Update on NAPs: Understanding biochemical interactions and pharmacokinetics

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SCIENCE OF HBV CURE

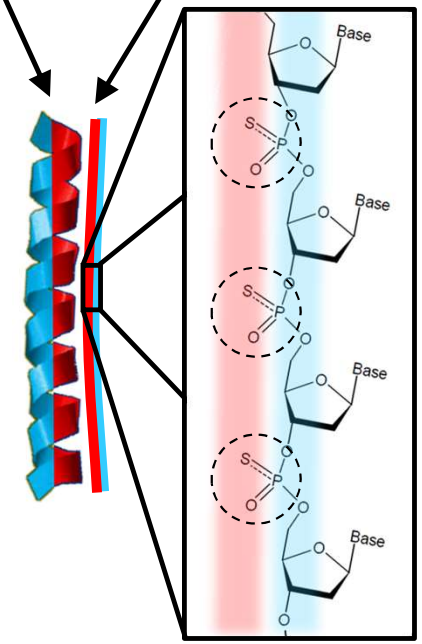
Conflict of interest

Employee / shareholder, Replicor Inc.

Understanding the NAP – target interface

Protein target
Amphipathic alpha helix

Nucleic acid polymer



An induced uncharged state is caused by a local hydrophobic environment

Driven by electron resonance in between sulfur and phosphorous in the phosphorothioate linkage

Hydrophobic interactions are greatly strengthened by lengthening the polymer

Antisense (20mer) = weak

NAP (40mer) = strong

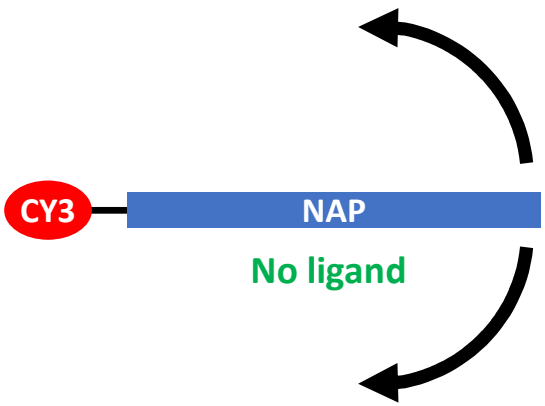
Activity is sequence independent

Poly AC sequence:
Removes host genome interactions
Removes secondary structure
Prevents immunostimulation

Naturally occurring modifications increase selectivity, safety and solubility

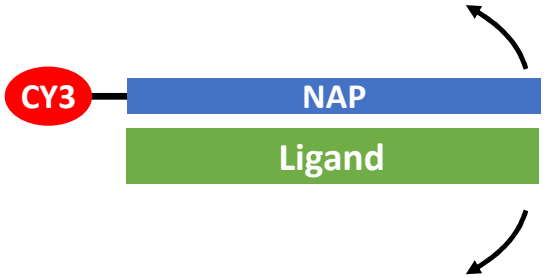
Vaillant. ACS Inf Dis 2019; 10: 675-687

Fluorescence polarization identifies biochemical parameters of NAP interactions



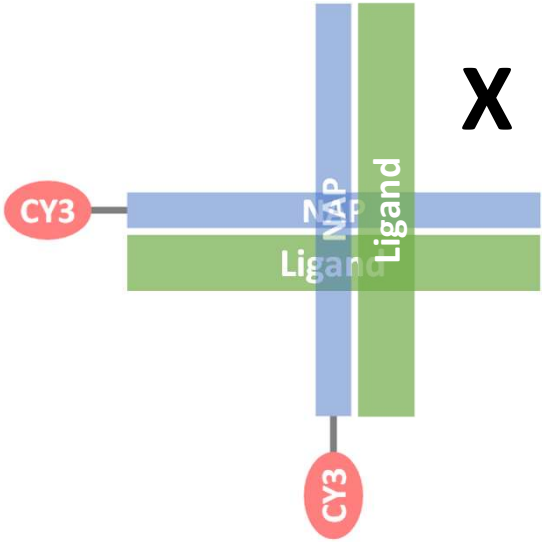
Rapid Brownian motion in solution

Minimal baseline polarization of emitted fluorescence



Retarded Brownian motion in solution

Increased polarization of emitted fluorescence



Perpendicular alignment of NAPs quenches polarization

Validated with numerous established phosphorothioate dependent and sequence independent protein / oligonucleotide interactions
Using known antisense oligonucleotides and NAPs

