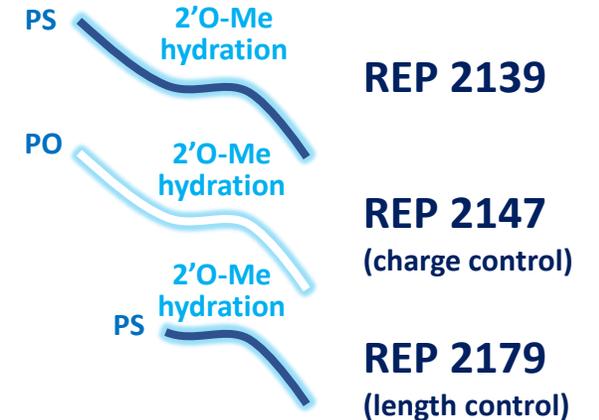
Bait used for interaction studies:
BioTEG-REP 2139



REP 2139

Validated *in vivo* and in humans
Hydration blocks non-specific protein interactions
Flexible b-form DNA structure for optimal target interaction

Bait structures



Control baits:

BioTEG-REP 2147: phosphodiester version of REP 2139 (validated control for non-specific charge interactions)

BioTEG-REP 2179: 20mer version of REP 2139 (validated control for length dependent interaction)

NAP target identification in HBV

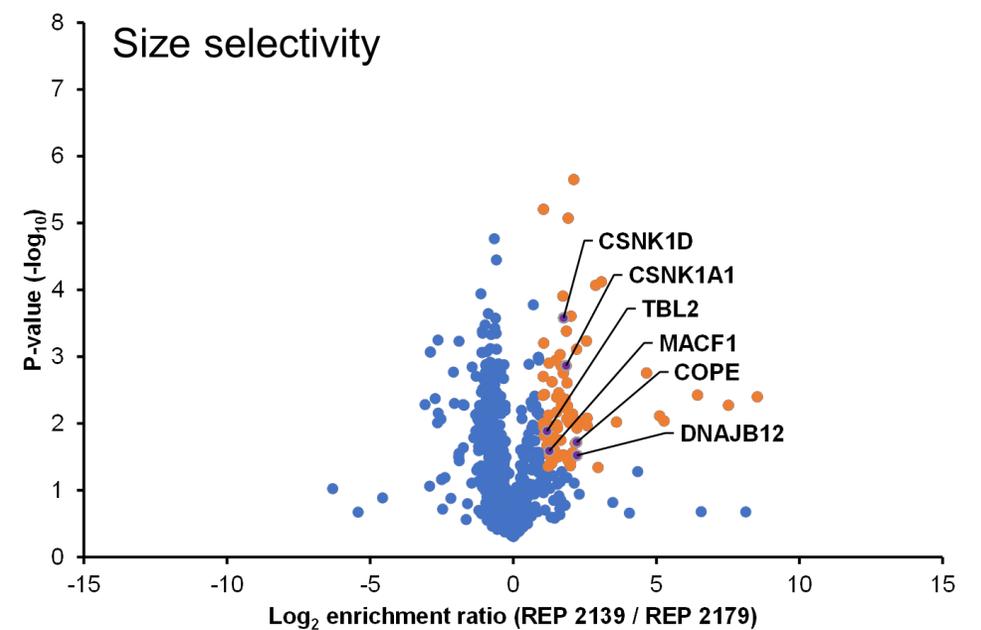
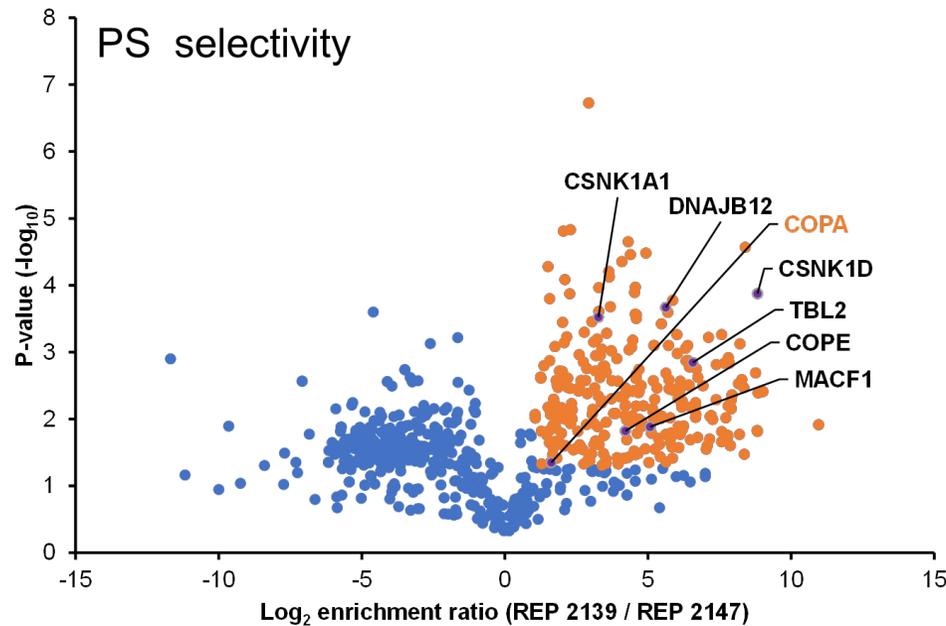
Bio-TEG NAPs used to pull out interactors from HepG2.2.15 lysate at neutral pH

