Oligonucleotide-based strategies for targeting HBsAg: Understanding mechanisms and effects

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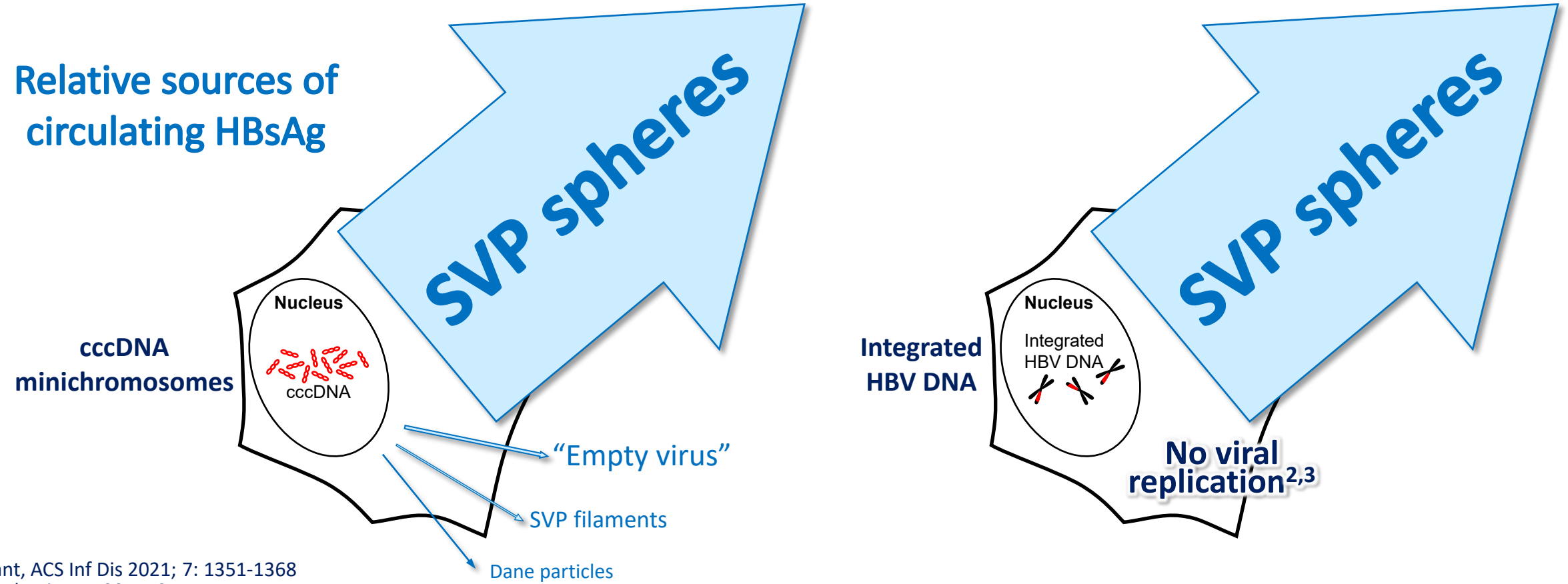
Disclosures

Employee and shareholder, Replicor Inc.

Targeting HBsAg means targeting subviral particles

Clearance of HBsAg is essential to restore immune control in chronic HBV infection[1]

Subviral particles (SVP) constitute 99.99% of circulating HBsAg[1]



1. Vaillant, ACS Inf Dis 2021; 7: 1351-1368
2. Tu et al., Viruses 2017; 9: 75
3. Yang et al., J Cancer 2018; 9: 3225-3235

Current clinical approaches to targeting SVP

All oligonucleotide-based
Specific activity requires delivery to hepatocytes

RNAi

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

Antisense

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

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

- **Rapid mutation rate of HBV!**
- **HBV quasispecies!**
- **Rapid turnover of active cccDNA!**

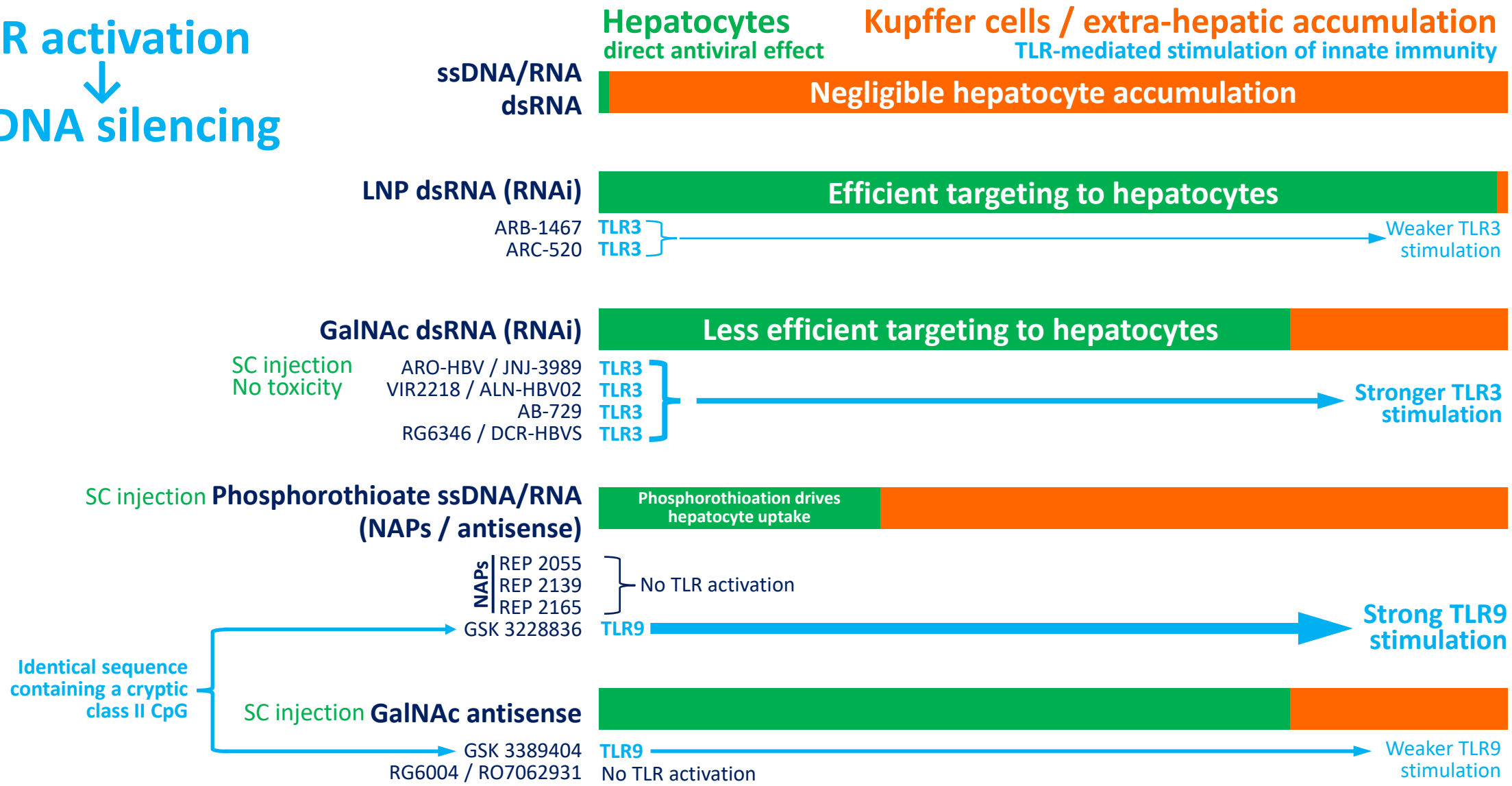
Nucleic acid polymers

Inhibition of subviral particle assembly

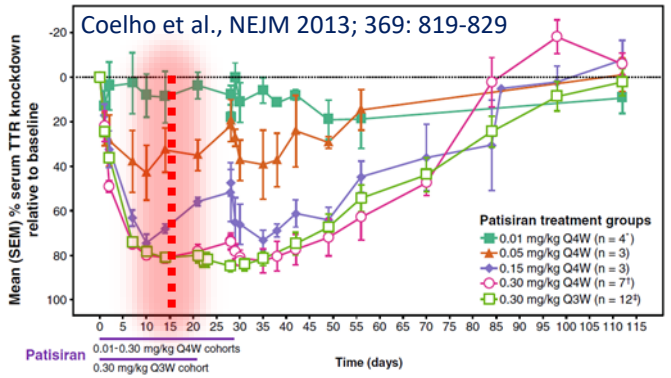
1. Vickers et al., J. Biol. Chem. 2003; 278: 7108-7118
2. Kawai and Akira Int Immunol 2009; 21: 317-337.
3. Robbins et al., Oligonucleotides 2009; 19: 89-101
4. Robbins et al., Hum Gene Ther 2008; 19: 991-999
5. Leuschner et al., EMBO Reports 2006; 7: 314-320

Oligonucleotide liver partitioning influences TLR activation

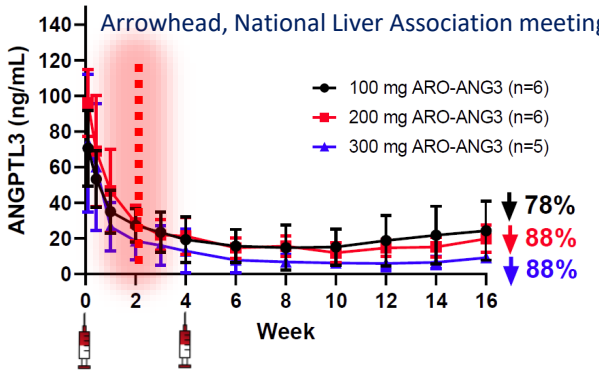
TLR activation
↓
cccDNA silencing



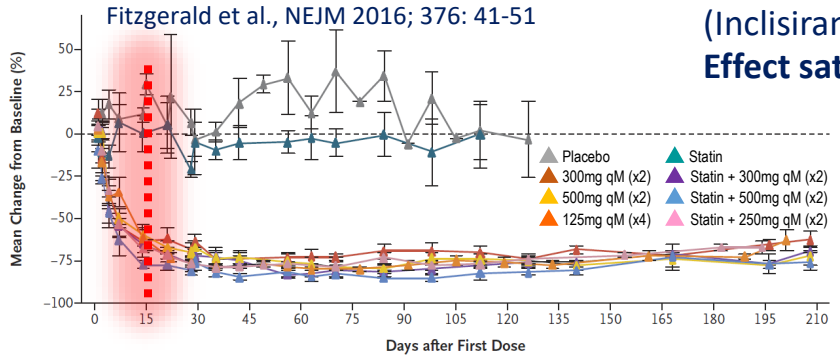
True RNAi pharmacodynamic signatures
(TLR3-independent)