Validation of FP assay for PS-ON / NAP protein interactions with known class interactors

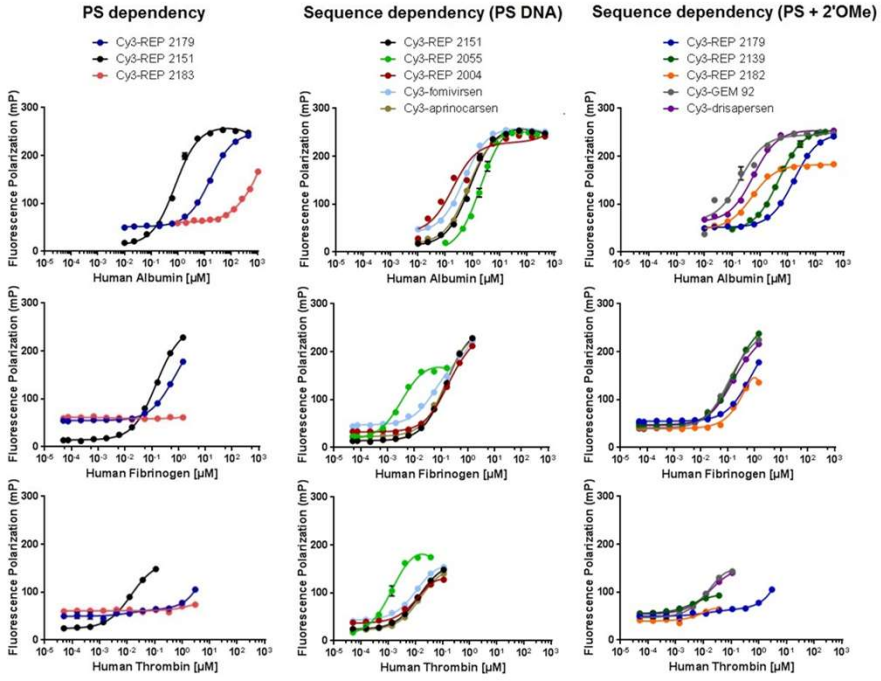
PS-ONs

Oligonucleotide	Sequence (5'-3')	Length	PS	2'OMe	5-MeC
fomiversen[a]	GCGTTTGCTCTTCTTCTTGCG	21	+	-	-
aprinocarsen[a]	GTTCTCGCTGGTGAGTTTCA	20	+	-	-
GEM 92[a]	UCGCACCCATCTCTCTCCUUC	21	+	+	-
drisapersen[a]	UCAAGGAAGAUGGCAUUUCU	20	+	+	-
REP 2004	NNNNNNNNNNNNNNNNNNNN	20	+	-	-
REP 2182	NNNNNNNNNNNNNNNNNNNN	20	+	+	-
REP 2183	ACACACACACACACACACAC	20	-	+	+
REP 2151	ACACACACACACACACACAC	20	+	-	-
REP 2055	ACACACACACACACACACACACACACACACACACACAC	40	+	-	-
REP 2184	ACACACACAC	10	+	+	+
REP 2179	ACACACACACACACACACAC	20	+	+	+
REP 2169	ACACACACACACACACACACACACACAC	30	+	+	+
REP 2139	ACACACACACACACACACACACACACACACACACACAC	40	+	+	+
REP 2147	ACACACACACACACACACACACACACACACACACACAC	40	-	+	+

[a] Clinically evaluated PS-ONs for other indications.
N = random incorporation of A, G, T (or 2'OMeT) and C.
Nucleotide positions with 2'O-methyl modified ribose are underlined.
NAPs outlined in green have clinically validated antiviral activity against HBV infection and or HBV / HDV co-infection.