Interactors are identified by MS/MS

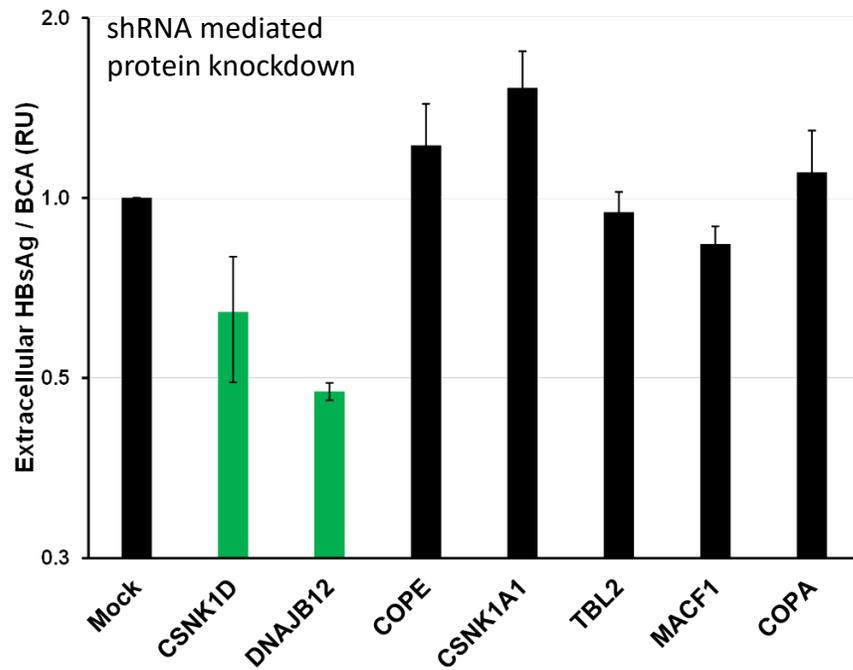
Candidate targets are ranked by the following criteria:

1. Exclusion of DNA / RNA binding proteins (non-specific interactions)
2. Preferential binding to REP 2139 vs REP 2147
3. Preferential binding to REP 2139 vs REP 2179