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



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



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

Low interpatient variability

1 $\log_{10}$ protein decline within 2 weeks saturated with multiple dosing

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

Upper respiratory tract inflammation / infection with RNAi consistent with TLR3-mediated lung inflammation[1-3]

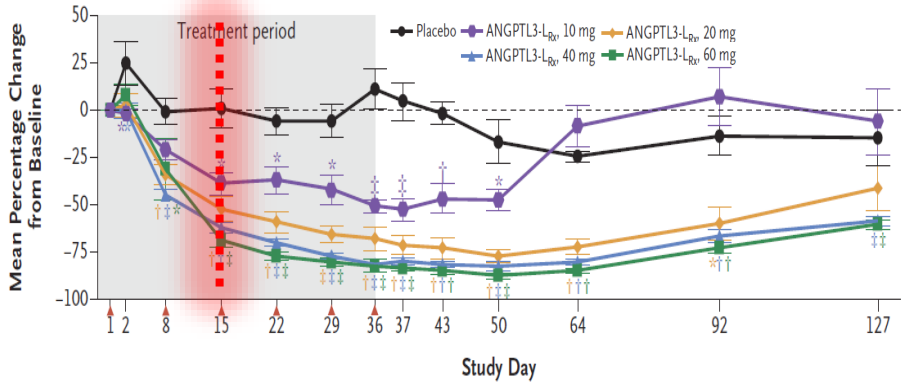
Sköld et al., Blood 2012; 120: 768-777
Murray et al., Am J Resp Care Med. 2008; 178: 1227-1237
Stowell et al., Respir Res. 2009; 10: 43

Patisiran, fitusiran, inclisiran, lumasiran, ARO-AAT, ARO-APOC3, ALN-AAT

ARC-520, VIR-2218 (ALN-HBV), JNJ-3938 (ARO-HBV)

True GalNAc-antisense pharmacodynamic signature (TLR9-independent)

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

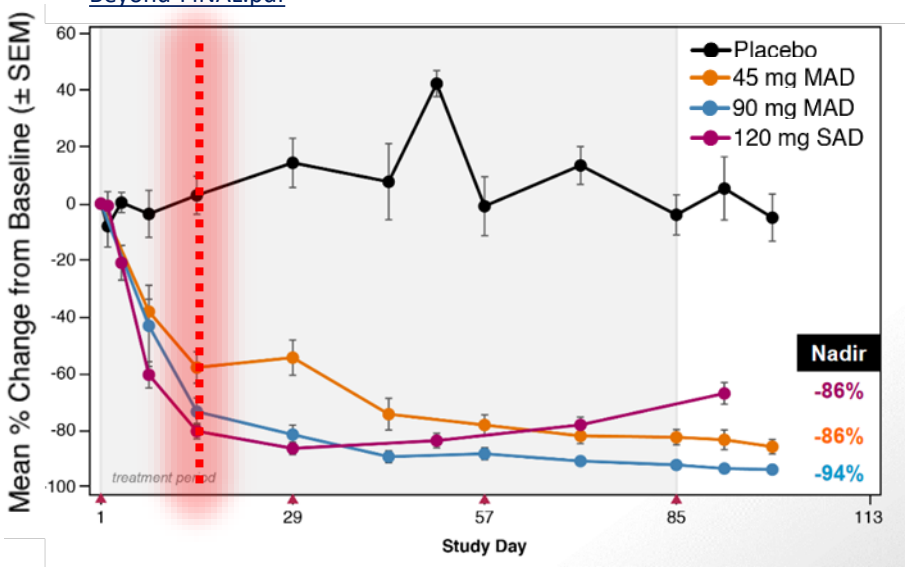


GalNAc-antisense
(Ionis / Akcea = ANGPTL3)
Effect saturated at 40mg

Low interpatient variability

1 $\log_{10}$ protein decline within 2 weeks saturated with multiple dosing

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

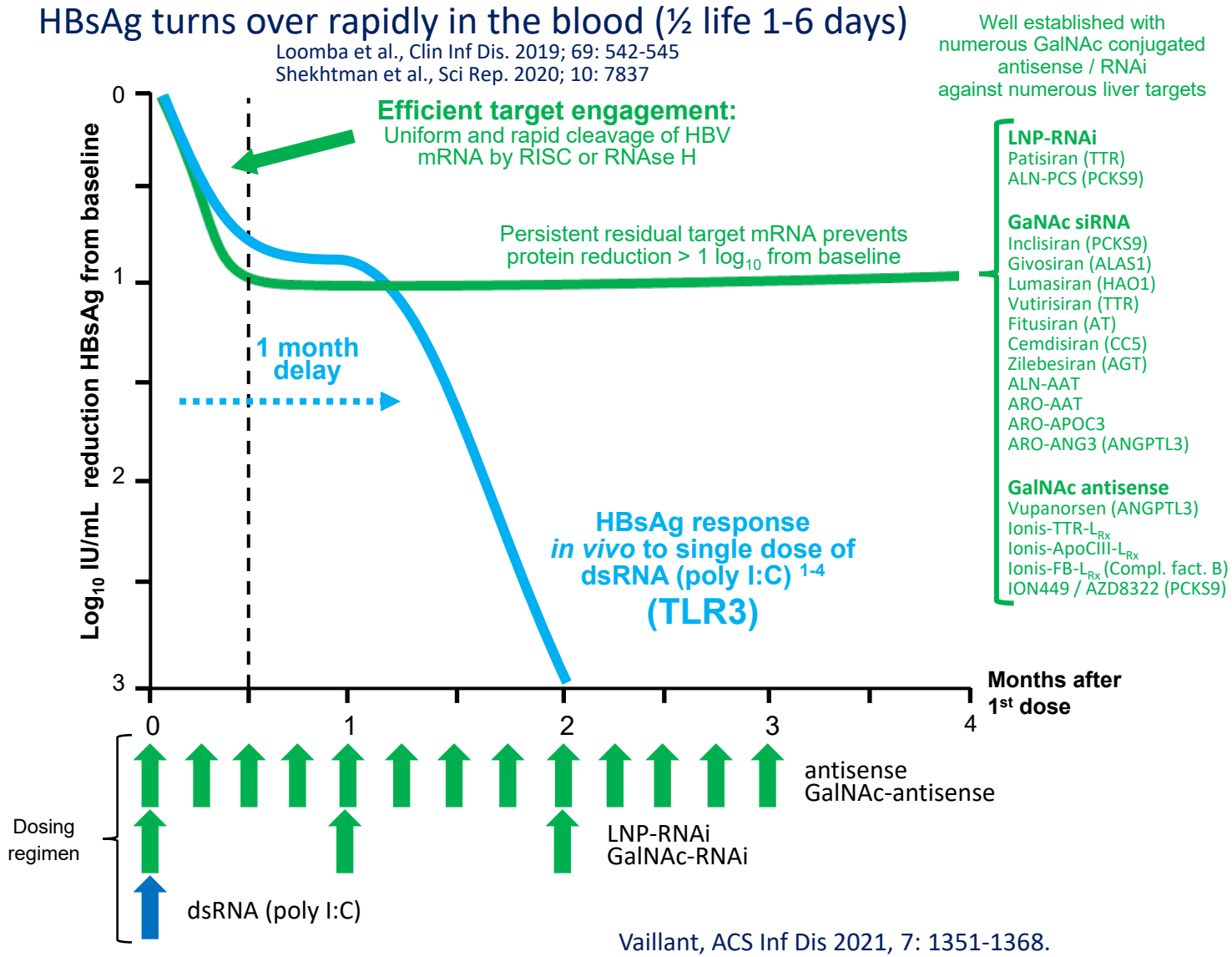


GalNAc-antisene
(Ionis / Akcea = TTR)
Effect saturated at 90mg

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

Pharmacodynamic response equivalent to RNAi

Expected HBsAg response with true antisense / RNAi effect



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

< 1 log_{10} HBsAg decline in 2 weeks:
Poor target engagement
Escape mutations present

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

Delayed and persistent HBsAg response :
dsRNA stimulation of TLR3

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

Which *in vivo* models are appropriate to assess RNAi effect?