Also validated for numerous other NAP targets

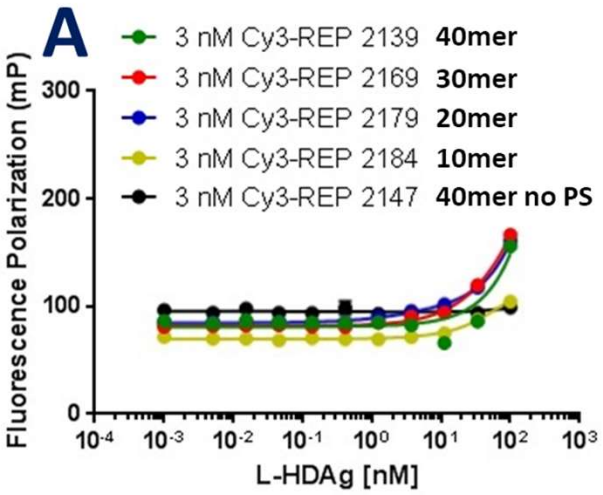
Shamur et al., Hepatol. 2017; 66: 504A

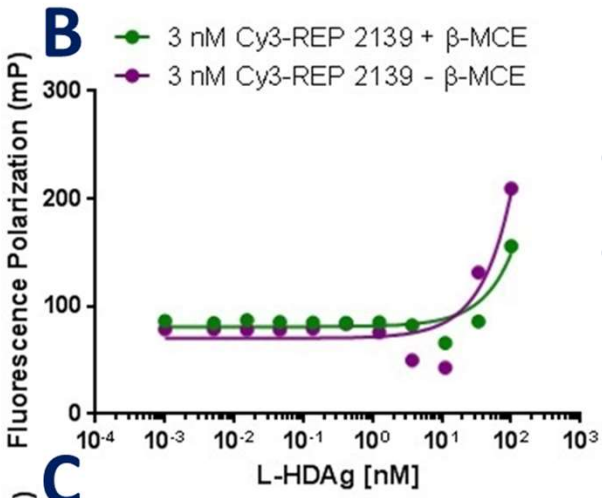


FP-generated PS-ON protein binding isotherms replicate previously described, class conserved phosphorothioate-dependent, sequence-independent interactions for a variety of clinically evaluated PS-ONs and NAPs with the known PS-ON interactors albumin, thrombin and fibrinogen. The clinical NAPs REP 2055 and REP 2139 are provided for reference in green.

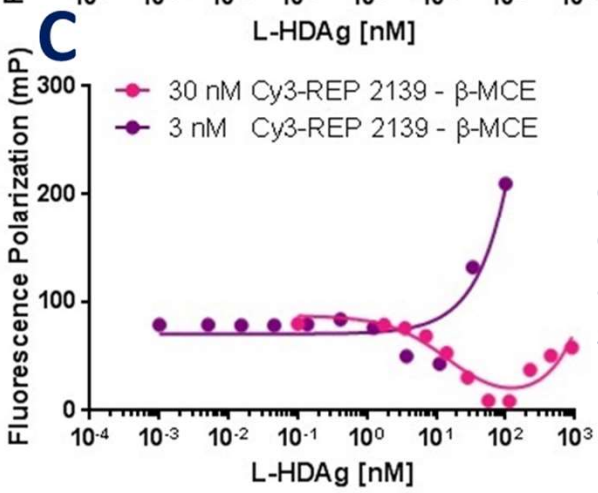
NAP interaction with L-HDAg

Size dependent
PS-dependent





Unique FP quenching is observed and is more pronounced in the absence of β-MCE



Increased REP 2139 concentration leads to complete FP quenching and blocks the FP supershift

