siRNAs and Antisense Oligonucleotides

HBV-TAG

2021 CONFERENCE

Ed Gane , University of Auckland, NZ

- Dr Gane discloses the following financial relationships with a commercial interest:
  - Advisor and/or speaker for AbbVie, Aligos, Arbutus, Arrowhead, Assembly, Avalia, Clear B Therapeutics, Dicerna, DrugFarm, Enanta, Finch Therapeutics, Gilead Sciences, GlaxoSmithKline, Janssen, Merck, Novartis, Roche and Vir Bio.
- Dr Gane will present investigational use of many drugs in development and also off-label use of tenofovir and entecavir
- The opinions expressed are entirely those of Dr Gane

Functional Cure
the New Treatment Goal

Clinical Practice Guidelines

JOURNAL OF HEPATOLOGY

EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection☆

European Association for the Study of the Liver*

"**HBsAg loss**, with or without anti-HBs seroconversion, is an optimal endpoint, as it indicates profound suppression of HBV replication and viral protein expression"

(Evidence level II-1, grade of recommendation 1).

Draft FDA Guidance

Chronic Hepatitis B Virus Infection: Developing Drugs for Treatment

Guidance for Industry

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

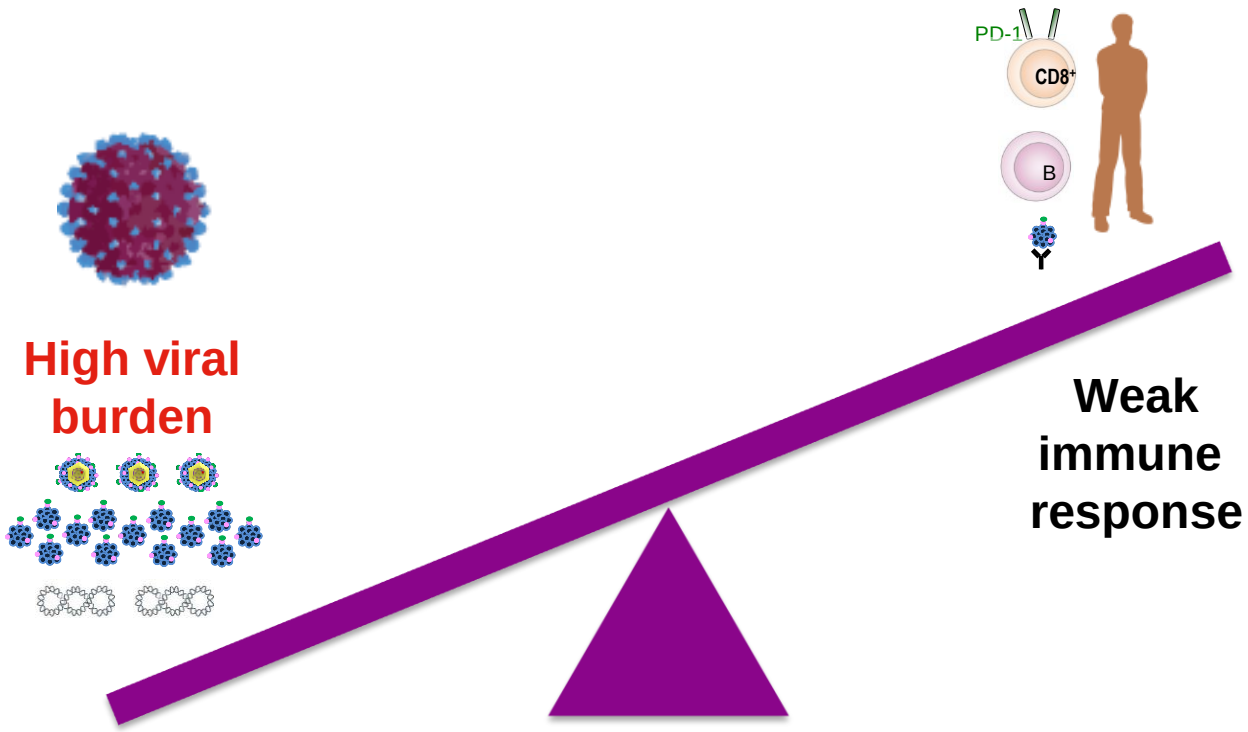
For questions regarding this draft document, contact Poonam Mishra at 301-796-1500.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