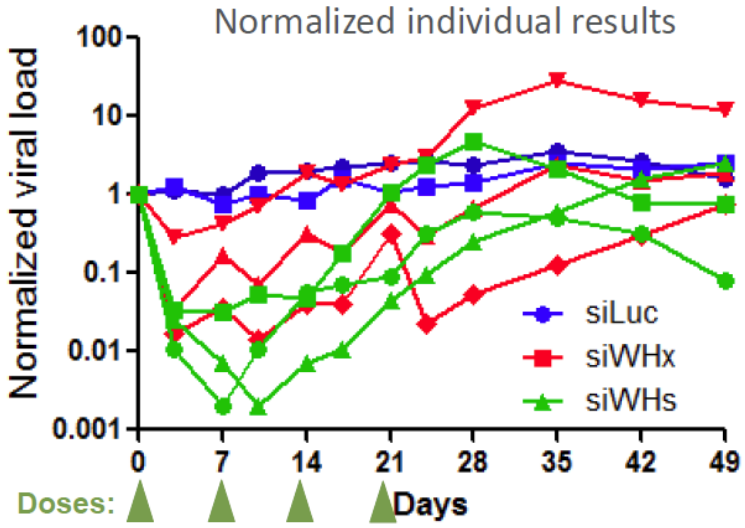
Model	Genetic diversity (pre-existing RNAi escape mutants)	Rapid turnover of cccDNA (evolution of RNAi escape mutants)	Response to TLR3 (off-target effect of RNAi)
Human	**Yes**	**Yes**	**Yes**
Transgenic mice	**No**	**Present but turnover unknown**	**Stronger vs primate[1,2]**
AAV / HDI-mice	**No**	**Present but turnover unknown**	**Stronger vs primate[1,2]**
Scid-Hu mice	**Yes** (limited due to short term infection)	**Yes**	**Stronger vs primate[1,2]**
Ducks	**Yes** (limited due to short term infection)	**Yes**	**Similar to primate**
Woodchucks	**Yes** (chronic infection)	**Yes**	**Similar to primate**

Poorly suited for RNAi analysis

Better suited for RNAi analysis

***In vivo* analysis of LNP-RNAi in WHBV infected woodchucks**

Tekmira, DIA 2015



Rapid response is consistent with RNAi effect

BUT

1. **Weak or null response in some animals** (escape mutants highly prevalent at baseline)
2. **Rapid rebound during treatment in all animals** (rapid evolution of escape mutants)

1. Mitchell et al., Am J Pathol 2014; 184: 1062-1072
2. Zshaler et al., Crit Rev Immunol 2014; 34: 433-454

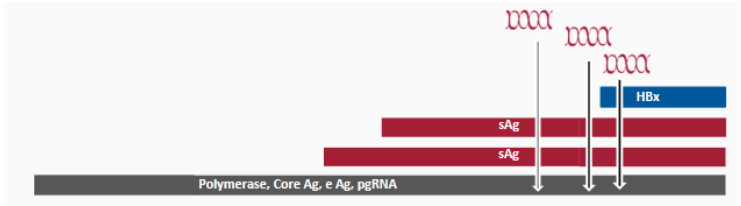
TKM-HBV (ARB-1467)

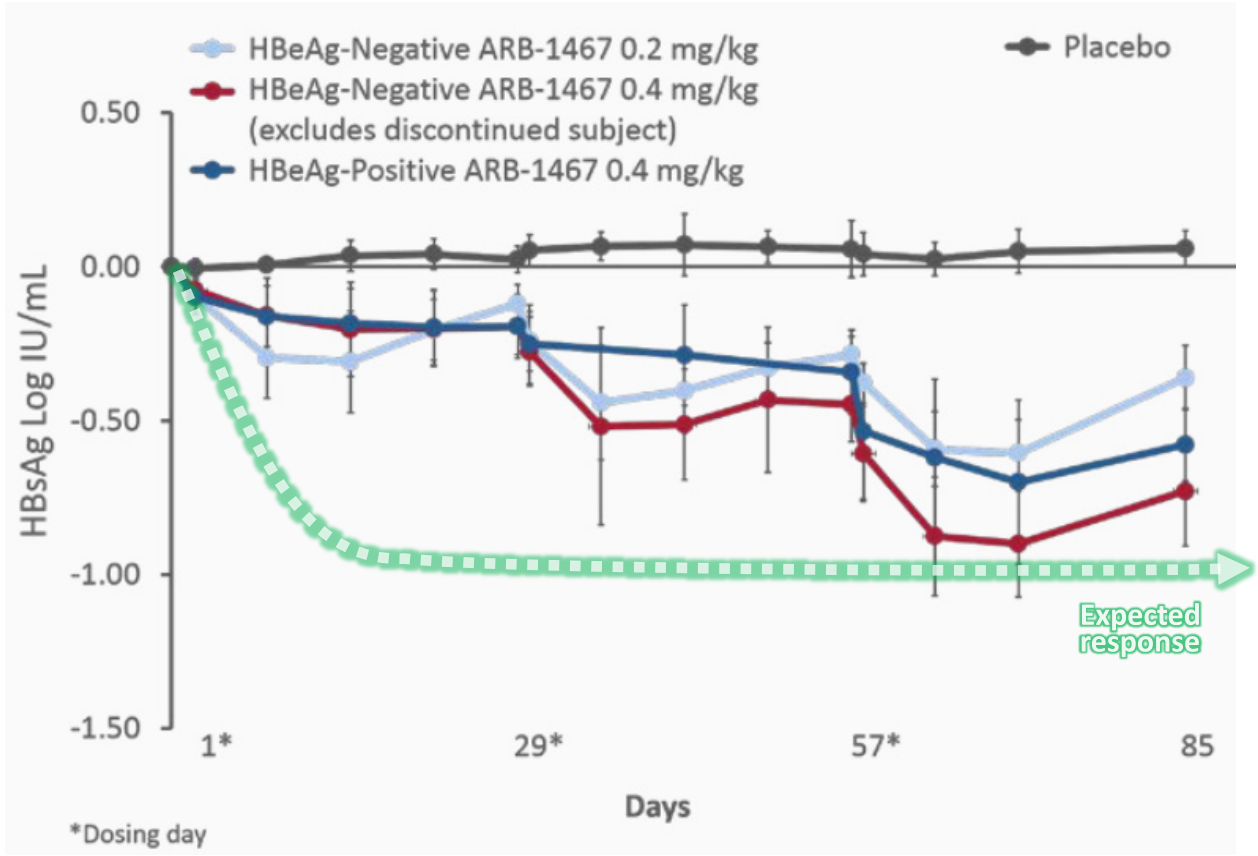
Optimum design for efficacy:

High efficiency targeting to hepatocytes (LNP)

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

best effort against mutational escape





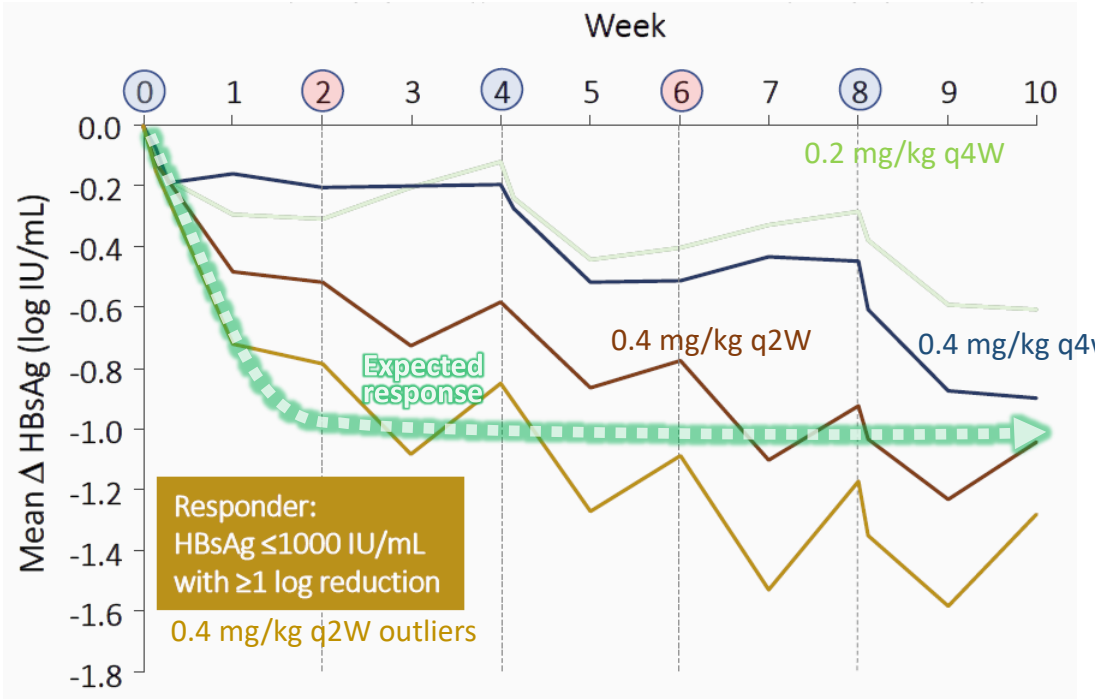
Highly variable and weak HBsAg response indicates minimal cleavage of HBV mRNA

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

TKM-HBV (ARB-1467)

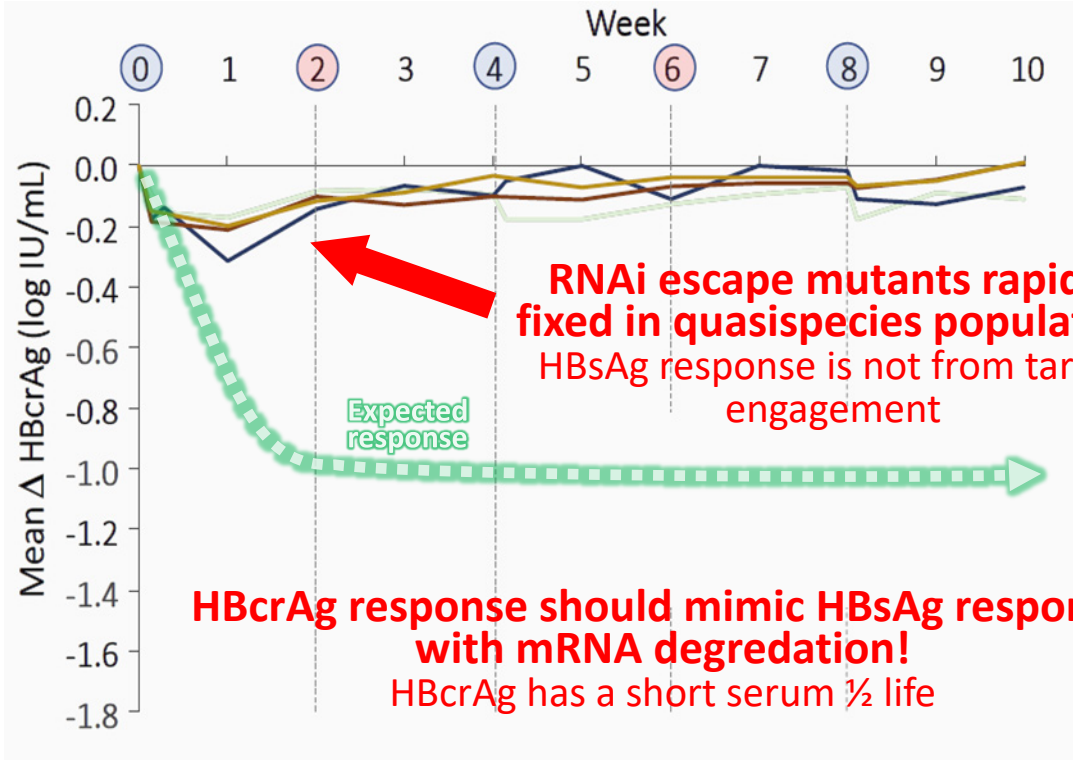
LNP: one RNAi targeting HBx and two RNAi targeting HBsAg (+ETV or TDF)