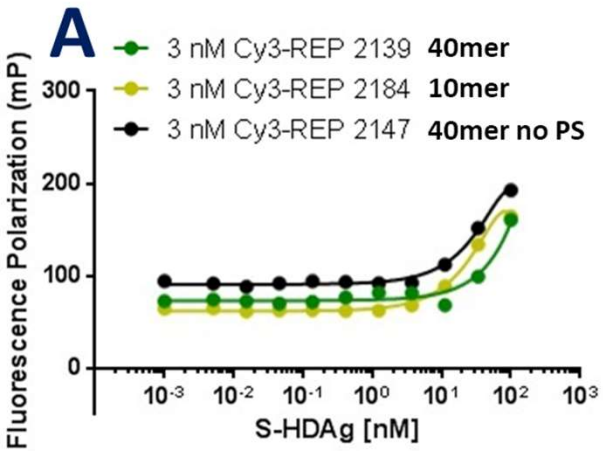
Shamur et al., Hepatol. 2017; 66: 504A

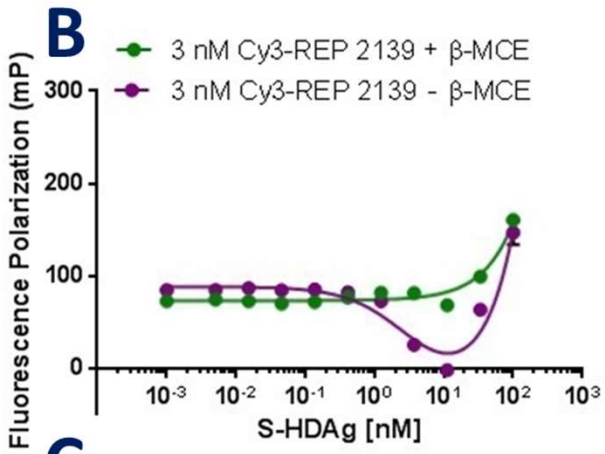
NAP interaction with S-HDAg

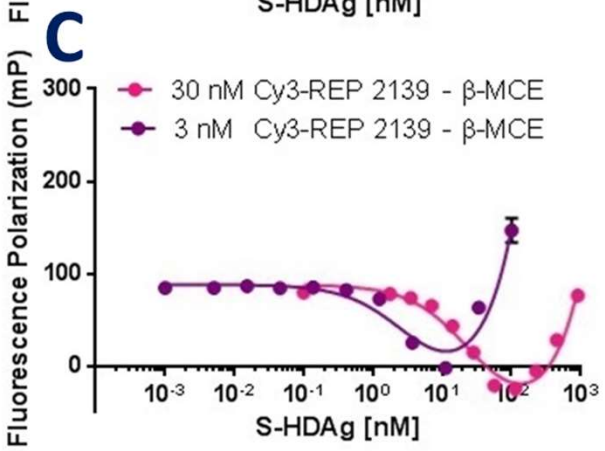
size independent
PS independent

Chaperone domain of HDAg (exposed in S-HDAg) binds promiscuously to a variety of oligonucleotides (PS and non-PS) in a sequence independent manner

Wang et al., Nuc Acids Res 2003; 31: 6481-6492





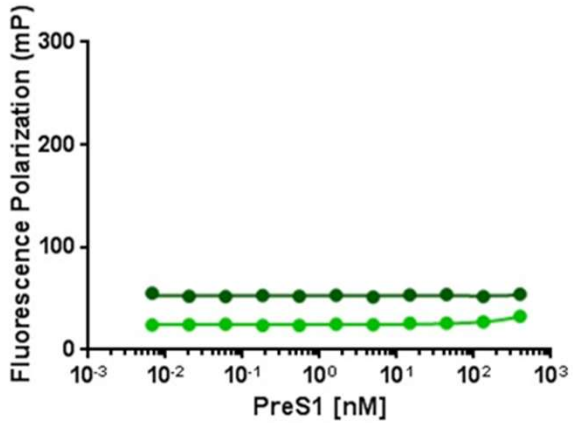


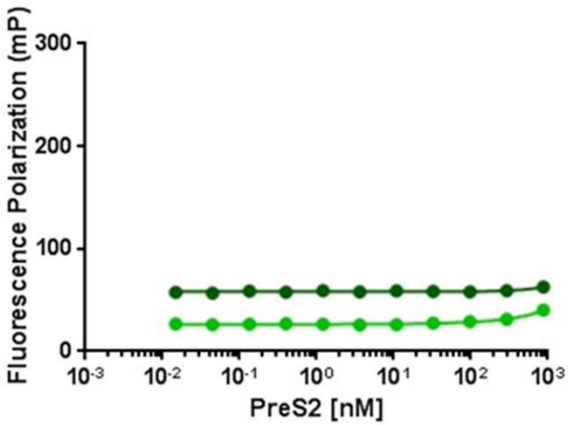
Unique FP quenching is observed only with REP 2139 and is more pronounced in the absence of β-MCE