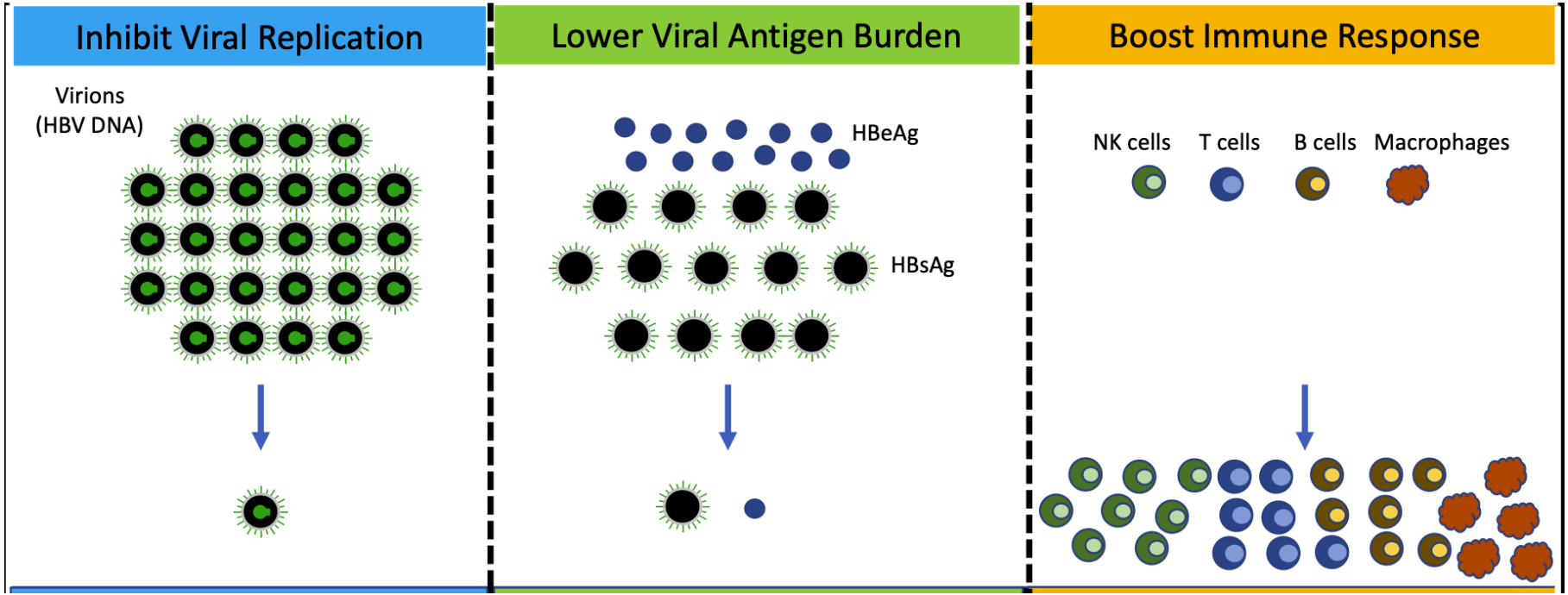
November 2018
Clinical/Antimicrobial

- Supports off-treatment HBsAg loss (± HBsAb seroconversion) after finite duration of therapy as a Phase 3 efficacy endpoint