HBsAg



Weak HBsAg response = poor target engagement

HBcrAg



RNAi escape mutants rapidly fixed in quasispecies population

HBsAg response is not from target engagement

HBcrAg response should mimic HBsAg response with mRNA degredation!

HBcrAg has a short serum ½ life

Lack of target engagement with three RNAi triggers demonstrates mutational escape for all sequence specific approaches in HBV

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

Development of ARB-1467 halted

Liver partitioning affects immunostimulatory properties of oligonucleotides

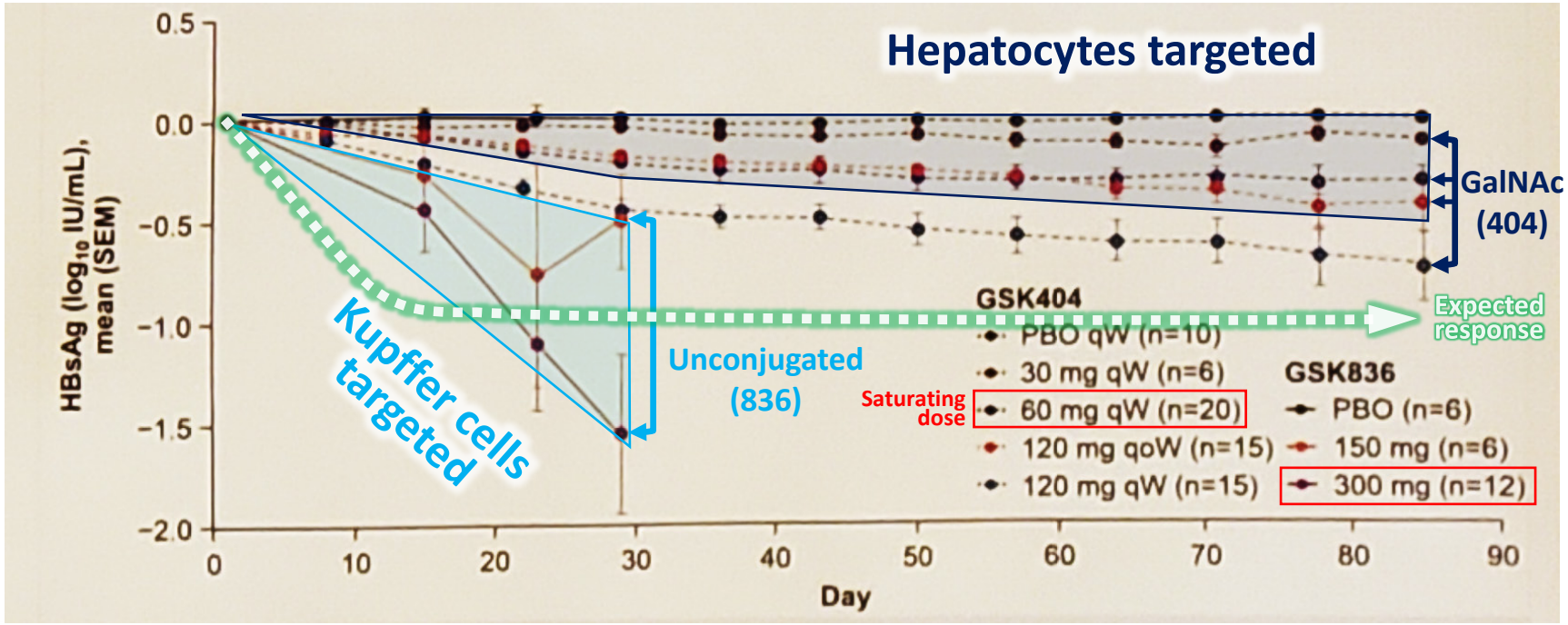
Cryptic class B CpG (TLR9 stimulation)

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

(HBx)



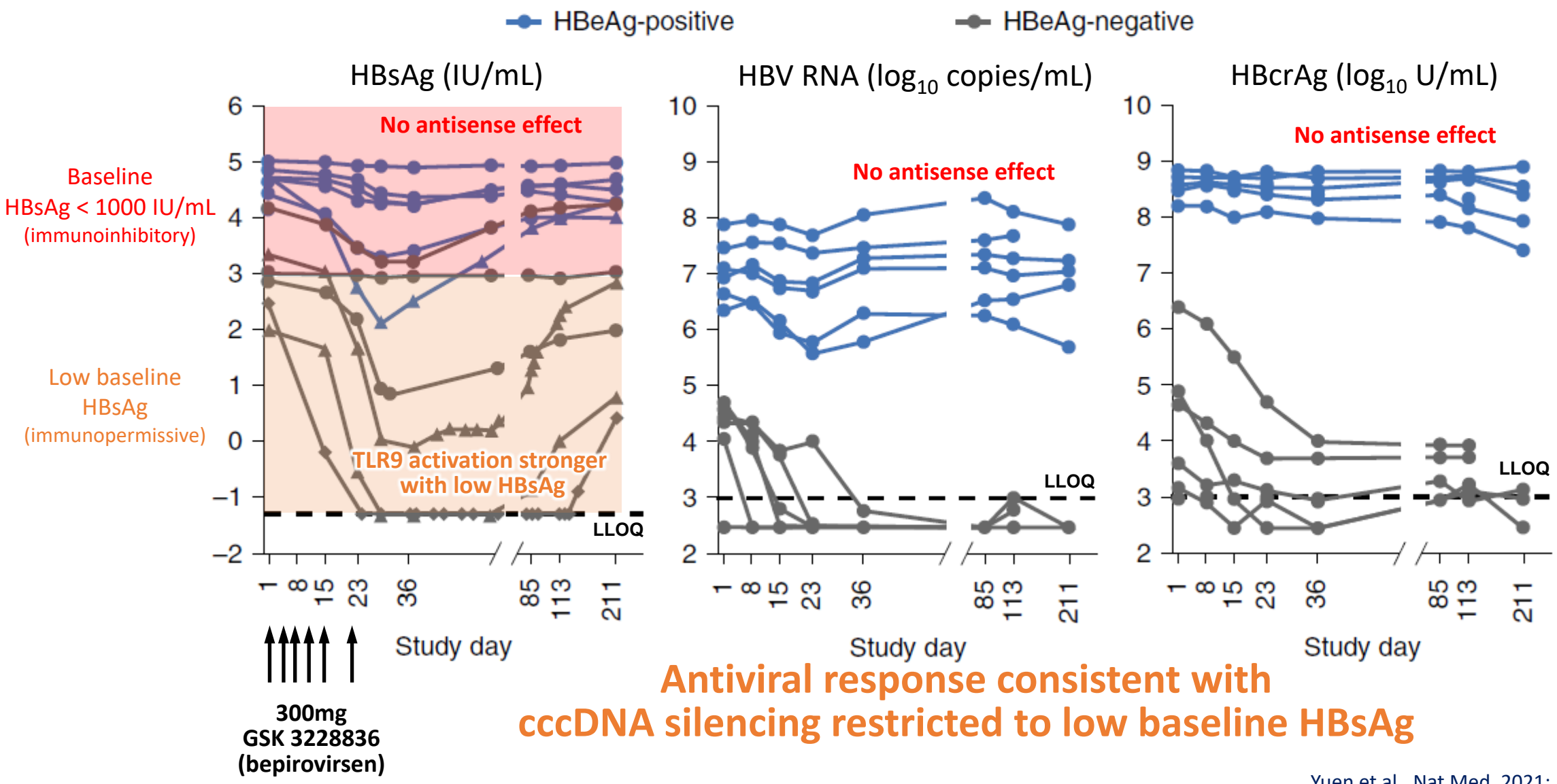
Theodore et al., HEPDART 2019 O15

GalNAc drives hepatocyte targeting
Weak HBsAg response indicates HBV mRNA is not efficiently cleaved
Similar to RG6004

GSK 404 and RG6004 development halted!