No HBV protein interactions observed

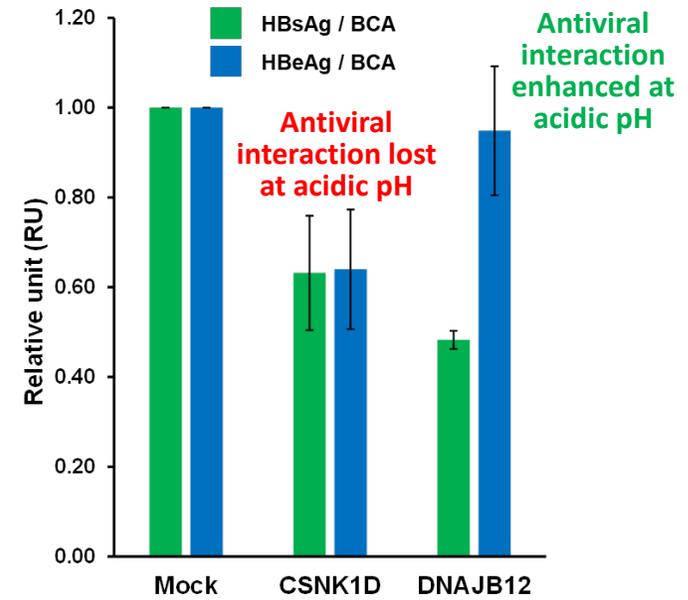


NAP target identification in HBV



Selectivity control
(no effect on HBeAg)

Selectivity control
(preferential interaction at acidic pH within the ERGIC)

