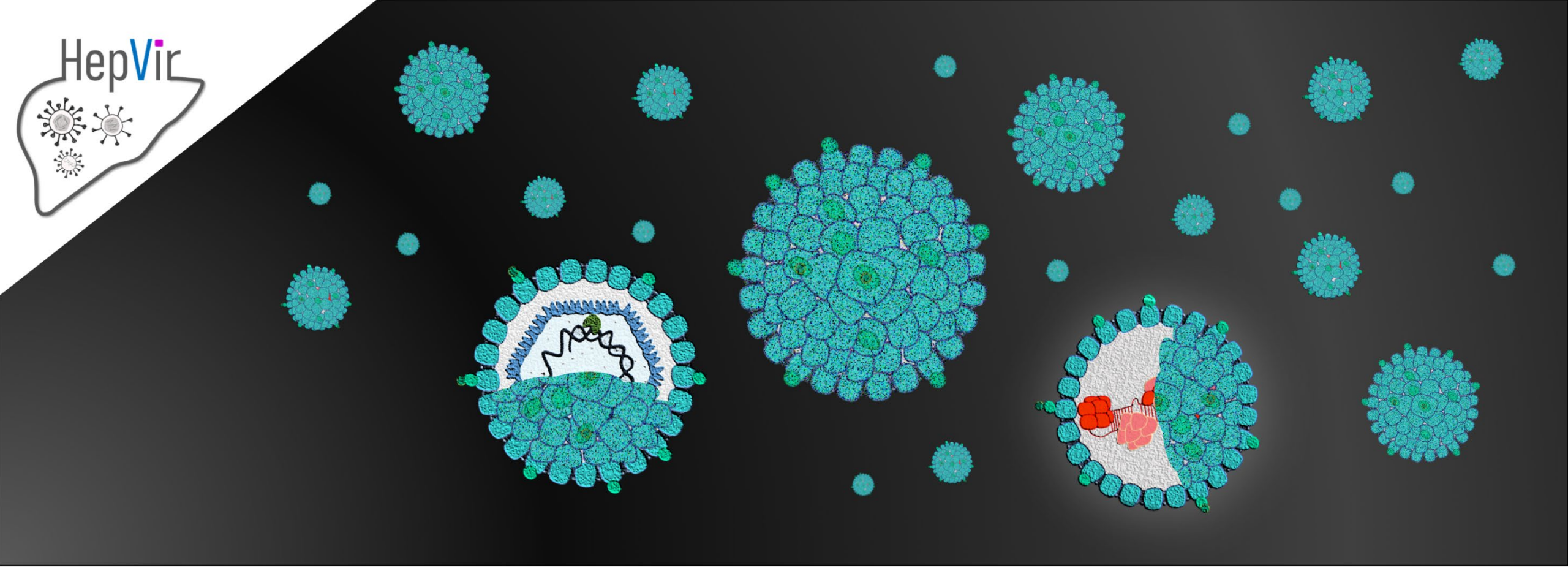


Antisense oligonucleotide-based strategy to target hepatitis delta virus infections

Julie Lucifora

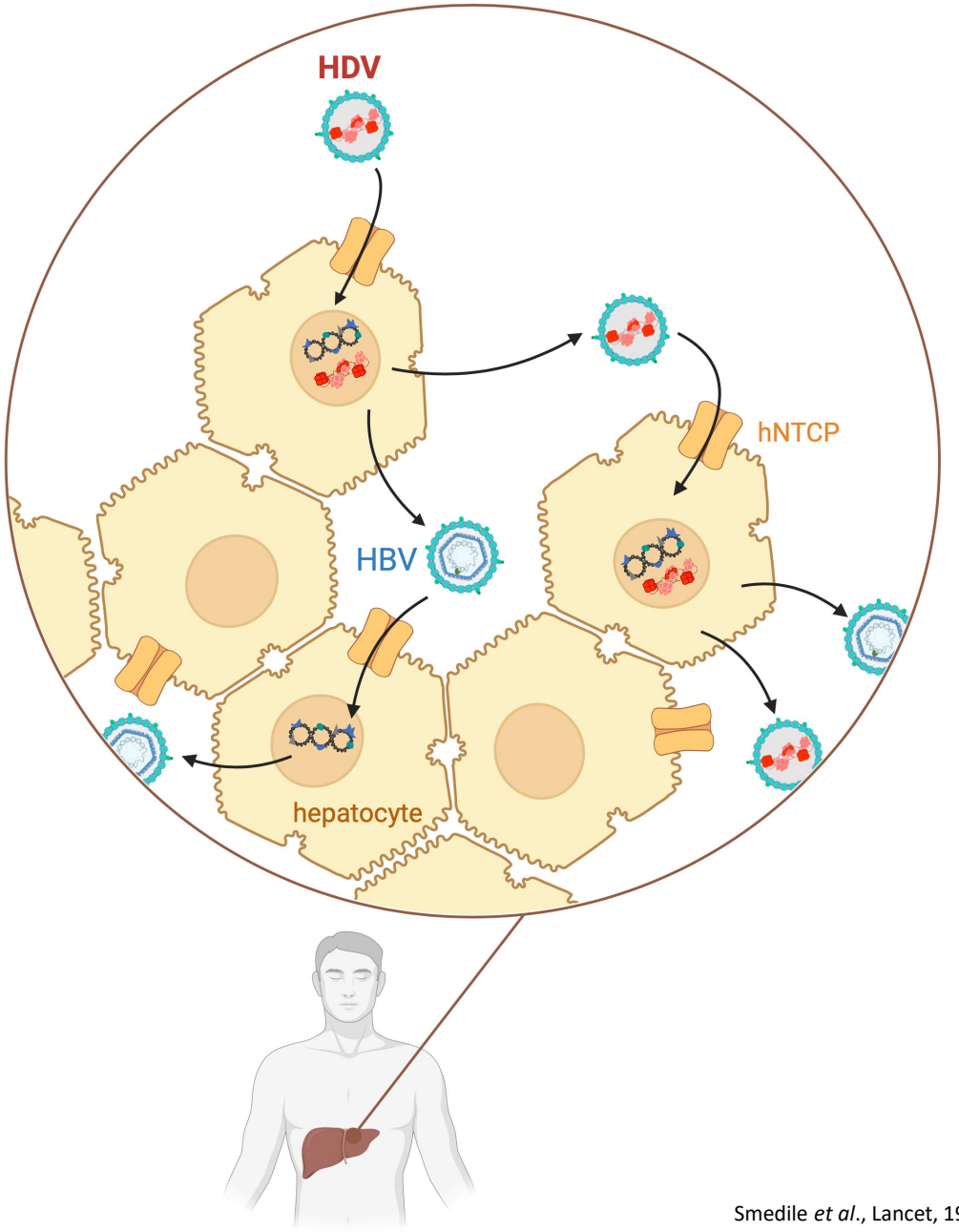
2025 International HBV meeting
September 2025