Kupffer cells targeted without GalNAc
Stronger but variable HBsAg response

GSK 3228836 – HBsAg response is dependent on baseline HBsAg



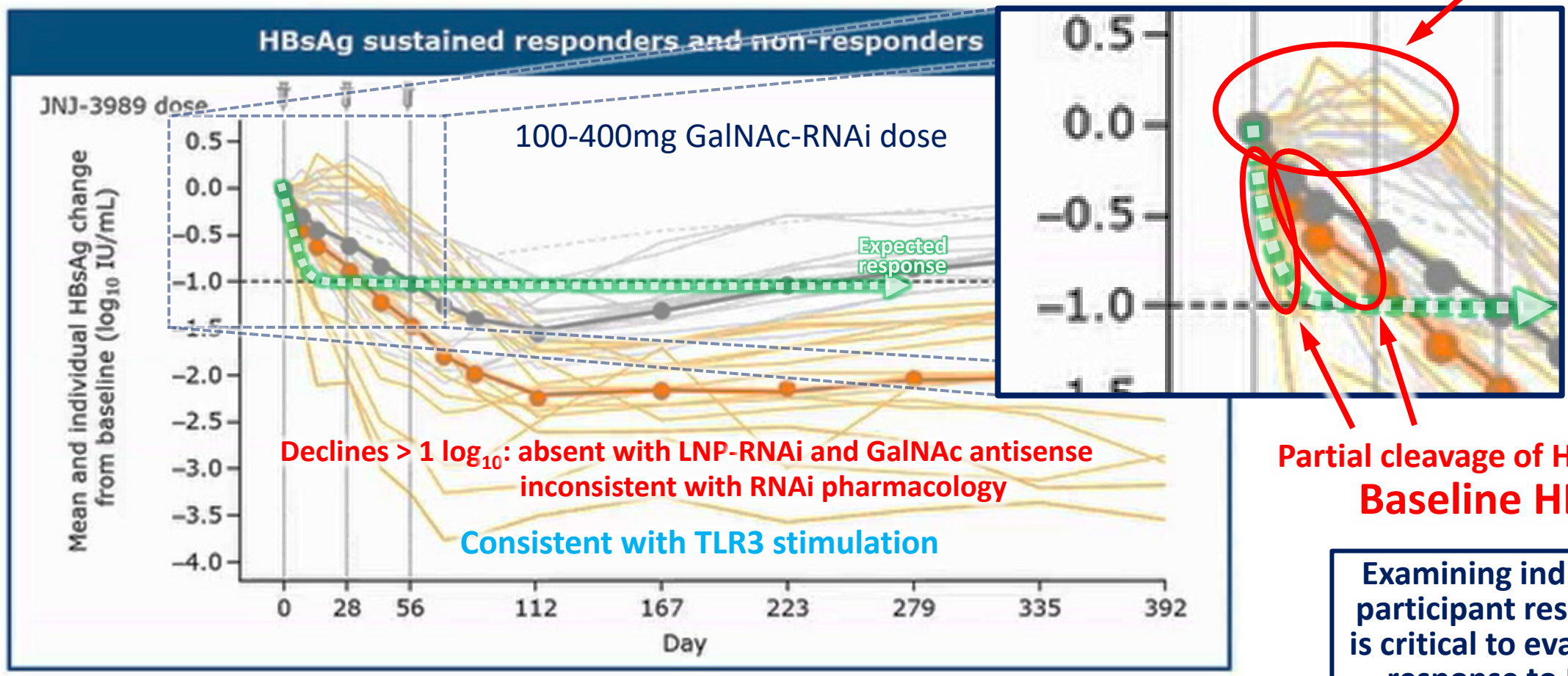
Antiviral response consistent with cccDNA silencing restricted to low baseline HBsAg

Yuen et al., Nat Med. 2021; 27: 1725-1734

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 (?)

Baseline HBsAg?

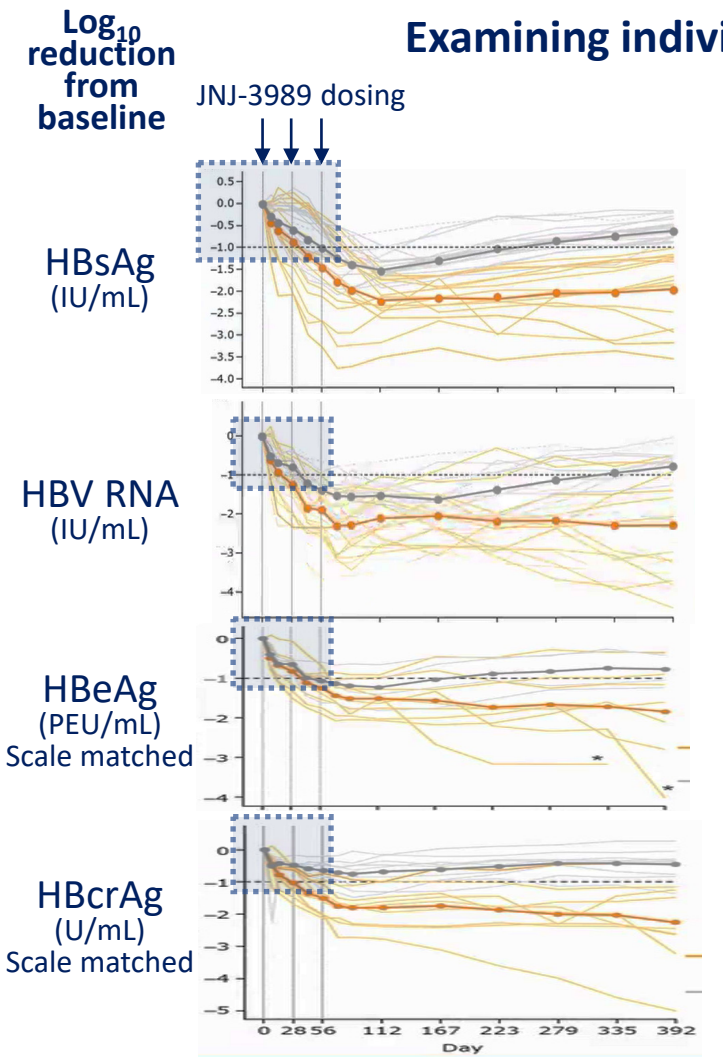
Examining individual participant responses is critical to evaluating response to RNAi

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

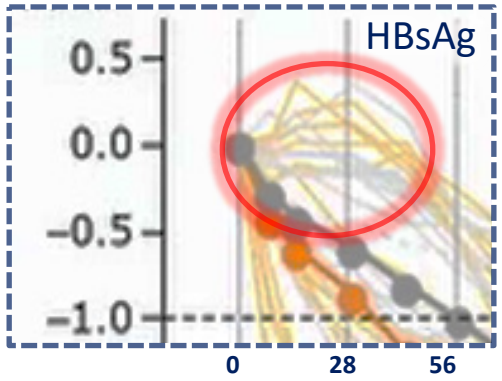
JNJ-3989 (ARO-HBV)

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

Examining individual participant responses is critical to evaluating response to RNAi