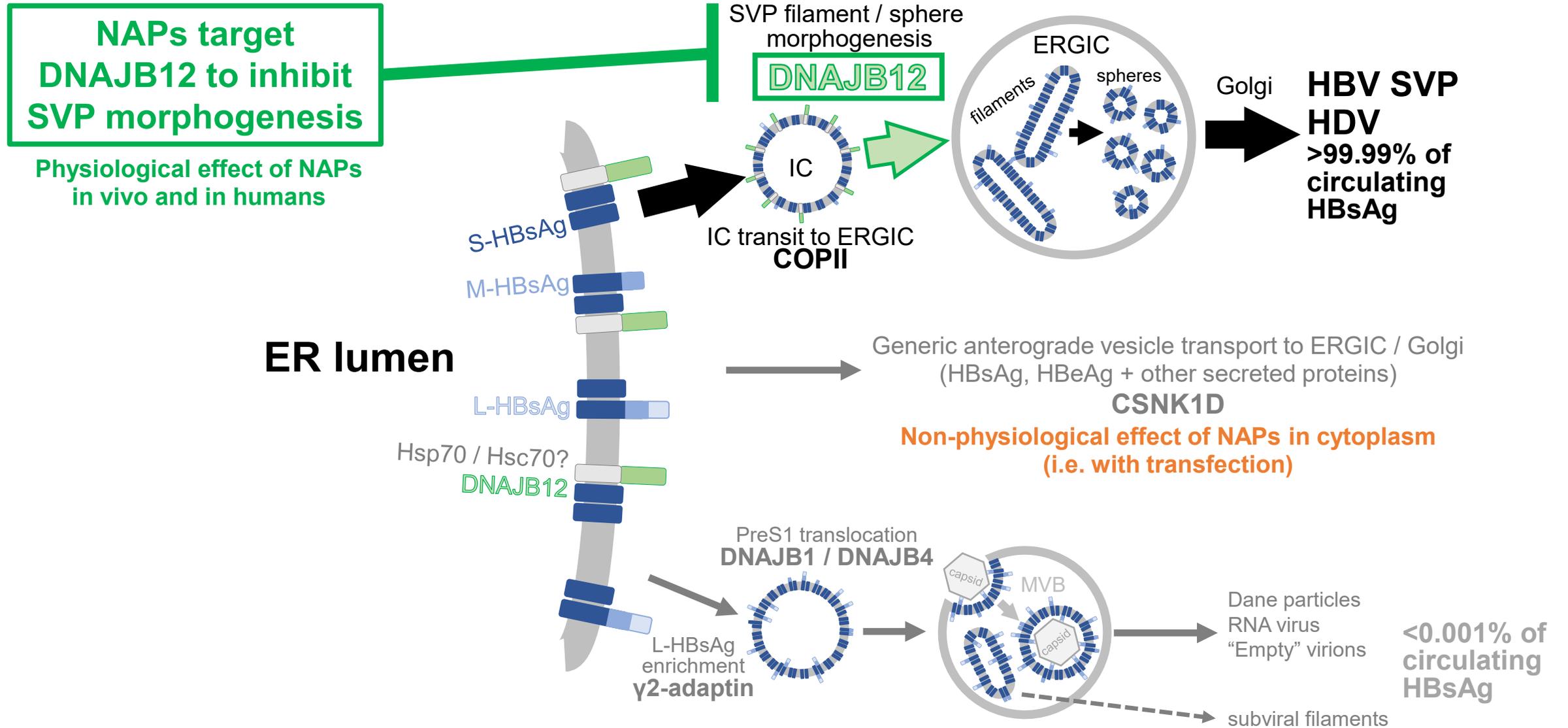


Antiviral interaction lost at acidic pH

Antiviral interaction enhanced at acidic pH

Non-physiological cytoplasmic interaction

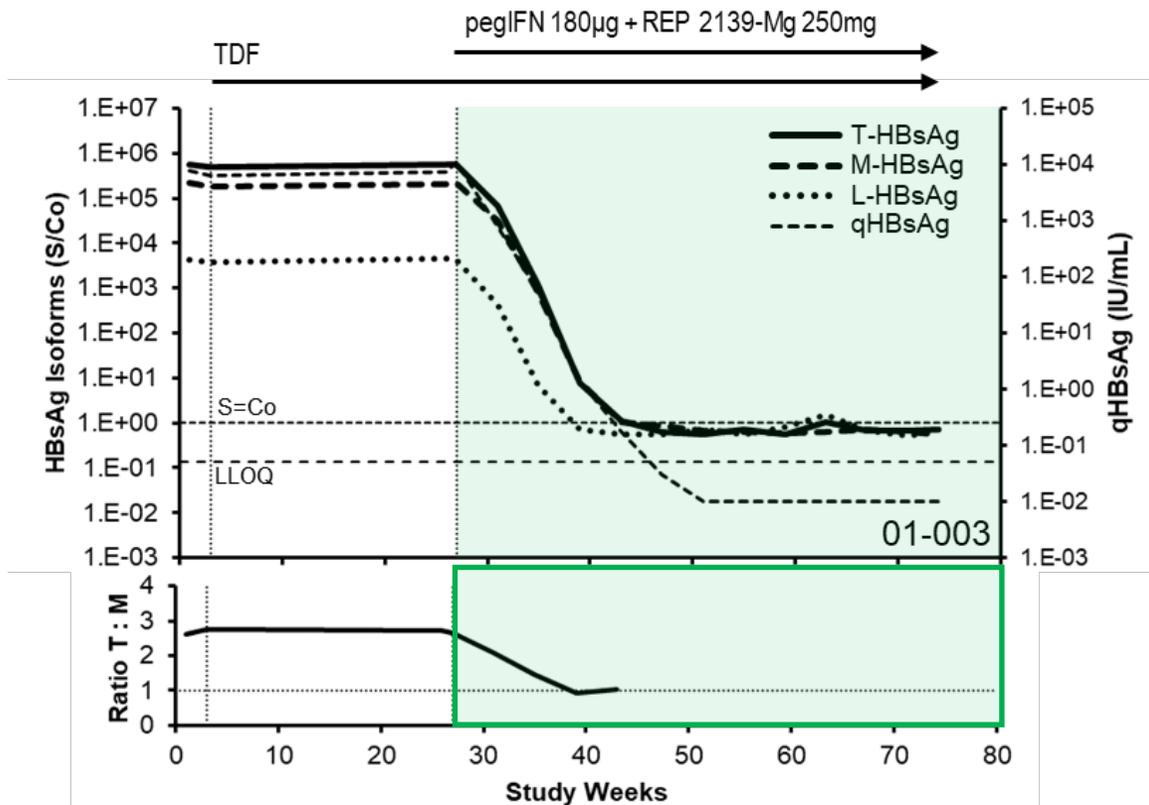
NAP molecular mechanism in HBV



REP 301 / 401: Validating the NAP mechanism in the clinic

Change in S-HBsAg content: change in ratio of total HBsAg (S+M+L) : preS2 (M+L) over time