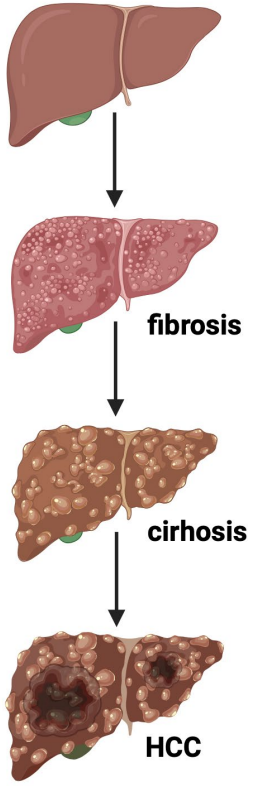
Disclosures

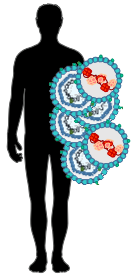
- Julie Lucifora signed collaborations agreements with:
  - Aligos Therapeutics, Inc.
  - EIT-Pharma

- Yannick Debing, Lars Degrauwe, Aneerban Bhattacharya, Kellan Passow, Antitsa Stoycheva, Lawrence Blatt, Julian Symons, Andreas Jekle, Vivek Rajwanshi and David B. Smith are employees of Aligos Therapeutics, Inc.