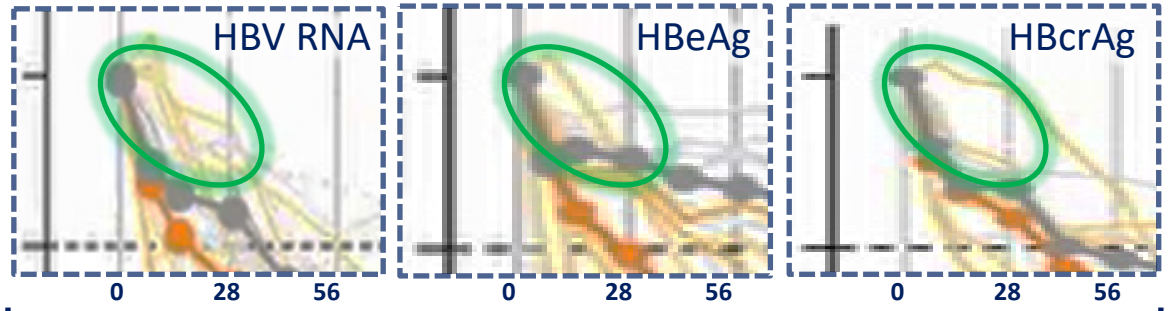


No HBsAg response
No cleavage of HBV mRNA



cccDNA + integrated HBV DNA

cccDNA silencing



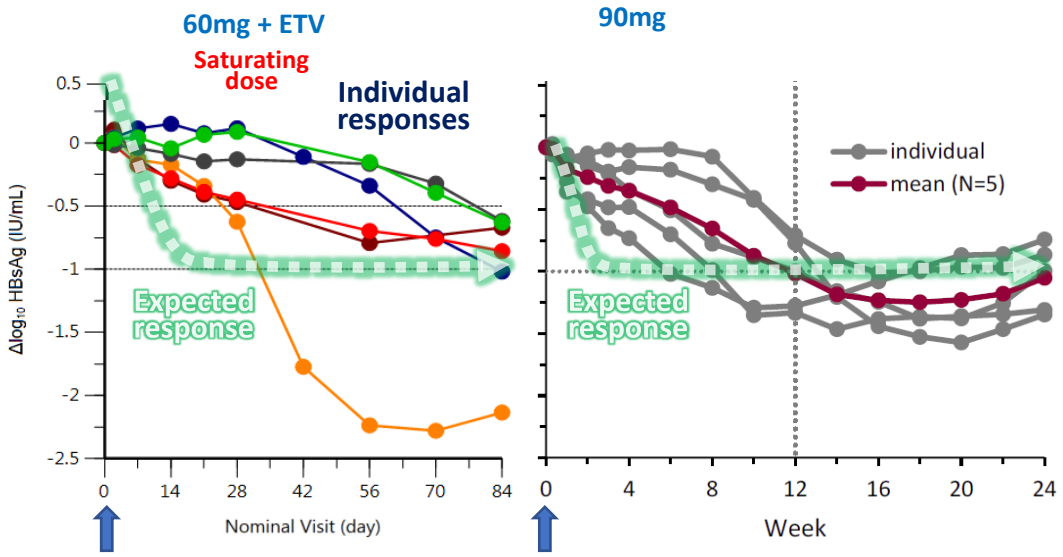
cccDNA

Initial HBV marker response consistent with inactivation of cccDNA

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

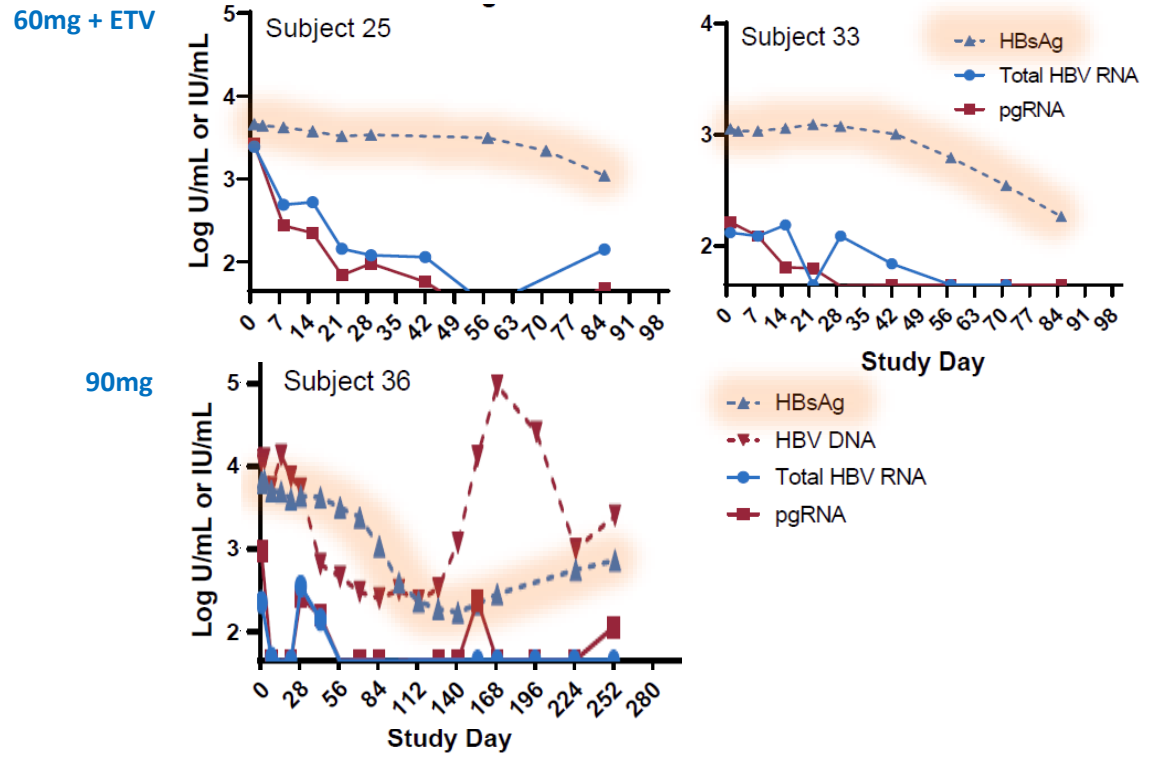
AB-729
GalNAc-RNAi targeting HBx

HBsAg reduction from baseline



HBV mRNA cleavage minimal or absent in most patients

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

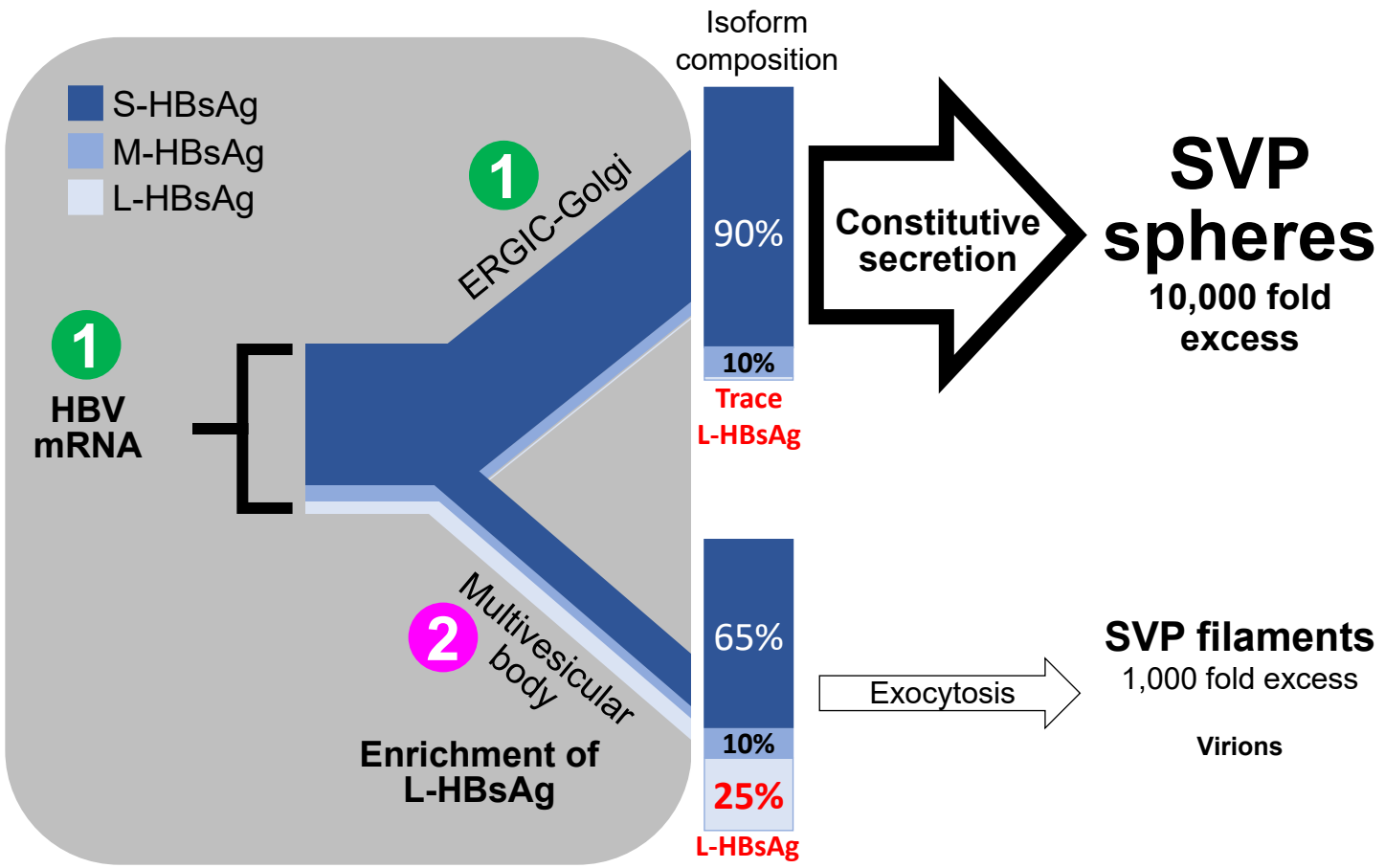


HBsAg response delayed relative to HBV DNA and HBV RNA (similar to JNJ 3989)

Consistent with inactivation of cccDNA without directly affecting integrated HBV DNA

http://www.arbutusbio.com/portfolio/ab-729-galnac-rnai.php
Gane et al., APASL 2021
Thi et al., J Hepatol 2021; 75: S760

HBsAg isoform response is dependent on antiviral effect



SVP turn over rapidly
99.99% of HBsAg

1 Selective decline in S-HBsAg

Signals inhibition of spherical SVP release

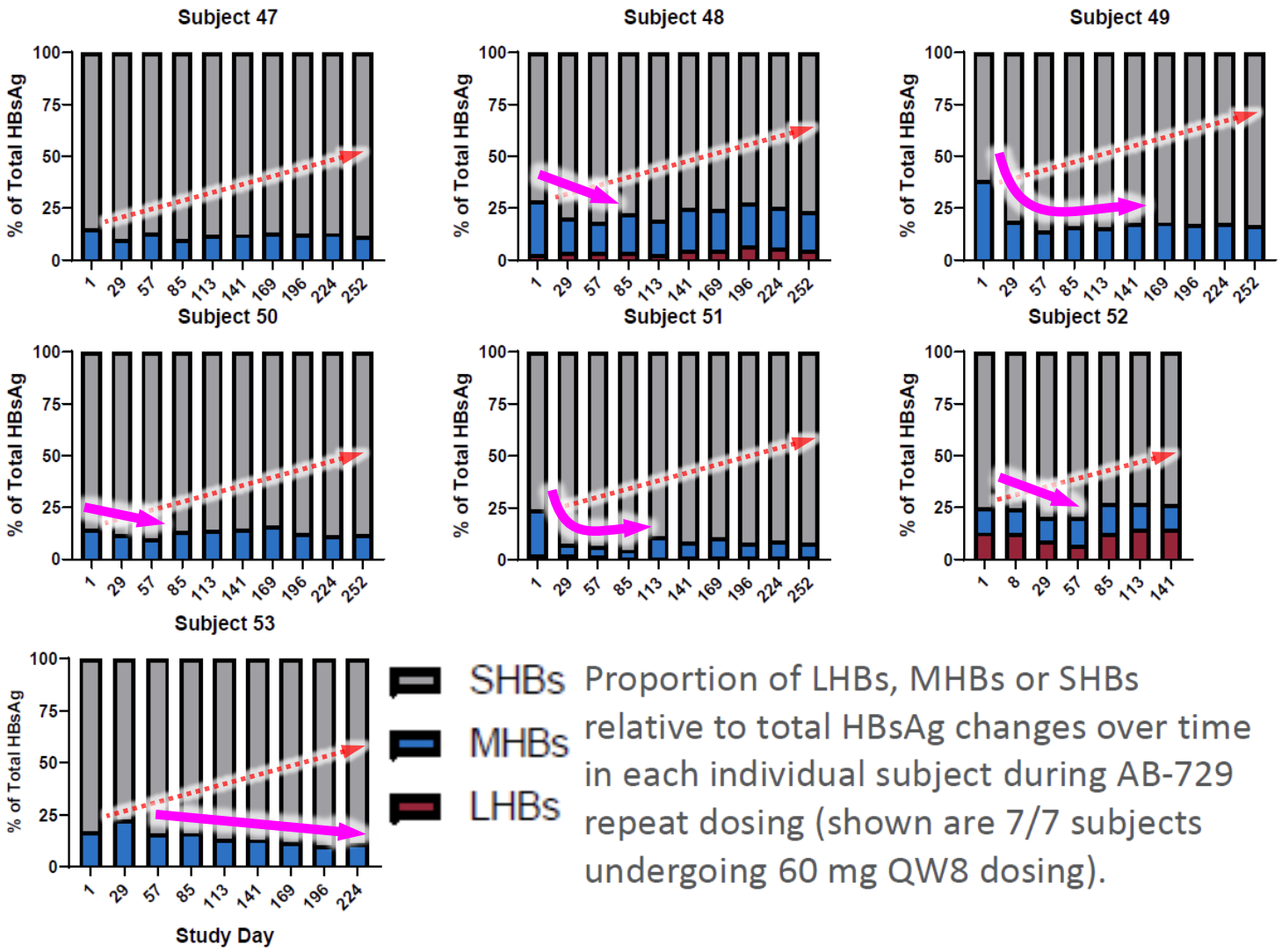
- HBV mRNA degradation
- Inhibition of SVP assembly / release

2 Selective decline in M/L-HBsAg

Inhibition of virion assembly?
Inhibition of exocytosis of virions / filaments?
Enhanced autophagy?