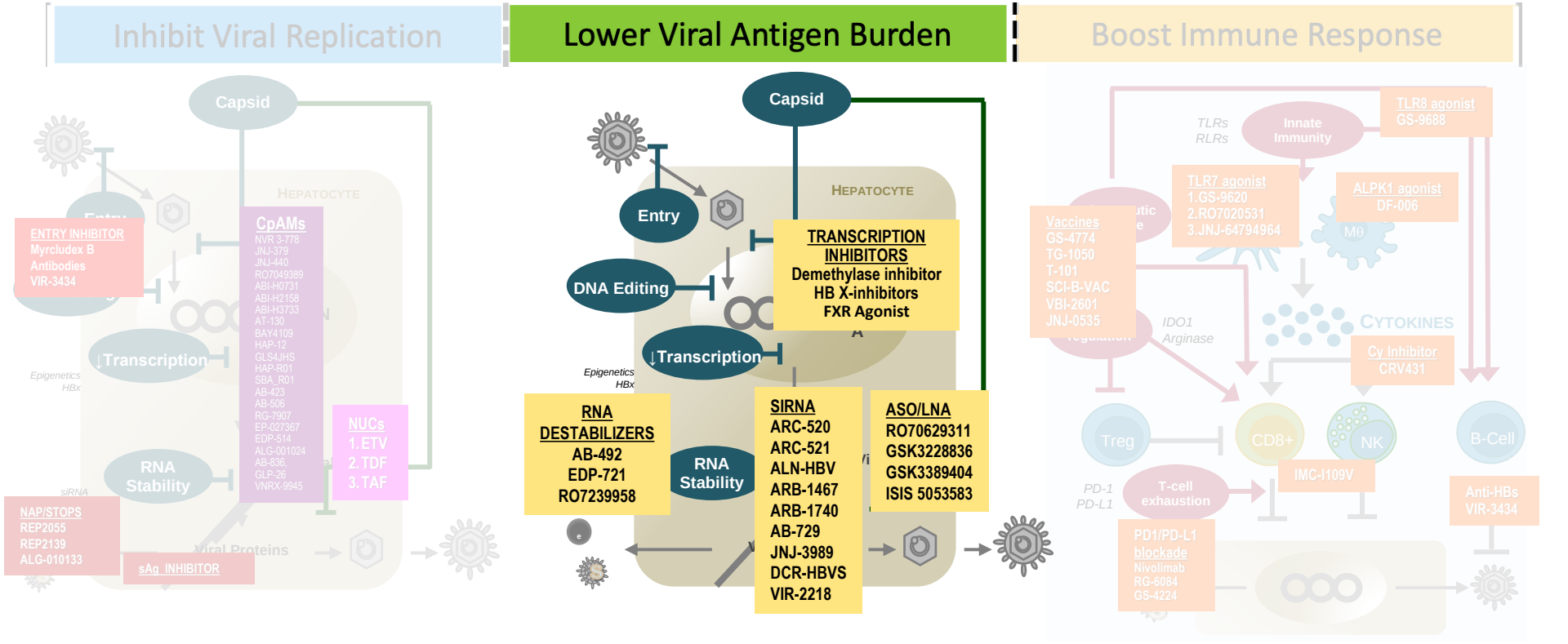
Barriers to HBV CURE



Therapeutic Approaches to HBV Cure



Therapeutic Approaches to HBV Cure

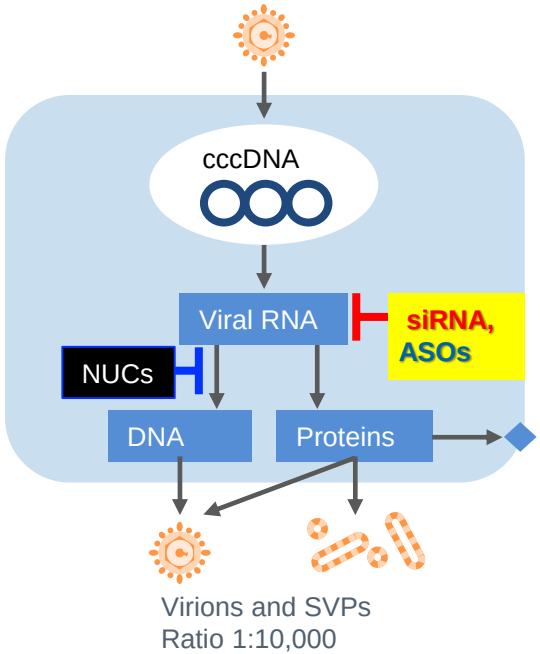


Translation Inhibitors block HBV protein synthesis

siRNAs

- Duplex (ds) RNA with guide (23nt) and passenger (21nt) RNA strands
- "Naked" siRNAs **cannot** enter cells so need carrier delivery system

1. NAG-MLP Chol-siRNA
   - ARC-520
   - ARC-521
2. Lipid nanoparticle (LNP)
   - ARB-1467
   - ARB-1740
3. GalNAC conjugated
   - ALN-HBV
   - ~~AB-729~~
   - ARO-B/JNJ-3989
   - DCR-HBVS/RG-6346
   - ALN HBV02/VIR-2218



ASO/LNAs

- Single strand DNA (8-10nt) modified to resist nucleases
- "Naked" ASOs enter all cells but require high doses

1. Naked
   - ISIS505358/GSK836
2. GalNAC conjugated
   - RO7062931
   - GSK3389404

➡ **knockdown viral protein synthesis**

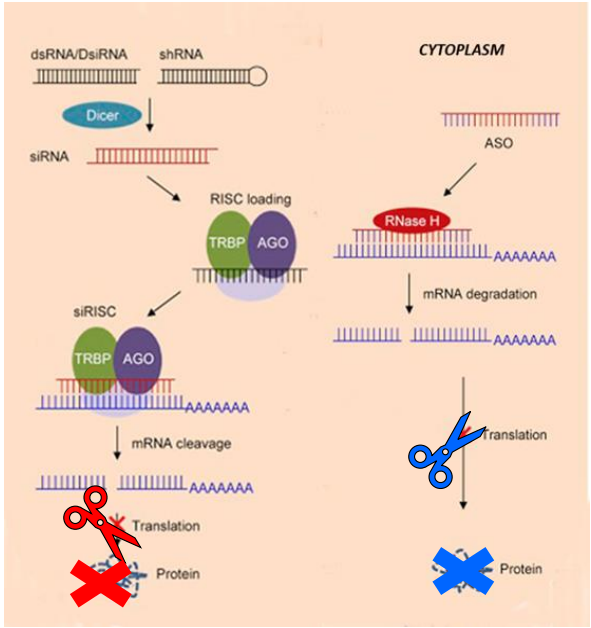
1. Directly inhibit virion & SVP production
2. *Indirectly boost host immune responses?*

Translation Inhibitors block HBV protein synthesis

siRNAs

- acid-stable siRNAs accumulate within endosomes which load RISC
- Stable guide RNA-AGO complex cleaves many target HBV mRNAs, amplifying gene silencing

➡ **Less frequent dosing but requires carrier system** *(lipid nanoparticles, GalNAc)*



ASO/LNAs

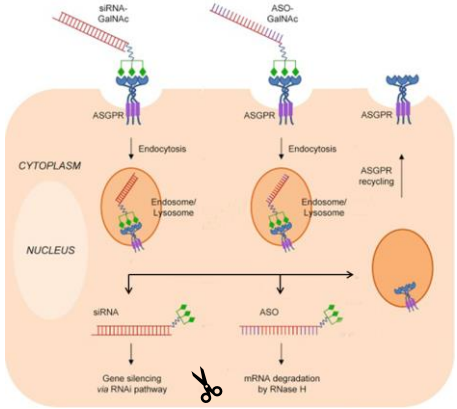
- Accumulate in liver, taken up by hepatocytes & Kupffer cells
- Simple enzyme-substrate reaction without amplification

➡ **Higher, more frequent dosing but no carrier required ⇨ less expensive production**

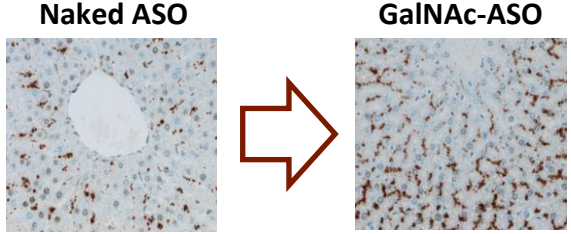
Liver-Targeting Drug Delivery (GalNAc or LNP)