Chronic HBV and HDV infections lead to the most severe form of viral hepatitis

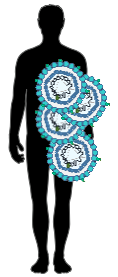






chronic HBV/HDV carrier

VS

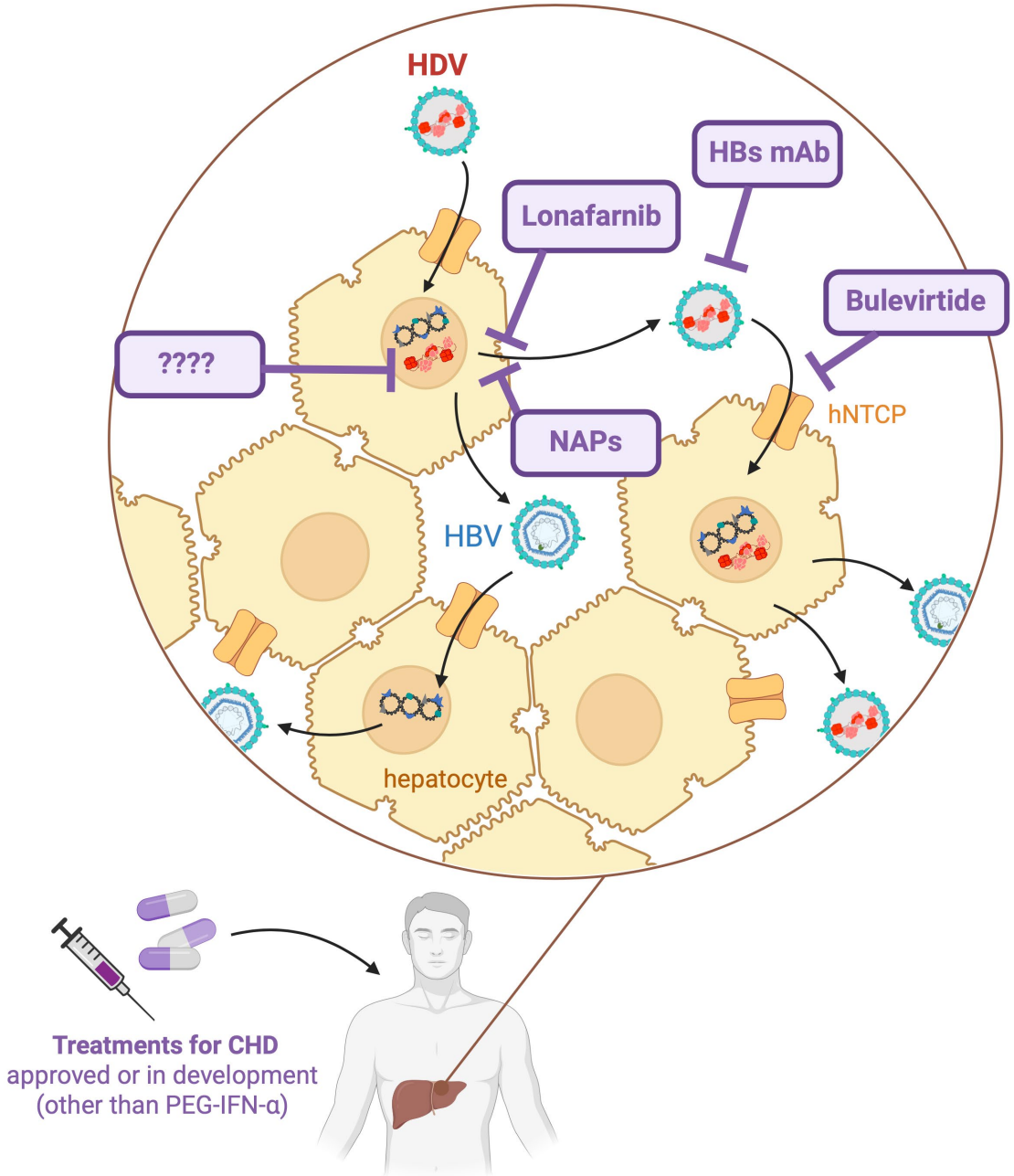


chronic HBV carrier

- ✓ **higher risk of severe hepatitis**
  - ➢ frequent evolution towards an acute liver failure requiring liver transplantation
- ✓ **faster progression to cirrhosis**
- ✓ **higher risk of HCC**

Smedile *et al.*, Lancet, 1982 / Govindarajan *et al.*, 1984 / Yurdaydin *et al.*, 2010 / Fattovich *et al.*, 2000 / Ji *et al.*, 2012 / Kushner *et al.*, 2015 / Beguelin *et al .*, 2017 / .....

Need to develop replication inhibitors to improve the therapeutic arsenal against HDV



Treatments for CHD:

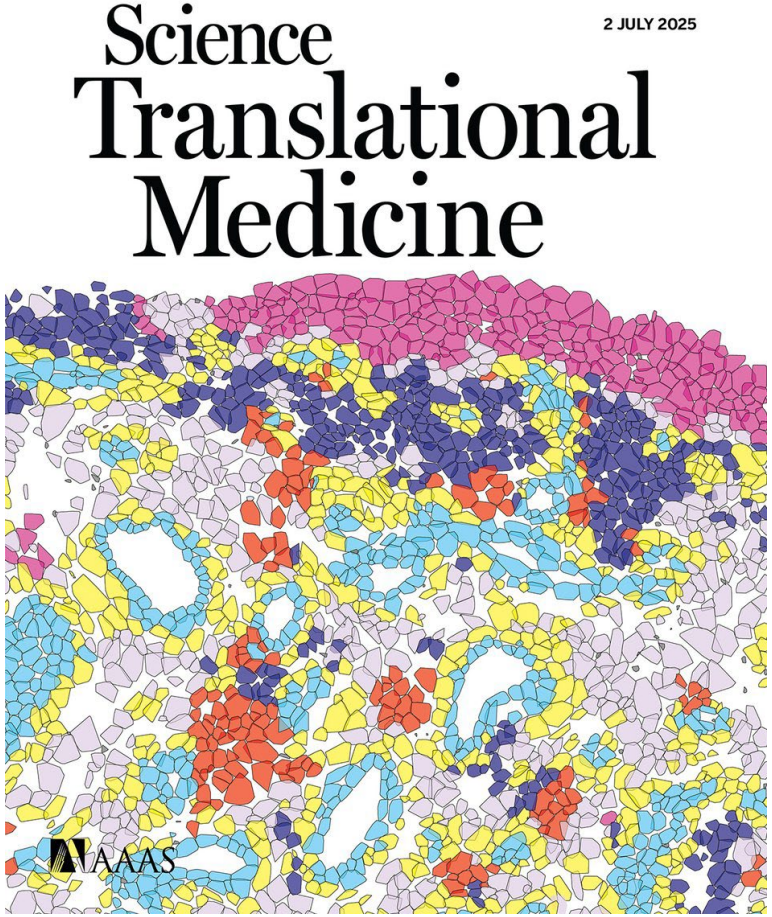
- **PEG-IFN-α**
- **Bulevirtide**

Treatments in clinical trials:

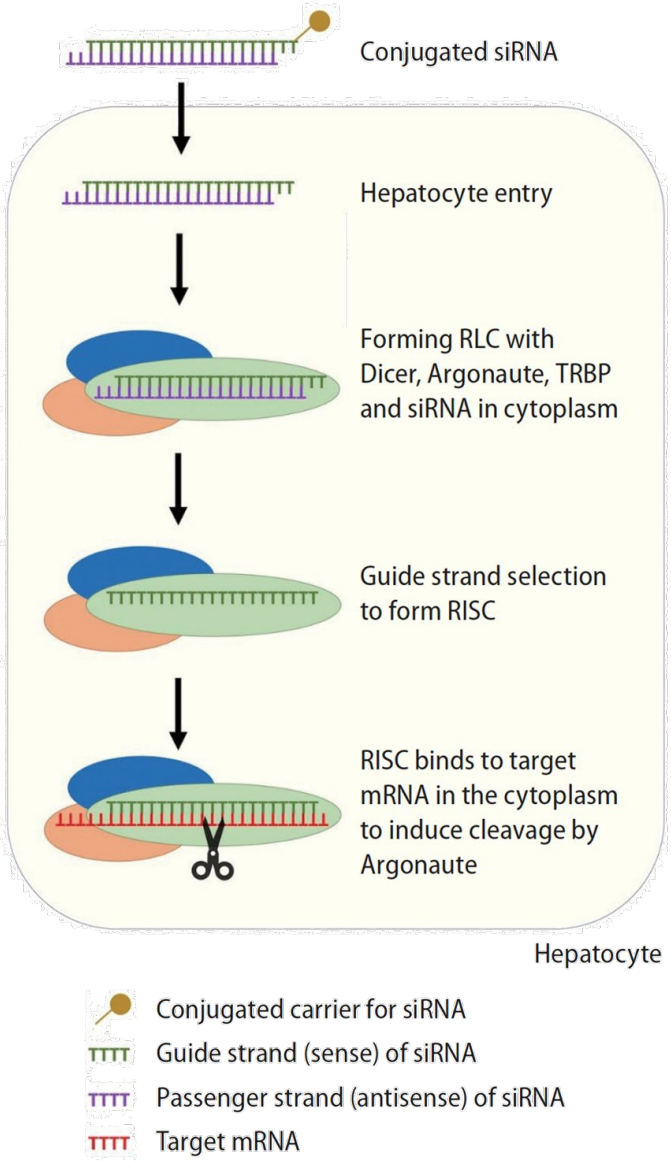
- **Lonafarnib**
- **NAPs**
- **Anti-HBsAg mAb (+ siRNA against HBV)**

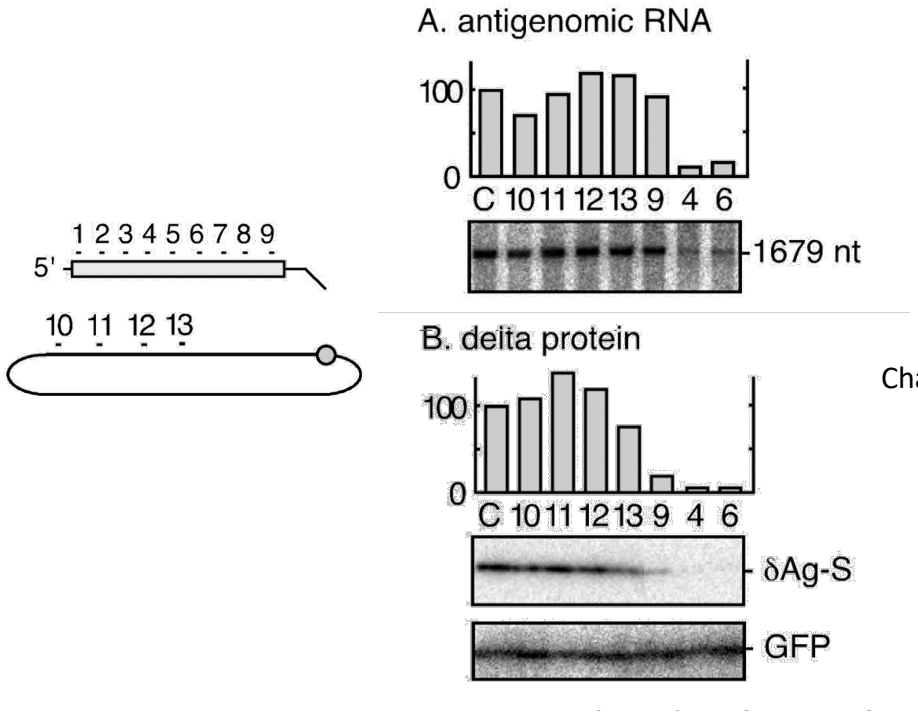
Abbas and Abbas, World J Hepatol 2025

RNAi strategies to target HBV (and HDV)