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

HBsAg isoform ratio response to AB-729



Proportion of LHBs, MHBs or SHBs relative to total HBsAg changes over time in each individual subject during AB-729 repeat dosing (shown are 7/7 subjects undergoing 60 mg QW8 dosing).

Thi et al., J Hepatol 2021; 75: S760

No selective decline in S-HBsAg

SVP sphere production is unaffected

Selective declines in M and L-HBsAg

Selective effects on SVP filaments and virions

TLR3-enhanced autophagy?

Delgado and Deretic, Cell Death Diff 2009; 16: 976-983
Lin et al., Cells 2020; 9: 2101

Inconsistent with mRNA degradation

Moving forward to correctly interpret clinical data with RNAi compounds in HBV/HDV

RNAi=dsRNA=TLR3 stimulation = strongest antiviral effect against HBV among TLRs[1-4]
TLR3 stimulation is common with RNAi but only effects protein response in HBV

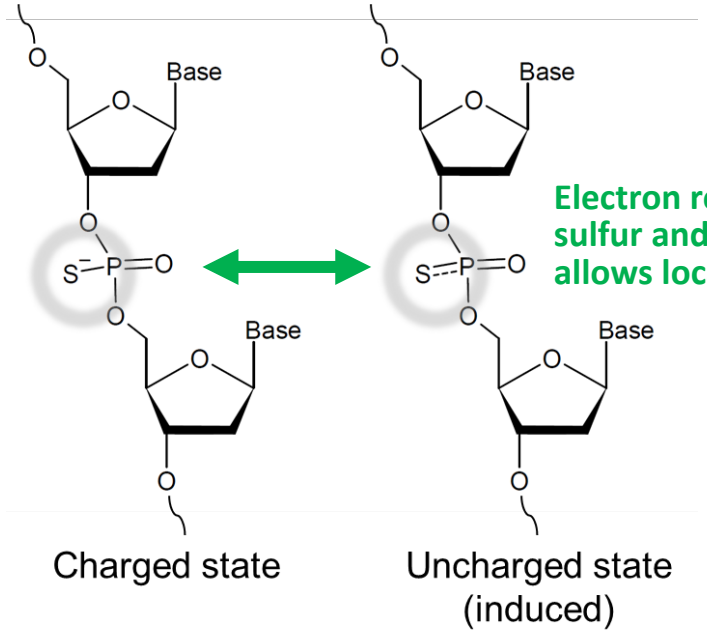
1. Exclude patients with HBsAg < 1000 IU/mL (these respond better to TLR stimulation)[5]
2. Disclose correlation between baseline HBsAg and HBsAg response on therapy
3. Disclose individual patient responses to HBsAg, HBV RNA, HBcrAg, and HBeAg
4. dsRNA-mediated TLR3 stimulation persists for several months

Delayed rebound during follow-up!

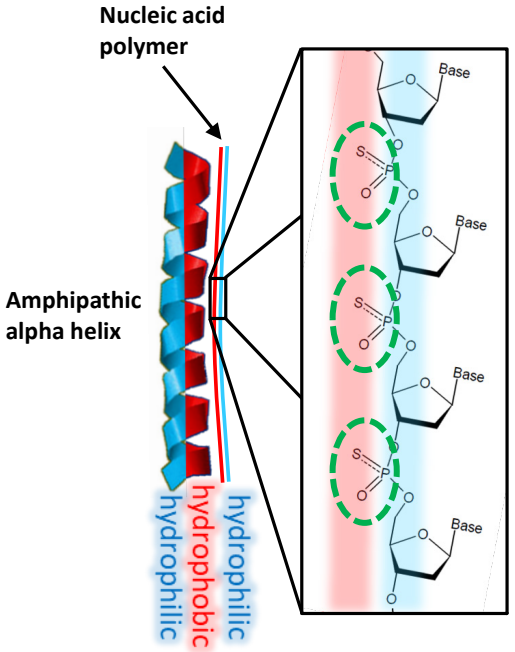
1. Isogawa et al., J Virol 2005; 79: 7269-7272
2. Wu et al., Hepatol 2007; 46: 1769-1778
3. Zhang et al., Front Immunol 2018; 9: 2921
4. Lucifora et al., Sci Rep 2018; 8: 5390
5. Real et al., Sci Rep 2016; 6: 24865

Nucleic acid polymers (NAPs)

Oligonucleotides with sequence independent activity[1]



Electron resonance between sulfur and phosphorous allows local hydrophobicity



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

LNA modified NAPs

ALG-10000 = fully LNA modified REP 2055
ALG-10093 = LNA altimer modified REP 2139
ALG-10133 = LNA altimer modified REP 2165

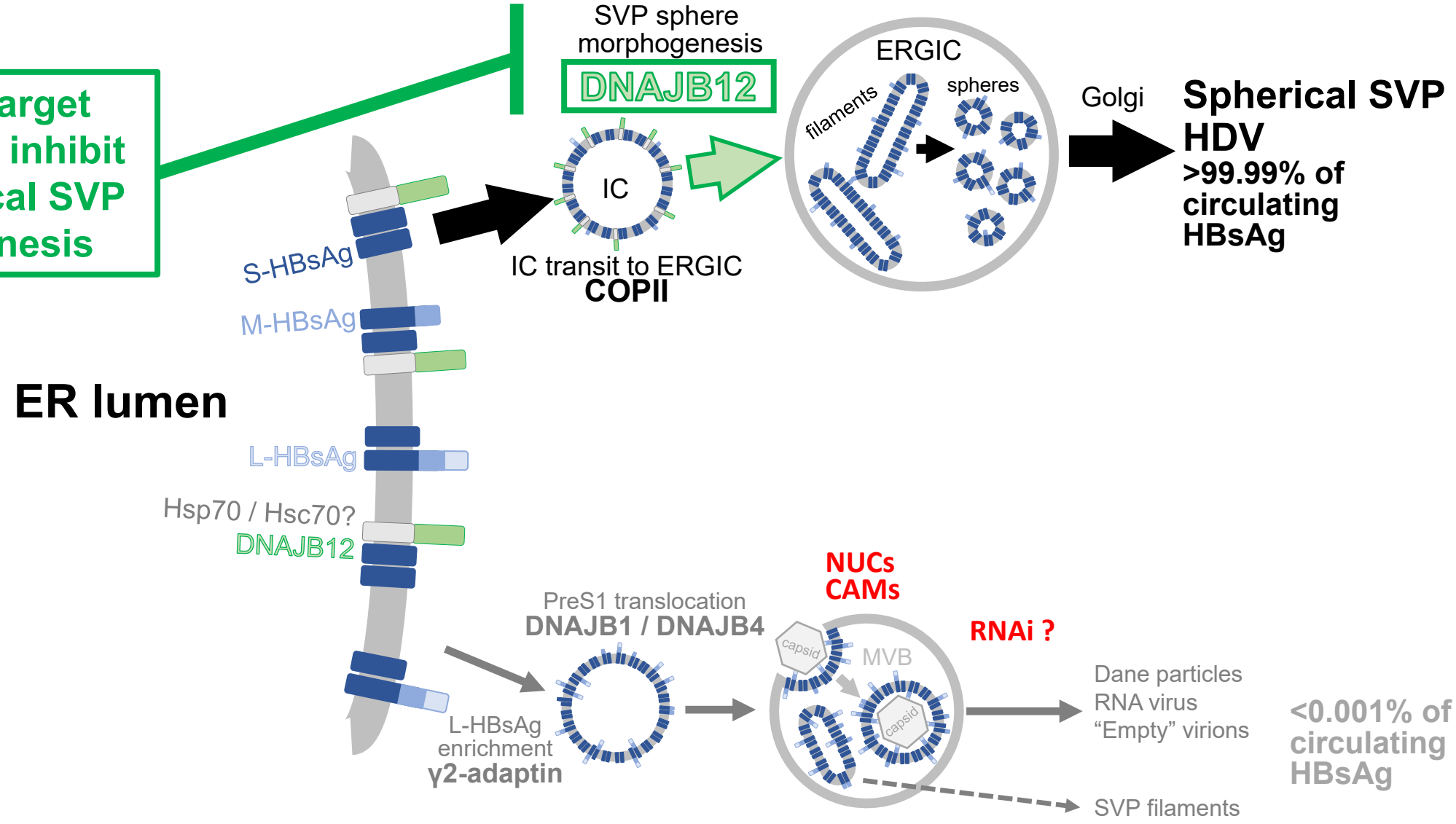
First described and protected by Replicor in 2002[2]
Abandoned early in NAP development!