Advantages of GalNAc conjugation

- Very high capacity ASGPR shuttle within the liver
  - 0.5 million receptors per hepatocyte
  - T½ receptor is 15 hours
  - Can administer >1 Gal-NAc therapy at same time
  - Minimal systemic exposure until very high doses
- GalNAc conjugation improves exposure
  - Required for siRNAs
  - Increases ASO/LNA uptake 10-fold
- GalNAc conjugation improves convenience
  - Subcutaneous vs. intravenous LNPs
  - Less infusion reactions than LNPs
  - Longer dosing intervals



Huang. Mol Ther Nucleic Acids, 2017



Javanbakht et al. Mol Ther Nucleic Acids, 2018

Efficacy of siRNAs/ASOs

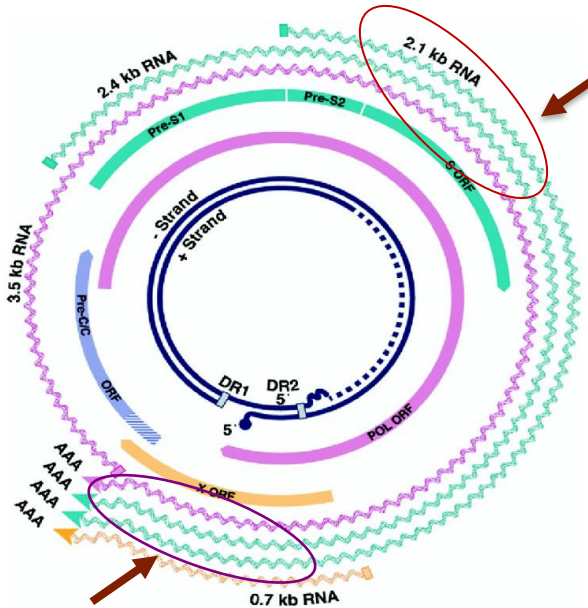
What are best HBV Targets for siRNAs/ASOs

- siRNA targeting overlapping region silences all transcripts

5 viral mRNAs
- 3.5 kb pre-genomic RNA
- 3.5 kb pre-core mRNA
- 2.4 kb pre-S1 mRNA
- 2.1 kb pre-S2/S mRNA
- 0.7 kb X mRNA

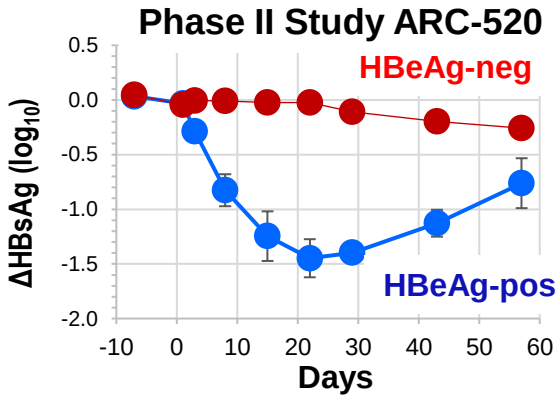
7 major proteins
- Polymerase/reverse transcriptase
- Core (HBcAg)
- e antigen (HBeAg)
- Large, medium and small surface proteins (HBsAg),
- X protein (Transactivator)

Ghany ***Gastro*** 2007; 132: 1574-85



"S" Trigger

- **1st gen siRNAs** target "S" transcripts from cccDNA. Less sAg decline in HBeAg neg CHB where most "S" from integrants



- **2nd gen siRNAs** triggers located down-stream from DR1-DR2. Silence "S" from cccDNA and integrants ⇨similar sAg declines in HBeAg pos & HBeAg neg CHB

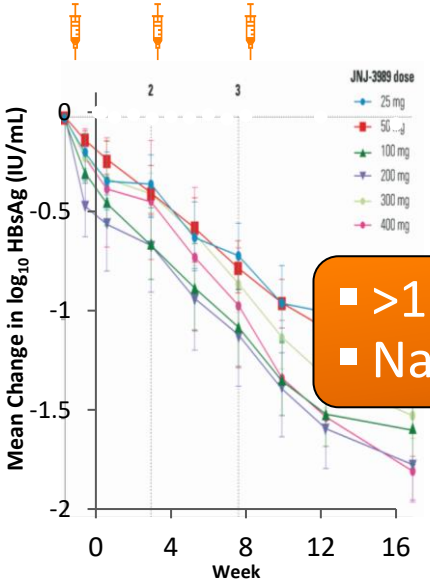
Woodell C. ***Sci. Transl. Med***. 9 2017; ,eaan0241

"X" Trigger

- Covers all HBV transcripts
- Should inhibit cccDNA transcription (via Smc5/6)

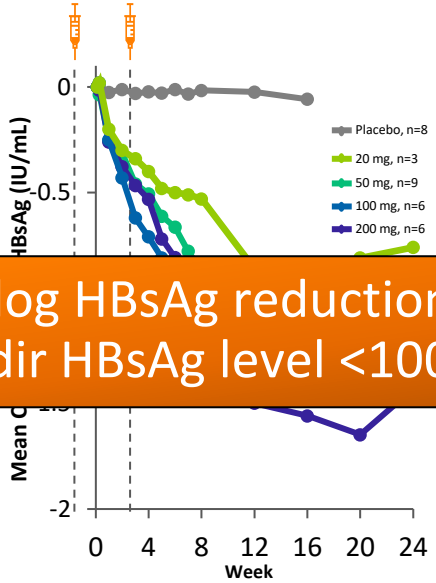
siRNAs achieve potent on-treatment HBsAg responses