HBsAg response



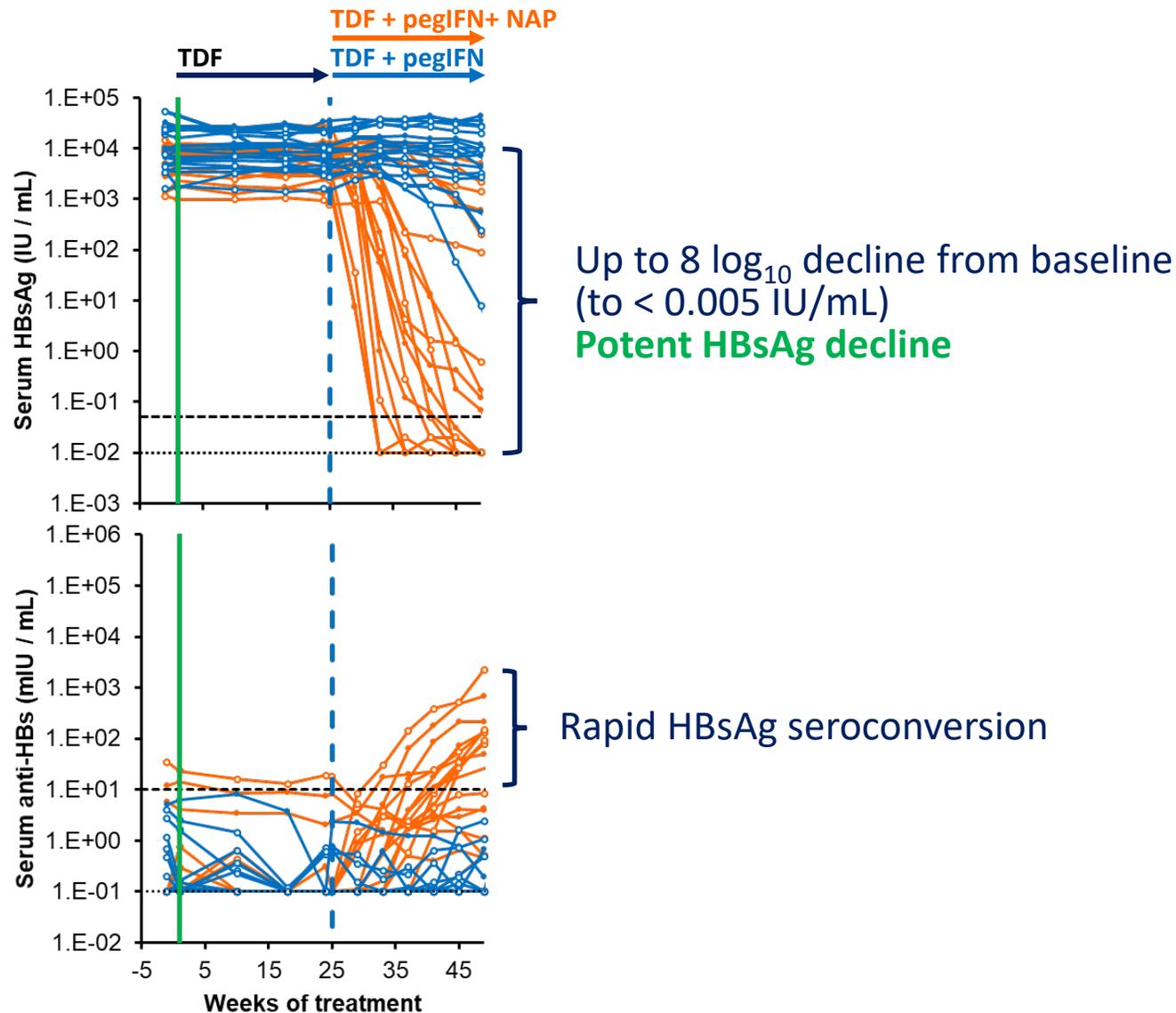
Strong HBsAg declines with NAPs are accompanied by selective decline of S-HBsAg

Correlation between selective S-HBsAg clearance during therapy and qHBsAg response (all 52 participants in REP 301+REP 401)

qHBsAg response during therapy (decline from baseline)	Total	Selective S-HBsAg decline	p-value
< 2 log ₁₀ IU/mL	10	1	< 0.01
> 2 log ₁₀ IU/mL	42	39	

Strong HBsAg decline with NAPs is accompanied by clearance of SVP (from cccDNA and integrated HBV DNA)