HDV mRNAs are sensitive to siRNA but not not HDV-AG and HDV-G



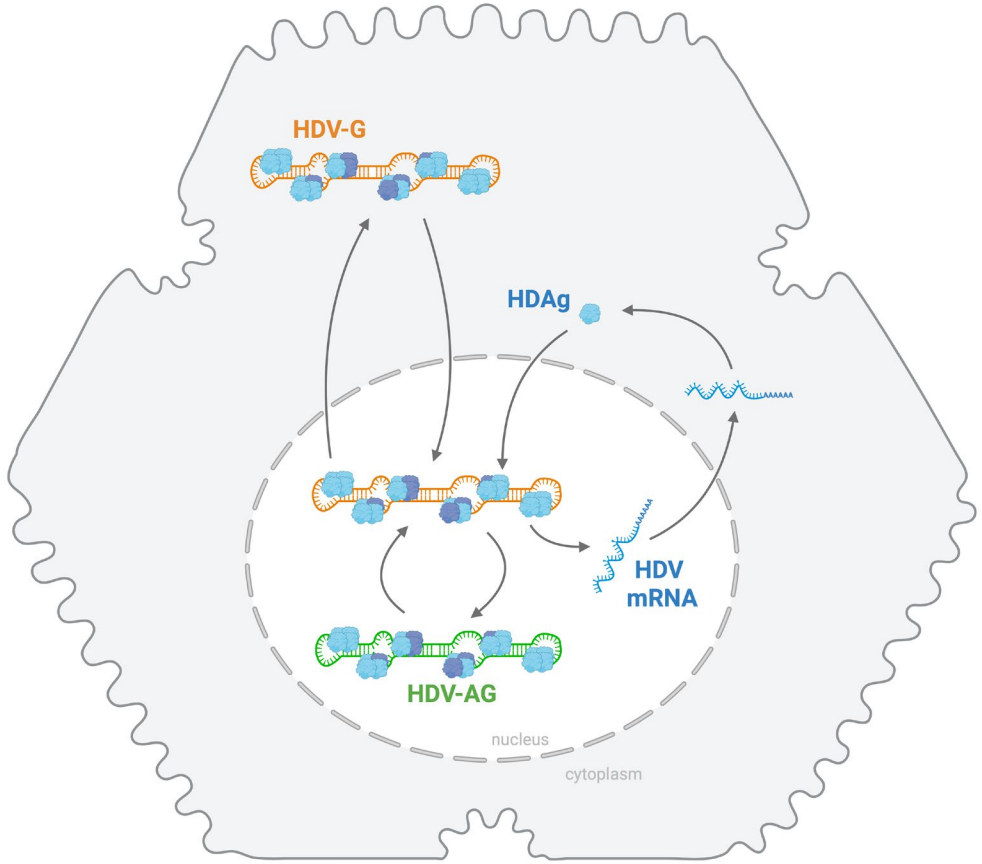


Chang and Taylor, J Virol, 2003

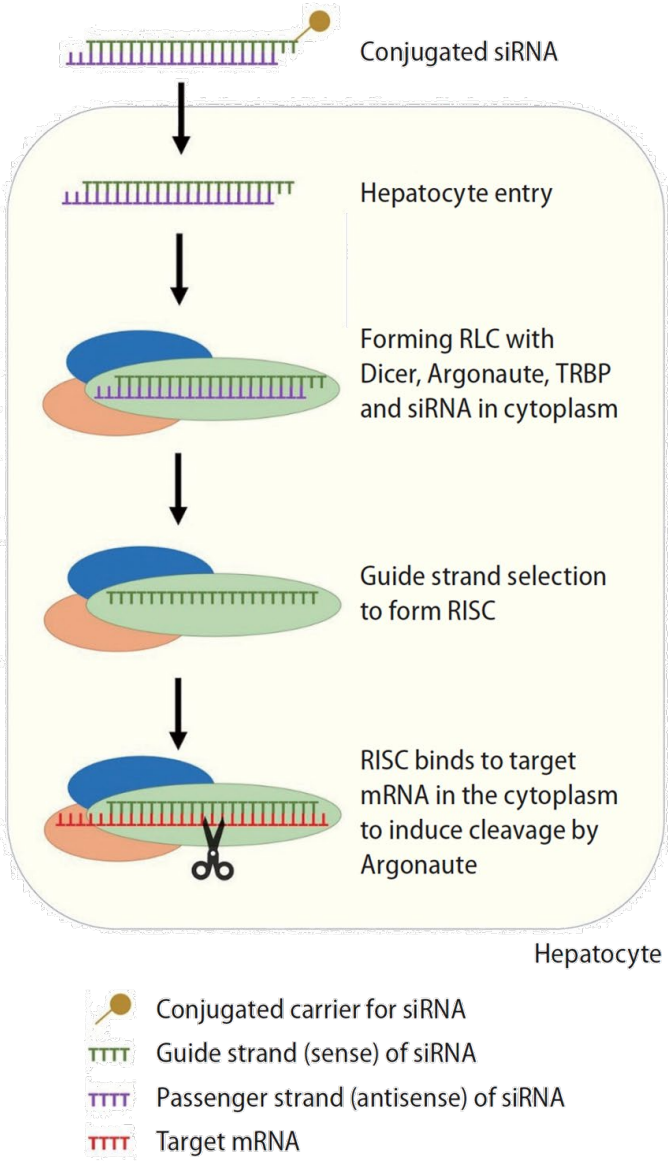
- ✓ **HDV mRNA sensitive to siRNA**
- ✓ **HDV-G and HDV-AG resistant to siRNA independently of:**
  - ➢ **RNA structure**
  - ➢ **RNA conformation**
  - ➢ **Presence of S-HDAg**
- ➢ **Hypothesis: resistance due to nuclear localization**