1. JNJ-3989/ARO-HBV
X and S triggers
3x monthly doses



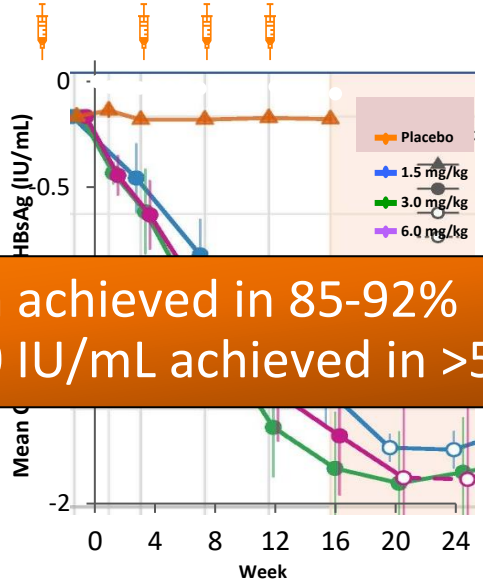
Gane E, et al. AASLD 2019 #0696
Gane E, et al. EASL dILC2020. #GS10

2. VIR-2218/ALN HBV02
Single X trigger
2x monthly doses



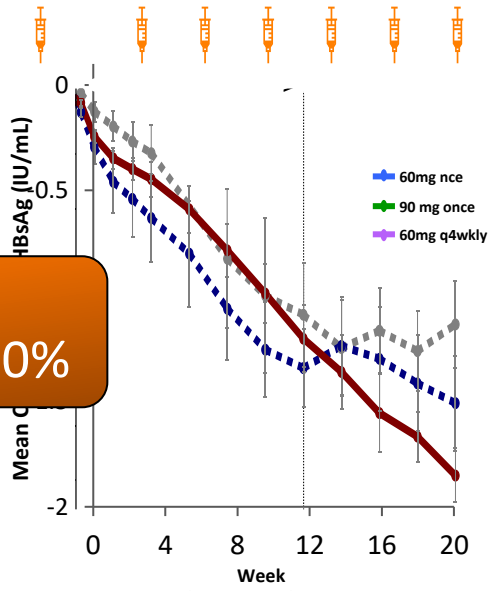
Gane E, et al. EASL dILC 2020 #AS068

3. RG-6346/DCR-HBVS
Single S trigger
4x monthly doses



Yuen M-F, et al. AASLD TLMdX2020. #LB

4. AB-729
Single X trigger
1-6x monthly doses



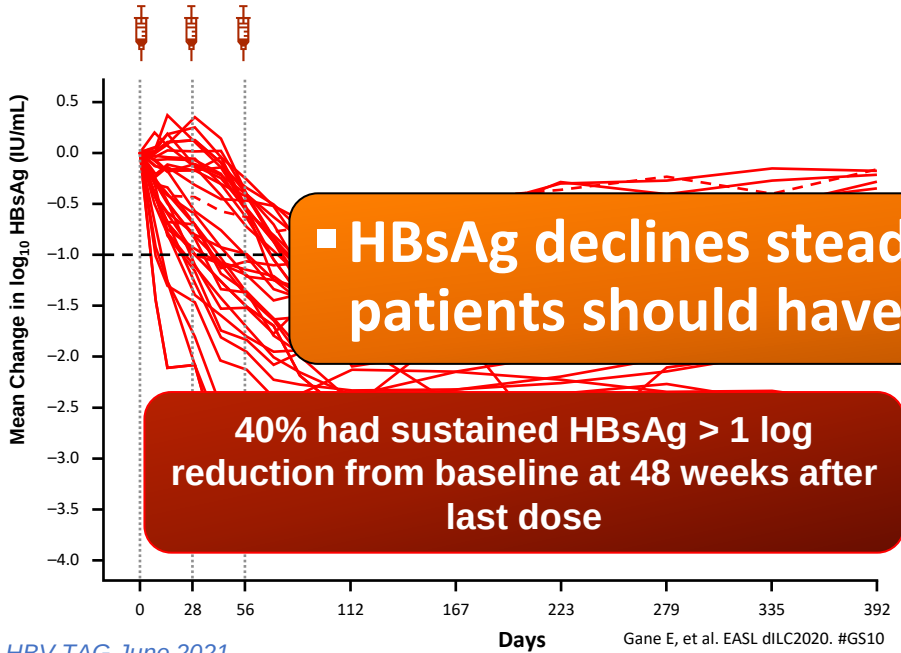
Yuen M-F, et al. AASLD TLMdX2020. #83

- >1 log HBsAg reduction achieved in 85-92%
- Nadir HBsAg level <100 IU/mL achieved in >50%

siRNAs achieve durable post-treatment response

Janssen (JNJ-3989/ARO-HBV)