LNA negatively impacts antiviral effect of NAPs
- structural alteration blocks NAP activity
- poor stability *in vivo*
- hepatotoxic

1. Vaillant. ACS Inf Dis 2019; 10: 675-687
2. Replicor US 8008239, 8008270, 8067385

NAP molecular mechanism in HBV

All NAPs target DNAJB12 to inhibit HBV spherical SVP morphogenesis

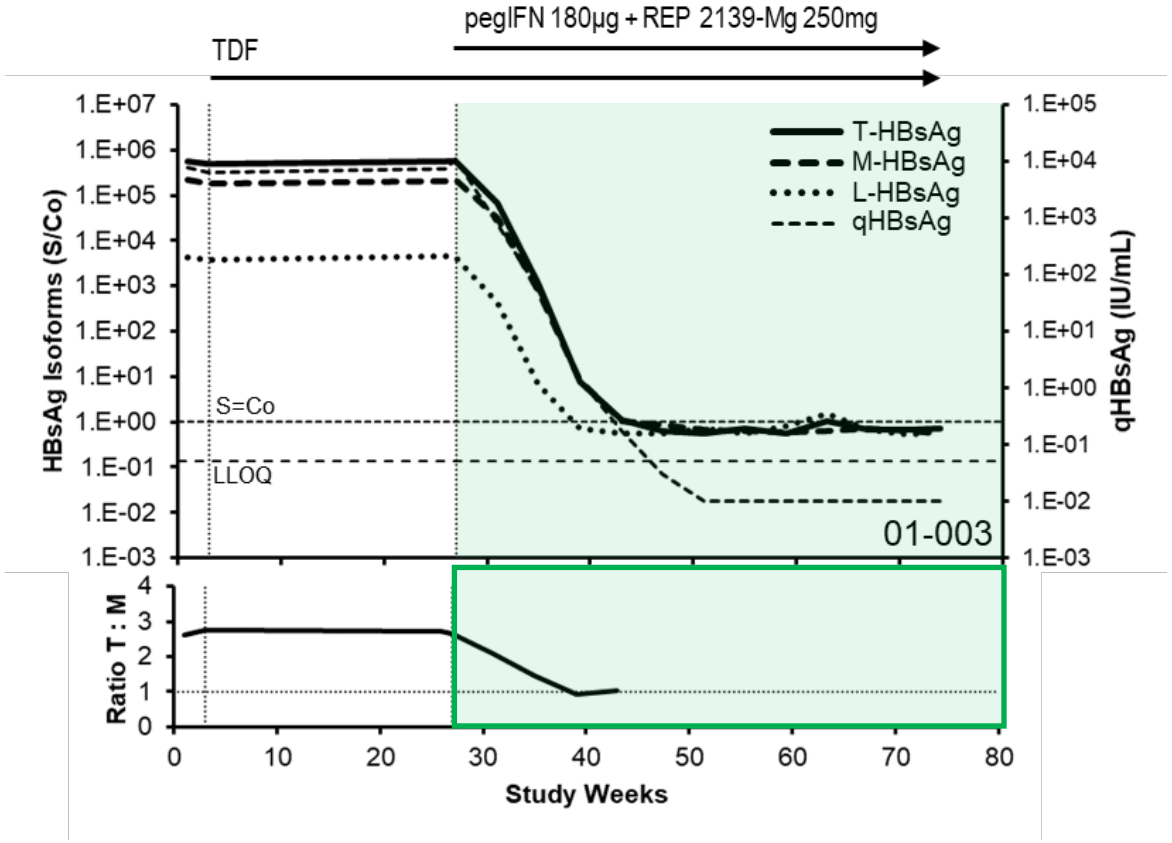


See Poster P1

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 (identical assay platform used for AB-729)

HBsAg response



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

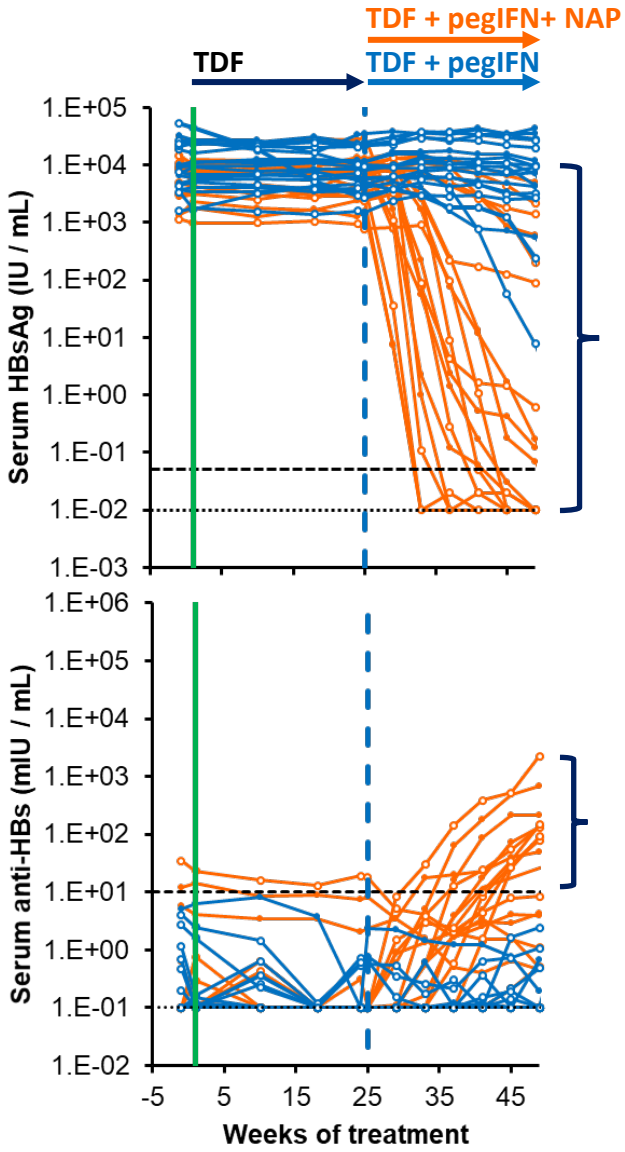
Correlation between selective S-HBsAg clearance during therapy and qHBsAg response
(all 52 participants in REP 301 + REP 401)

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)

Bazinet et al., Hepatology 2020; 72: 500A

REP 401: NAPs dramatically improve responses with TDF + pegIFN



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

Rapid HBsAg seroconversion

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

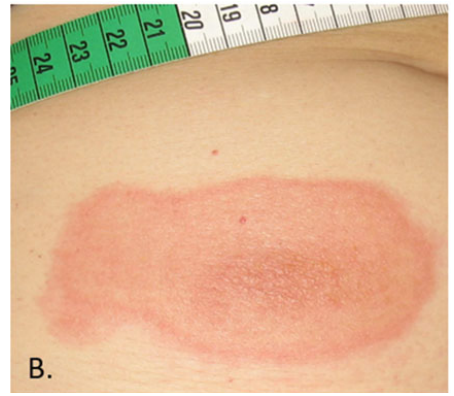
NAPs + TDF + pegIFN
HBsAg < 0.005 IU/mL (60%)
HBsAg seroconversion
Inactivation of cccDNA
Host mediated transaminase flares (95%)
High rates of HBV functional cure (39%)

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

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

Transition of REP 2139-Mg to subcutaneous administration

All oligonucleotides are accompanied by injection site reactions (ISR)[1]



RNAi (21-22mer)

Antisense (18-21mer)

NAPs – longer size (40mer) make ISRs **MUCH** stronger (even at doses as low as 25mg)[2,3]

regular sodium salt formulations of NAPs are not a suitable drug product[3,4]

Chelate complexes block administration reactivity (IV and SC)[2]

REP 2055 (sodium salt) – very poor administration tolerability (IV)[3] – also observed with ALG-10133 (SC)[4]

REP 2139-Ca (calcium chelate complex) – mild to moderate administration tolerability (IV)[4,5]

REP 2139-Mg (magnesium chelate complex) – administration asymptomatic (IV) with no supportive therapy[6]

Recent compassionate use of REP 2139-Mg SC in cirrhotic HBV / HDV co-infection

Good SC administration tolerability

Rapid HBsAg clearance and seroconversion (12 weeks)

Rapid HDV RNA clearance (4 weeks)

Early host mediated transaminase flare with no alteration of liver function

See poster P-7

1. Van Meer et al., Brit J Clin Pharmacol 2016; 82: 340-351
2. Replicor US 8,513,211
3. Al-Mahtab et al., PLoS One 2016; 11: e0156667
4. Gane et al., 2021, EASL ILC Poster 1004
5. Bazinet et al., Lancet Gastro Hepatol 2017; 2: 877-889
6. Bazinet et al., Gastroenterol 2020; 158: 2180-2194

Summary

Subviral particles (SVP): > 99.99% of circulating HBsAg, block immune control and function of immunotherapy
Removal during therapy is essential for functional cure

Integrated HBV DNA: Bulk of SVP production in HBeAg negative infection
Therapeutic transaminase flares signal removal of integrated HBV DNA from the liver

RNAi appears to act like dsRNA in human HBV infection:

HBsAg response consistent with TLR3-mediated effects:

- cccDNA inactivation
- TLR3-mediated enhancement of autophagy[1,2]? Inhibition of SVP filament/virion secretion from MVB?

Production of spherical SVP appears unaffected

Can TLR3 activation play a role in partial / functional cure?

1. Delgado and Deretic, Cell Death Diff 2009; 16: 976-983
2. Lin et al., Cells 2020; 9: 2101

NAPs: Inhibit SVP assembly from cccDNA and integrated HBV DNA
Chelate complex formulation key to good SC tolerability

Immunotherapy used in combination is associated with:

- **high rates of asymptomatic host-mediated transaminase flares**
- **high rates of partial cure and functional cure**

REP 2139-Mg successfully transitioned to SC administration