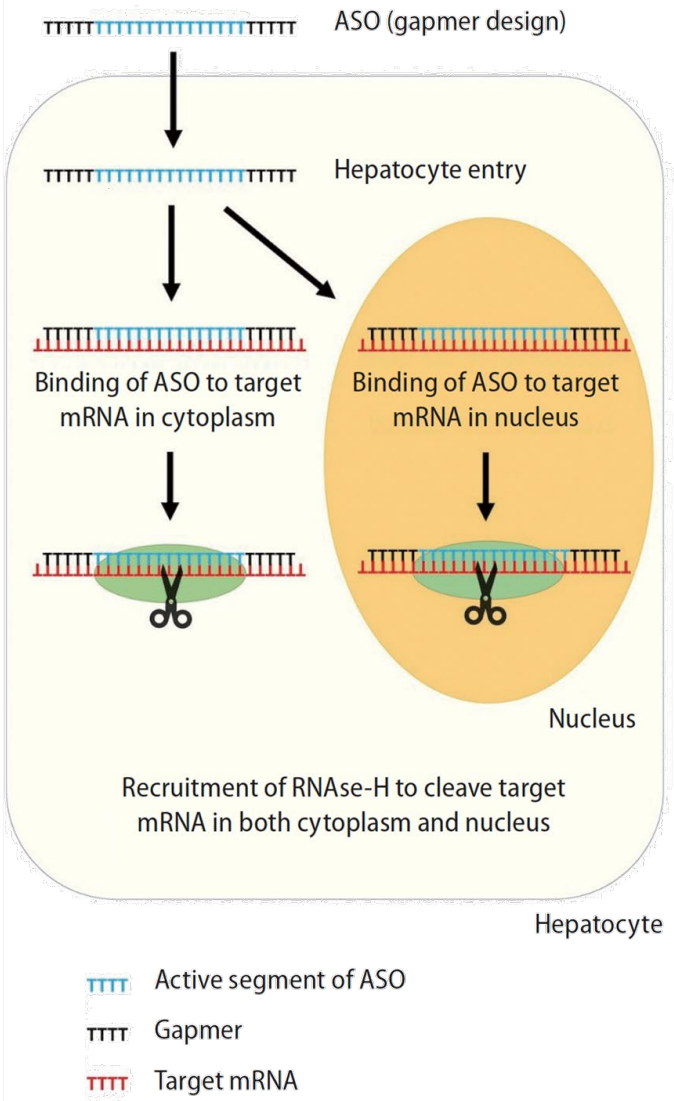
Hui *et al*., CMGH, 2022

HDV RNAs replication occurs in the nucleus of hepatocytes

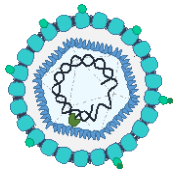


Mechanism of small-interfering RNA and antisense oligonucleotides (ASO)