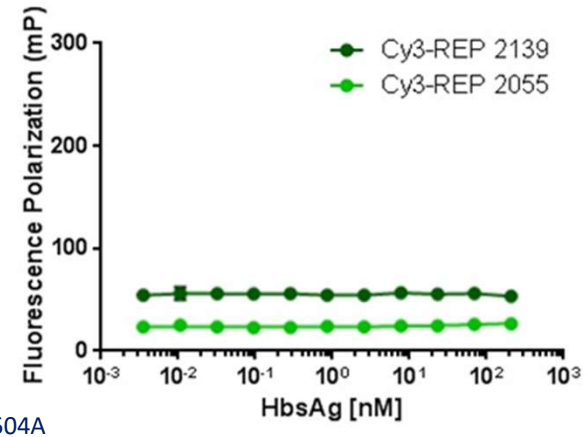
Increased REP 2139 concentration leads to complete FP quenching and blocks the FP supershift

Shamur et al., Hepatol. 2017; 66: 504A

NAP interaction with HBsAg







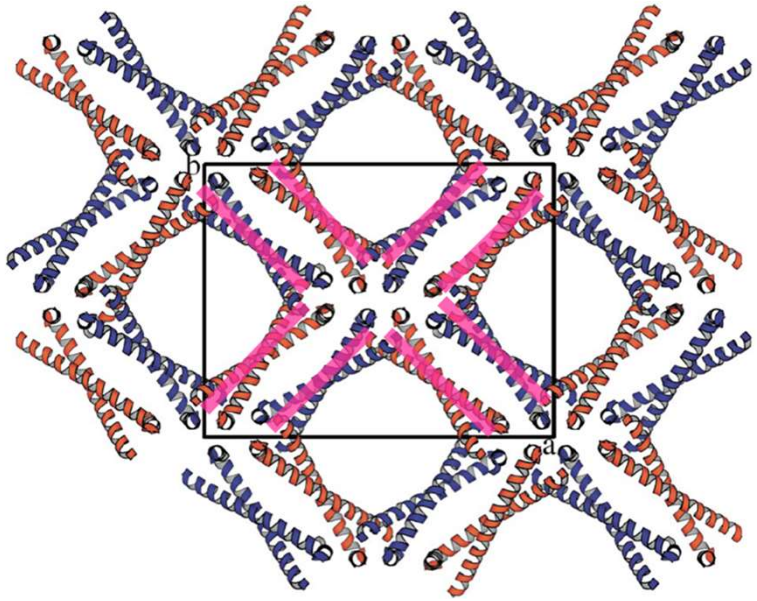
No interaction between REP 2055 or REP 2139 and preS1, preS2 or full length strain ayw HBsAg.

Shamur et al., Hepatol. 2017; 66: 504A

Model for NAP interaction with HDAg

Alpha helices in S-HDAg and L-HDAg drive HDAg oligomerization by perpendicular cooperative interactions

Zuccola et al. Structure. 1998;6:821-830.
Cromwell et al. J Virol. 2003;77:10213-10326.



NAPs (pink) anneal to amphipathic alpha helices in oligomerizing HDAg in the nucleus (consistent with FP quenching)

NAP annealing destroys biochemical functionality of HDAg
(HDV RNA replication? HDV RNP assembly?)