- X and S triggers
- 3x monthly doses

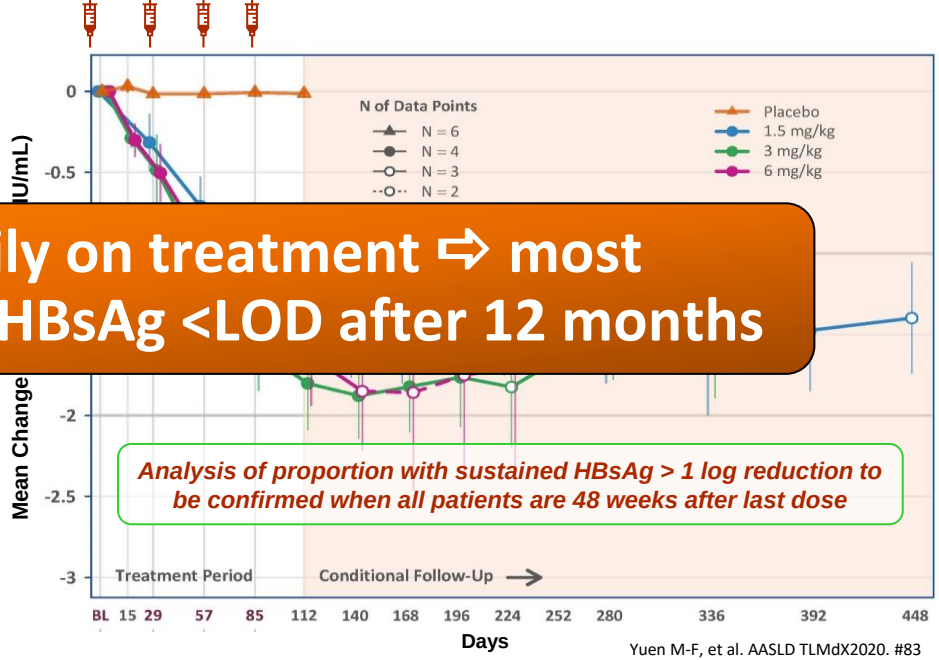


Gane E, et al. EASL dILC2020. #GS10

40% had sustained HBsAg > 1 log reduction from baseline at 48 weeks after last dose

Roche (RG-6346/DCR-HBVS)

- Single S trigger
- 4x monthly doses



Analysis of proportion with sustained HBsAg > 1 log reduction to be confirmed when all patients are 48 weeks after last dose

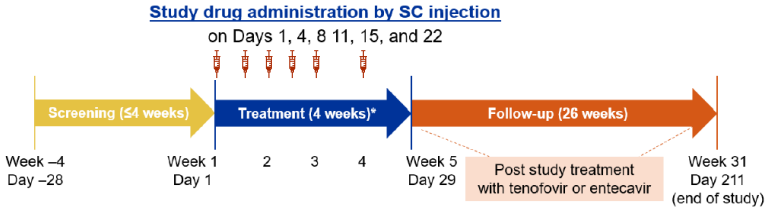
Yuen M-F, et al. AASLD TLMdX2020. #83

HBsAg declines steadily on treatment ⇨ most patients should have HBsAg <LOD after 12 months

ASOs achieve potent ontreatment HBsAg responses

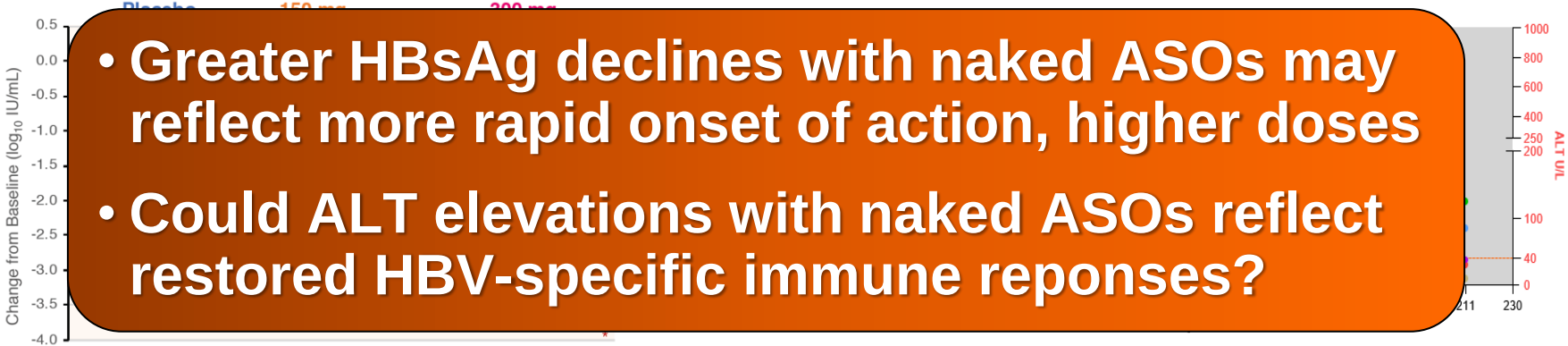
ASO ISIS 505358 (GSK-328836)

- Naked (non-Gal-NAC) ASO with X-trigger
  - 300mg Loading and frequent dosing over 28 days
  - NUC-naïve (12) & suppressed (4); HBeAg pos & neg



HBsAg Reduction

ALT Flares



- **Greater HBsAg declines with naked ASOs may reflect more rapid onset of action, higher doses**
- **Could ALT elevations with naked ASOs reflect restored HBV-specific immune reponses?**