HBV

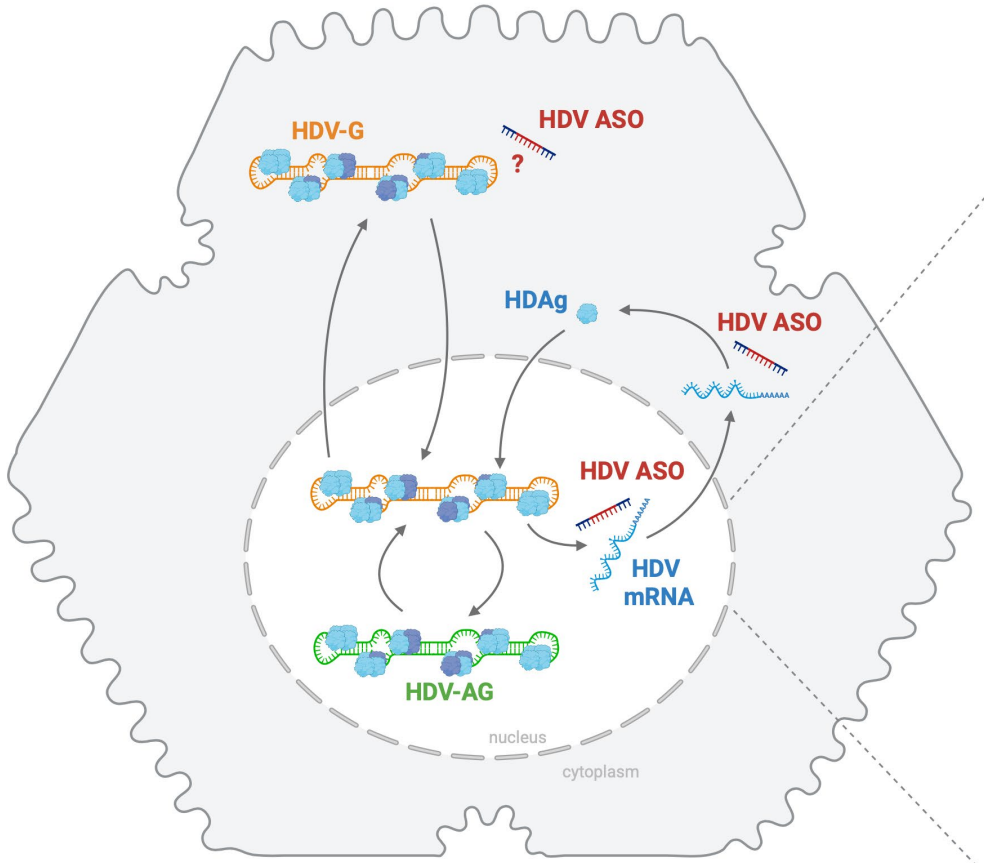


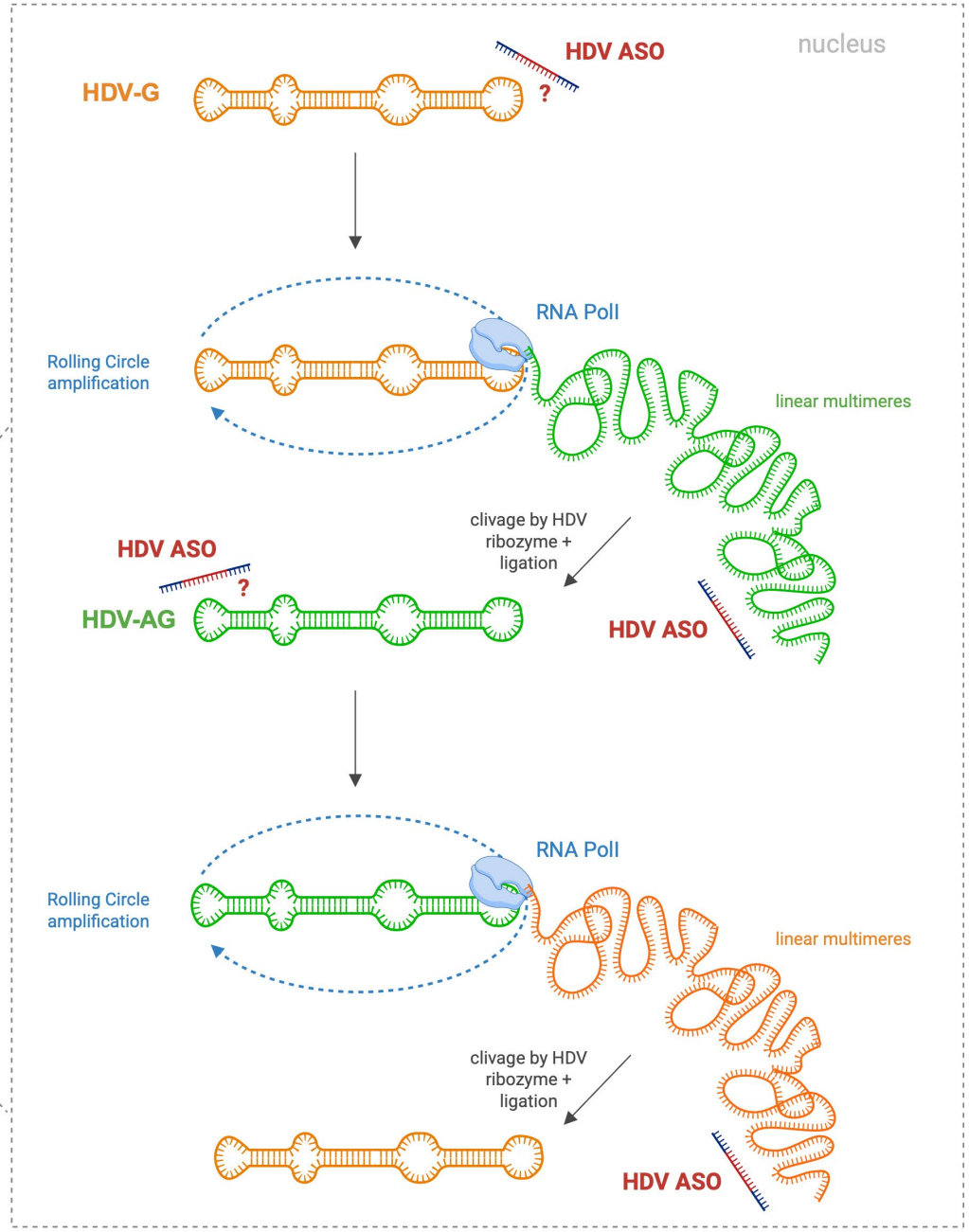
ASO against HBV in clinical trial

- bepirovirsen
- ....