REP 401: NAPs dramatically improve HBsAg clearance with TDF + pegIFN



NAP monotherapy¹

REP 2055 = REP 2139

Up to 7 log₁₀ HBsAg reduction at 12 weeks

HBsAg seroconversion

Low rates of HBV functional cure

NAPs + TDF + pegIFN²

HBsAg < 0.005 IU/mL

HBsAg seroconversion

Inactivation of cccDNA

Host mediated transaminase flares (95%)

High rates of HBV functional cure (39%)

GT D functional cure rate

PegIFN + TDF = 0%

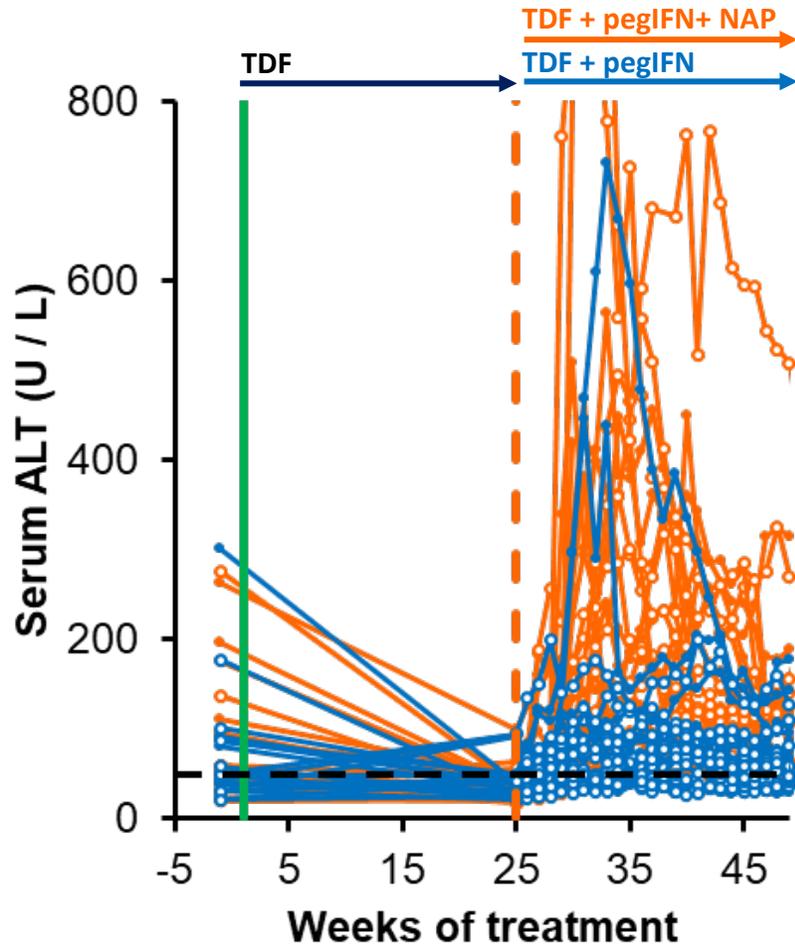
(Marcellin et al, Gastroenterology 2016; 150: 134-144)

PegIFN + TDF + NAPs = 39%²

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

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

REP 401: NAPs dramatically improve response over TDF + pegIFN



Dramatic increase in host mediated transaminase flares¹

- occur in 95% of participants²
- no alteration in liver function / asymptomatic²
- correlated with functional cure (when HBsAg is also < 1 IU/mL)²
- Signals the removal of integrated HBV DNA³

Consistent with the safe and beneficial nature of host mediated transaminase flares during therapy with approved agents with maintained viral suppression, even in cirrhotics⁴

1. Bazinet et al., Gastroenterol. 2020; 158: 2180-2194
2. Bazinet et al., J Viral Hep 2021; 28: 817-825
3. Bazinet et al., Hepatol Comm 2021; July 10
4. Vaillant, Viruses 2021; 131: 745

REP 2139-Mg: next steps

1. REP 2139-Mg via SC administration

- Proof of concept ongoing in cirrhotic HBV / HDV co-infection
- Magnesium chelate complex confers good subcutaneous injection tolerability
- Potent antiviral activity preserved (stay tuned!)

2. Verify safety of SC REP 2139-Mg in advanced fibrosis / cirrhosis

3. Assess efficacy / safety of pegIFN vs thymosin α 1 in combination

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