- HBsAg levels <LLOQ in 4 patients,
- Sustained post-treatment in 2

- ALT flares after profound HBsAg reduction
- No systemic inflammatory response

Yuen M-F, et al. EASL dILC2020. #AS067

Safety of siRNAs/ASOs

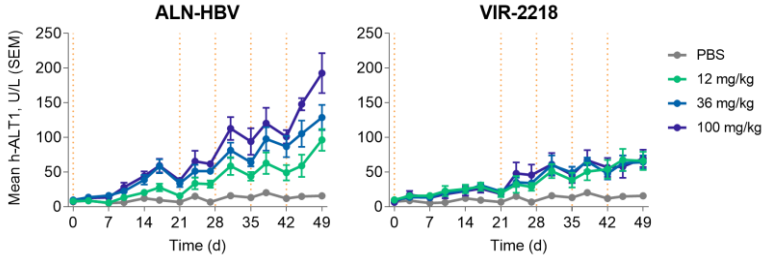
Mechanism of flares during ASO/siRNA therapy

- Delivery system-related ALT elevations
  - Lipid nanoparticles caused ALT elevations in healthy subjects
- Drug-induced ALT elevations *("Bad" Flares)*
  - Off target binding of guide RNA to host RNA
  - Nonspecific protein binding of siRNA/ASO
  - Systemic inflammatory response to ASOs?
- Immune-mediated ALT Elevations *("Good" Flares)*
  - Immune restoration following reduction in HBV antigen load?
  - Precursor to off-treatment immune control?

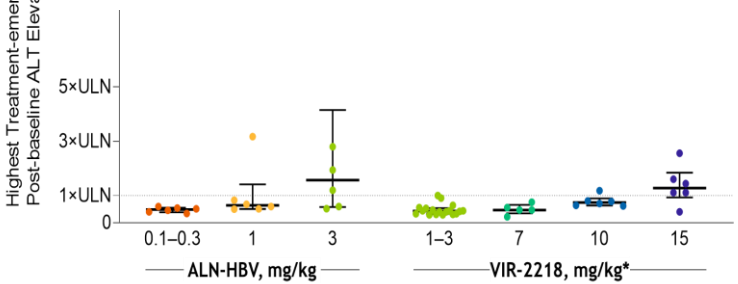
Preventing Drug-induced ALT elevations

- ALN-HBV caused ALT elevations in healthy subjects and patients due to off-target binding of guide RNA with human RNA
- ALN-HBV modified by Enhanced Stabilisation Chemistry to VIR-2218: single base substitution within guide RNA, which reduces off-target binding but maintains on-target activity against HBV transcripts

ALT elevations in humanized mice



ALT elevations in human volunteers



Combining siRNAs/ASOs with other novel therapies

Novel agents to be combined with siRNA/ASO

Antigen reduction	±	Replication inhibition	±	Immune stimulation
siRNA		NUC		Peg IFN-α
ASO/LNA		CAM		TLR7 agonist
NAP/STOPs		Entry inhibitor		TLR8 agonist
				Anti-PD-1/L1
				Ther Vaccines
				VIR-3434 mAb

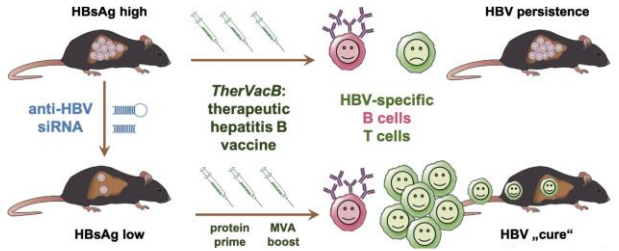
Combining siRNA/ASO with Immunotherapy

Antigen reduction ± **Immune stimulation**

RNAi and TherVacB (MVA) in the AAV mouse model

- Stable AAV-HBV infection (HBsAg >3 log IU/mL)

1. Group 1: 3x monthly subcut GalNAc-siRNA alone
2. Group 2: 3x monthly IM TherVacB alone
3. Group 3: 3x monthly GalNAc-siRNA **followed by** 3x monthly TherVacB



- Endpoints

1. HBV-specific T and B cell responses,
2. HBsAg reduction and loss

Michler T, et al. ***Gastroenterol*** 2020;158:1762–1775

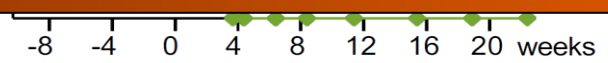
Combining siRNA/ASO with Immunotherapy

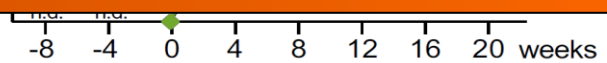
Antigen reduction ± **Immune stimulation**

RNAi and TherVacB (MVA) in the AAV mouse model

	HBsAg Responses		HBV-specific immune responses		
	On-treatment	Post-treatment	CD4+	CD8+	Anti-HBs
siRNA	2 – 3 log	nil	nil	nil	nil
TherVacB	1 log	nil	++	nil	nil
siRNA + TherVacB	**3 - 5 log**	**12/12 HBsAg loss**	**+++**	**+++**	**+++**

- **Sequential HBV antigen knockdown with siRNA plus therapeutic vaccination cured 12/12 animals**
- **HBV antigen suppression may be needed to boost therapeutic vaccine responses**