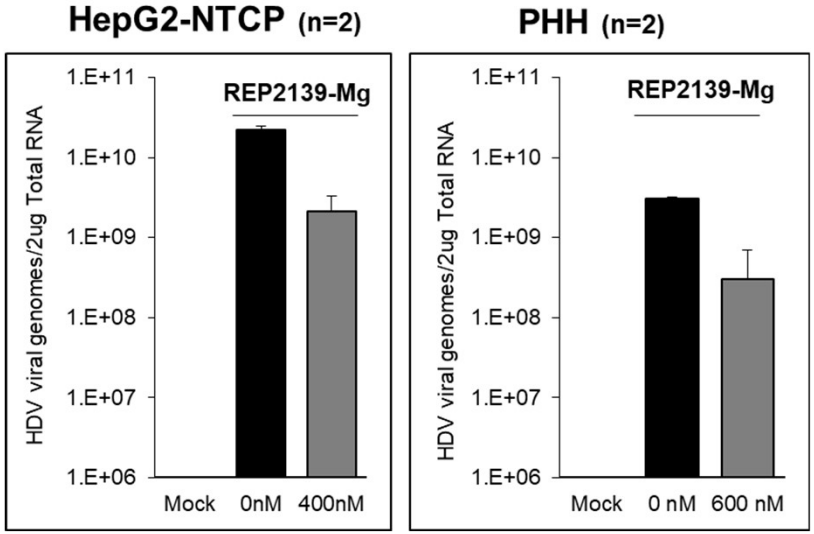
Shamur et al., Hepatol. 2017; 66: 504A

Visualizing the direct acting activity of NAPs against HDV

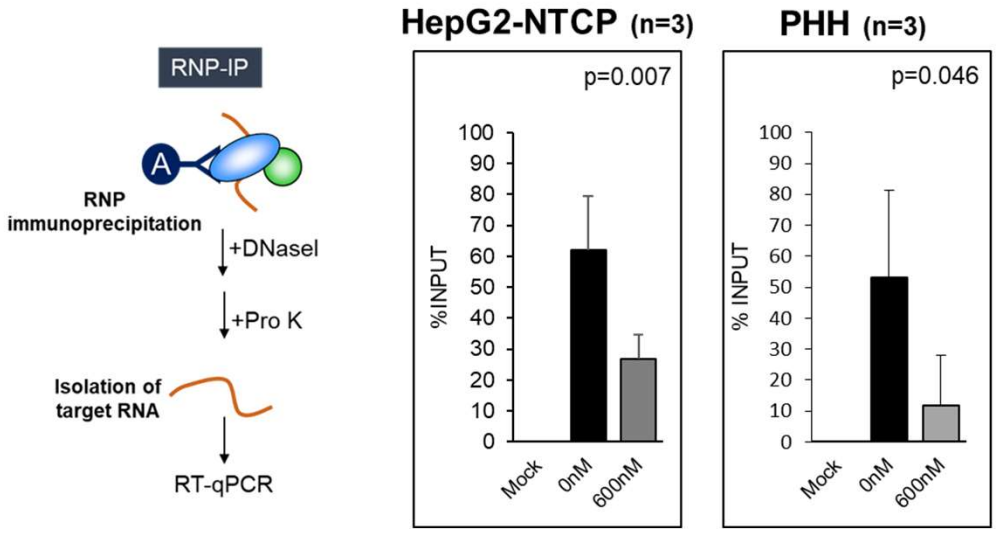
Data courtesy of Dr. Massimo Levrero, Lyon, France

Recall: NAPs bind to the small and large isoforms of HDAg suggest inhibition of HDV RNA replication and HDV RNP assembly

A single dose of REP 2139-Mg reduced intracellular HDV viral genome levels by ~1 log10 in HepG2-NTCP and PHH cells at 400nM and 600nM, respectively

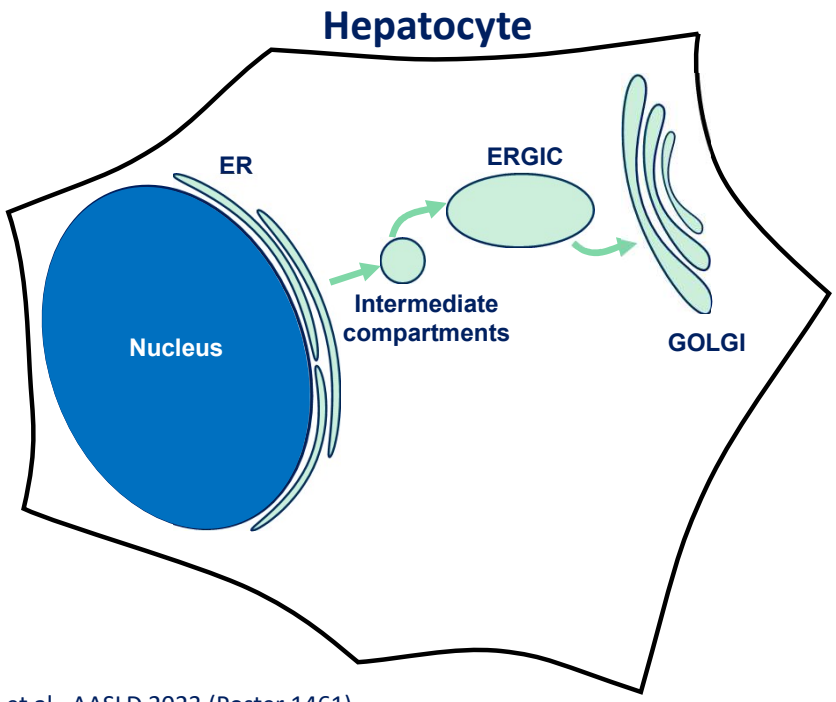


A single REP 2139-Mg dose also reduced the intranuclear association of HDV RNA with HDAg by ~60% in HepG2-NTCP and by 80% in PHH, without changing the HDAg protein levels



Fonte et al., AASLD 2023

Understanding how NAPs work in HBV / HDV co-infection



Passage through secretory pathway (transient)

- Activity occurs at acidic pH (post ER)
- Targets the host HSP40 chaperone DNAJB12
- Blocks inhibition of HBV SVP assembly
- Blocks envelopment of HDV RNP

Accumulation in nucleus

- Targets S-HDAg and L-HDAg
- Inhibits replication / morphogenesis of HDV upstream of RNP envelopment (mechanism under exploration)
- **Nuclear accumulation is more efficient**
  - ➢ Anti-HDV effects are easier to achieve

Fonte et al., AASLD 2023 (Poster 1461)
Boulon et al AASLD 2020 (Poster LP-42)
Boulon et al., Antiviral Res. 2020; 183: 104853
Blanchet et al., J Virol. 2019; 164: 97-105
Bazinet et al., Lancet Gastro. Hepatol. 2017; 2: 877-889
Shamur et al., Hepatol. 2017; 66: 504A

Antisense (and NAP) dosing: a well documented path

Knowledge base derived from 83 unconjugated (non-GalNAc) phosphorothioate oligonucleotides (PS-ONs) entering clinical development

78 ASOs against numerous liver targets (18-21 mer)
2 vaccine adjuvants (TLR9 agonists: 18 – 21 mer)
3 NAPs (REP 2055, REP 2139 and REP 2165, all 40mer)

Common dosing drives pharmacological effects in the liver with all unconjugated PS-ONs (including NAPs)