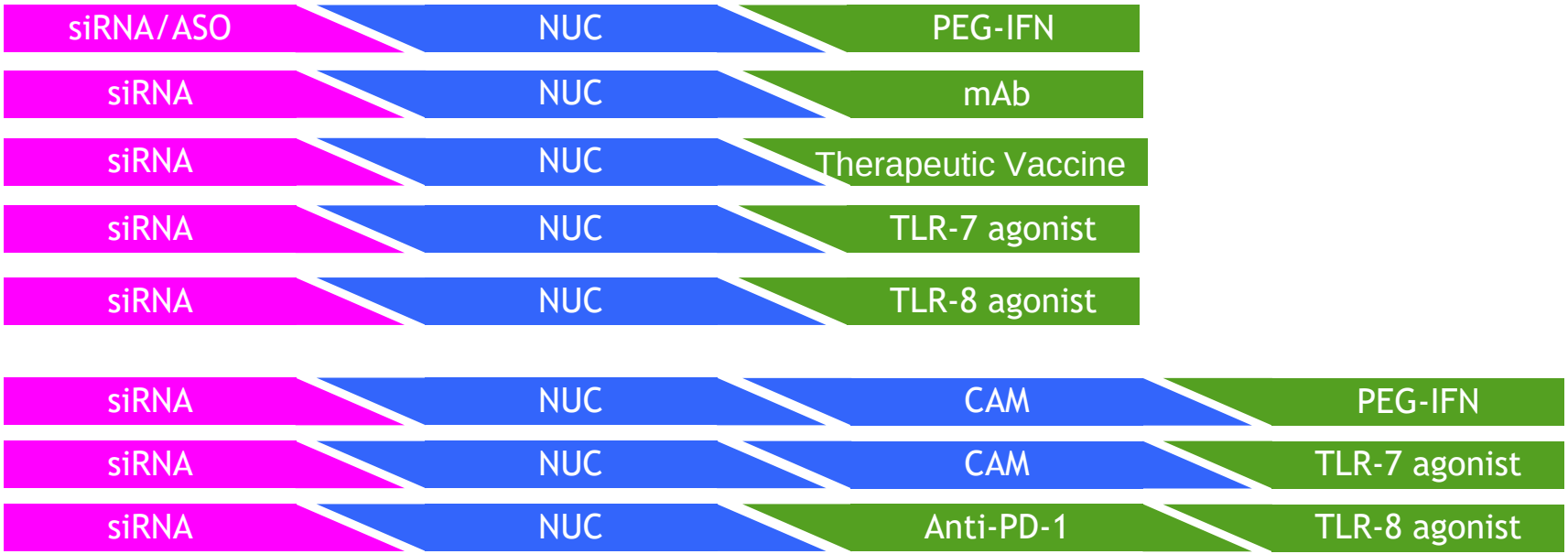




Michler T, et al. ***Gastroenterol*** 2020;158:1762–1775

Combining siRNA/ASO with Immunotherapy

Antigen reduction ± **Replication inhibition** ± **Immune stimulation**

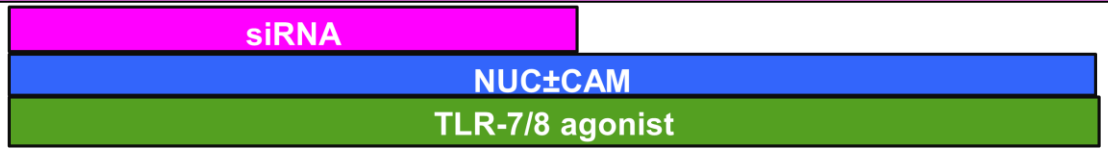


Combining siRNA/ASO with Immunotherapy

Antigen reduction + **Replication inhibition** + **Immune stimulation**

Unresolved questions

- When to start the ASOs/siRNAs?
  - *Induction or continuous?*
- When to add the immunotherapy?
  - *simultaneous, overlap, or sequential*
- When to stop the NUC?
  - *12, 24, or 48 weeks post-siRNA*



Translation Inhibitors – Conclusions

1. **GalNAc conjugated siRNAs**
   - Potent HBsAg reduction (>1 log after 4 weeks)
   - Durable responses for many months post-treatment
   - Safe, well-tolerated, convenient SC dosing (monthly or less)
2. **Naked Antisense Oligonucleotides**
   - More profound HBsAg reduction (>3 log after 4 weeks)
   - More rapid rebound post-treatment
   - Frequent ALT flares– restored HBV immunity or toxicity?

➡**Phase II studies will determine whether siRNAs/ASOs can achieve sustained HBsAg loss (i.e. Functional Cure)**

Translation Inhibitors – Unresolved Issues

1. Are both T- and B-cell responses restored by ASOs/siRNAs?
2. Will on-treatment ALT flares be important for maintaining off-treatment response?
3. Following ASO/siRNA therapy, when does “HBsAg negative” mean cure - End-of-treatment, SVR12, SVR24, or later?
4. Will a neutralising anti-HBs response be needed for cure?

Acknowledgements

- Dr M-F Yuen, Hong Kong
- Dr Stephen Locarnini, Melbourne
- Dr Kosh Agarwal, London
- Dr James Hamilton, Arrowhead, USA
- Dr Michael Biermer, Janssen, Belgium
- Dr Phil Pang, VIR Biotechnology, USA
- Dr Hardean Achneck, Dicerna Pharmaceuticals, USA
- Dr Gaston Picchio, Arbutus Biopharma, USA
- Dr Melanie Paff, GSK, USA