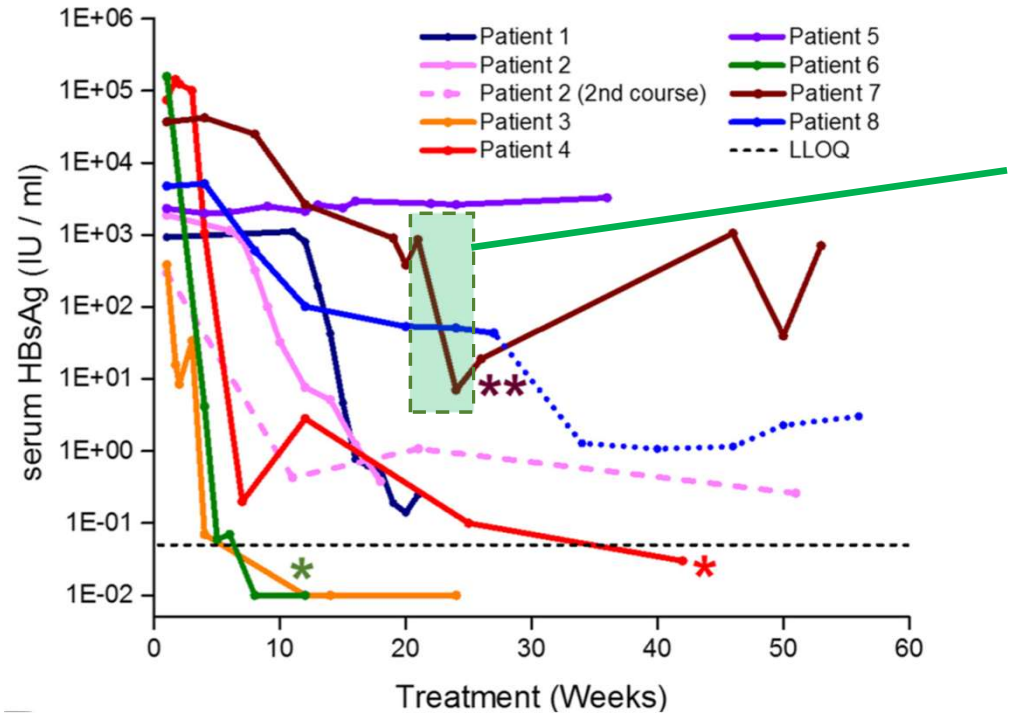
- Once weekly dosing (IV or SC) – IV infusion results in much higher C_{max}
- Rapid clearance from the blood (faster with IV infusion)
- Primary accumulation in the liver and kidney with repeated dosing (IV or SC), elimination via urine
- Hepatocyte accumulation in the liver is proportional to blood C_{max} following administration (IV or SC)
- 150-250mg required for pharmacologically effective hepatocyte accumulation of antisense in the liver (250-500mg for a 40mer = NAPs)

Roehl et al., Mol Ther Nuc Acids 2017; 8: 1-12
Yu et al., Mol Ther Nuc Acids 2015; 4: e218
Geary et al., Biochem. Pharmacol. 2009; 78: 284-291
Yu et al., Drug Metabol Disposit 2007; 35: 460-468
Geary et al., Drug Metabol Disposit 2003; 31: 1419-1428
Yu et al., J Pharmacol Sci; 2001; 90: 182-193

Recovery of NAP response with dose alteration (HBV)

HBsAg response in REP 101 trial (REP 2055 monotherapy)

Al-Mahtab et al. PLOS ONE. 2016;11:e0156667.



Transition of REP 2055 dose for one week:
IV 400mg QW to IV 400mg QD
Improves hepatocyte loading
Achieved rapid additional decline in HBsAg > 2 $\log_{10}$ IU/mL within three weeks

Slowly returned to original HBsAg response when REP 2055 dosing was retuned to QW

Non-response is a pharmacokinetic issue

Current phase II response data with existing NAP drug product
(chelate complex)

HBV / HDV (REP 301, 7.4 years post therapy):
Weekly IV infusion 500mg / 250mg (suboptimal therapy)

64% HDV cure
36% HBV functional cure

HBV (REP 401, 5.3 years post therapy):
Weekly IV infusion 250mg

78% partial cure (no therapy indicated)
56% functional cure

Ongoing RCAP program
Demonstrates increasing C_{max} improves antiviral response (HDV RNA and HBsAg) seen with weekly SC dosing

- Transition from 250mg SC to 500mg SC QW
- Transition from 250mg SC to 250mg IV or 500mg IV QW

A drug product optimized to improve C_{max} during administration will increase antiviral responses

Summary

NAPs are bifunctional agents against HBV and HDV infection via interactions with DNAJB12 and HDAg

Details of the HDV mechanism / antiviral effects are still under investigation

Suboptimal clinical responses are caused by suboptimal hepatocyte loading and can be rescued by increasing C_{max}

A novel REP 2139-Mg drug product achieving improved C_{max} during administration will be deployed in future phase II studies

Expected to improve rates of HBV functional cure and HDV cure