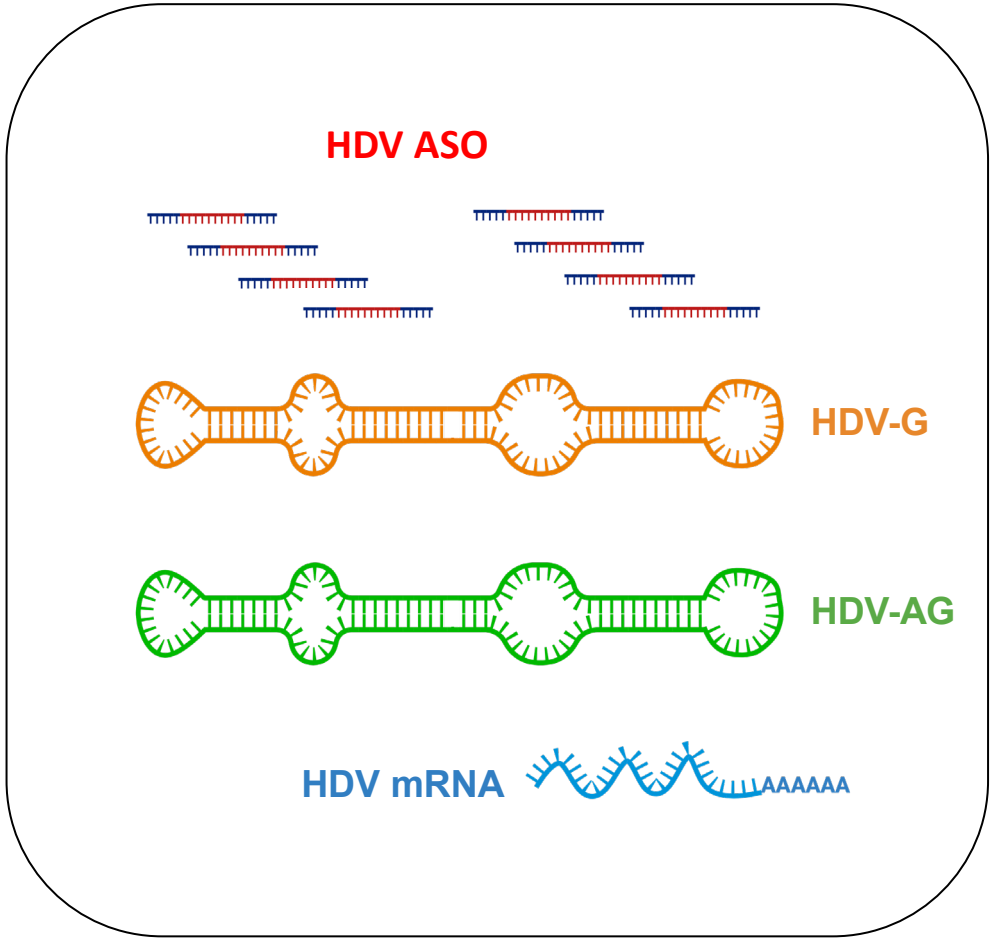
Hui *et al*., CMGH, 2022

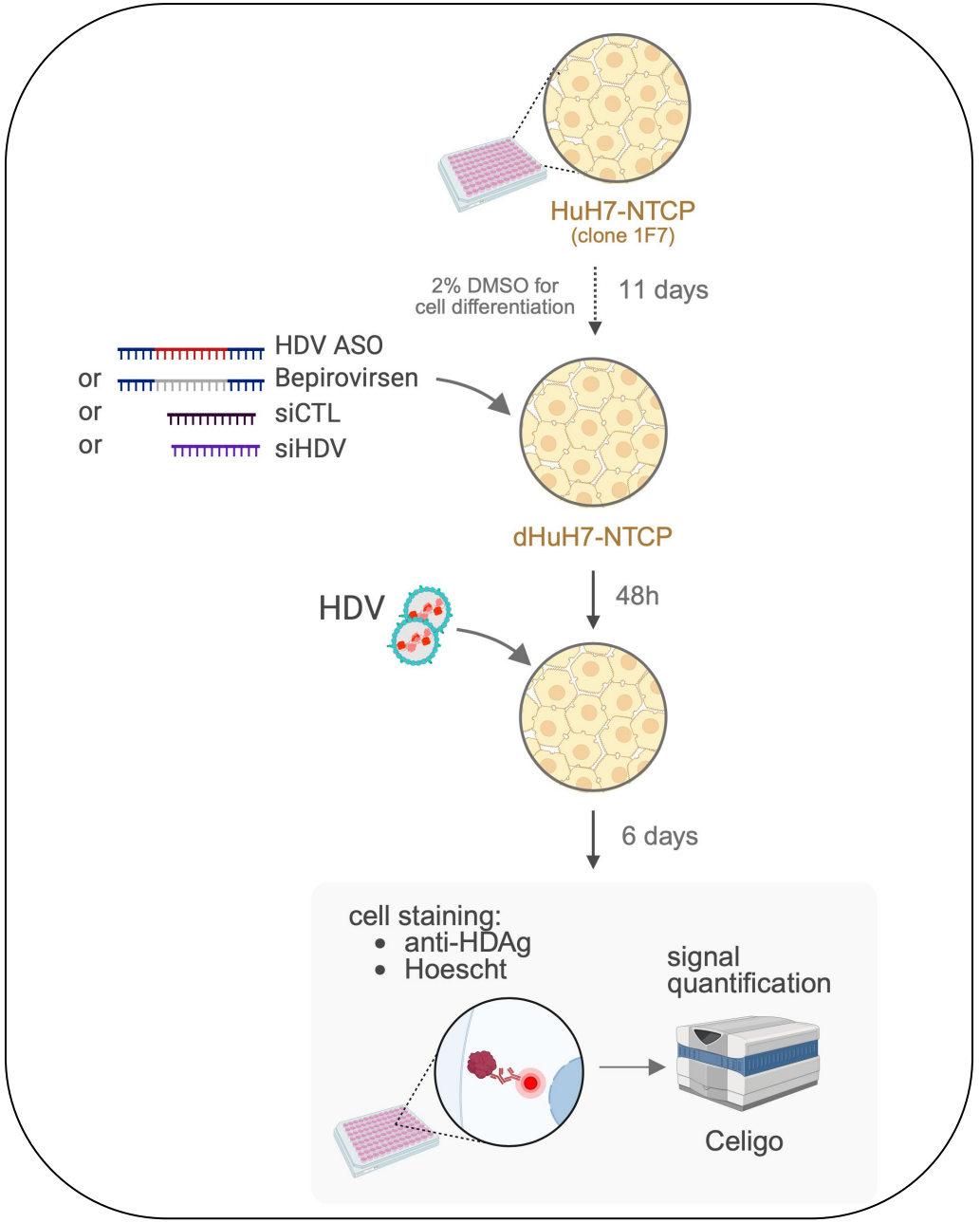
HDV RNAs replication occurs in the nucleus of hepatocytes



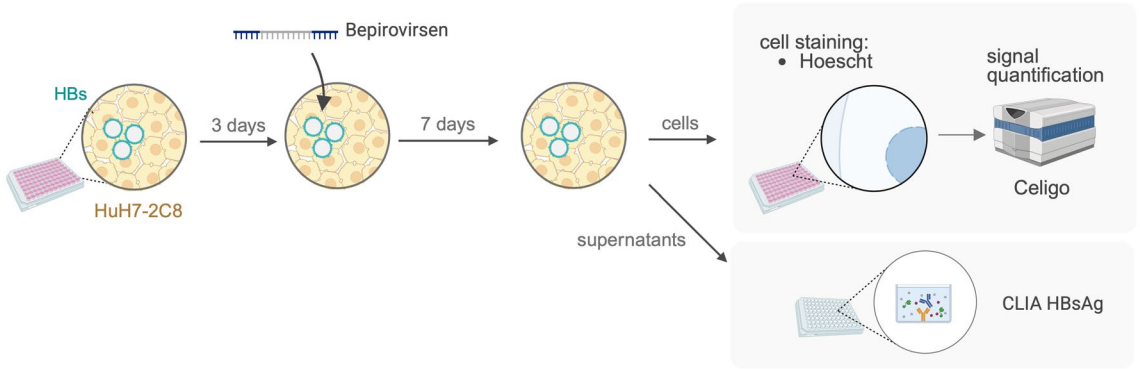


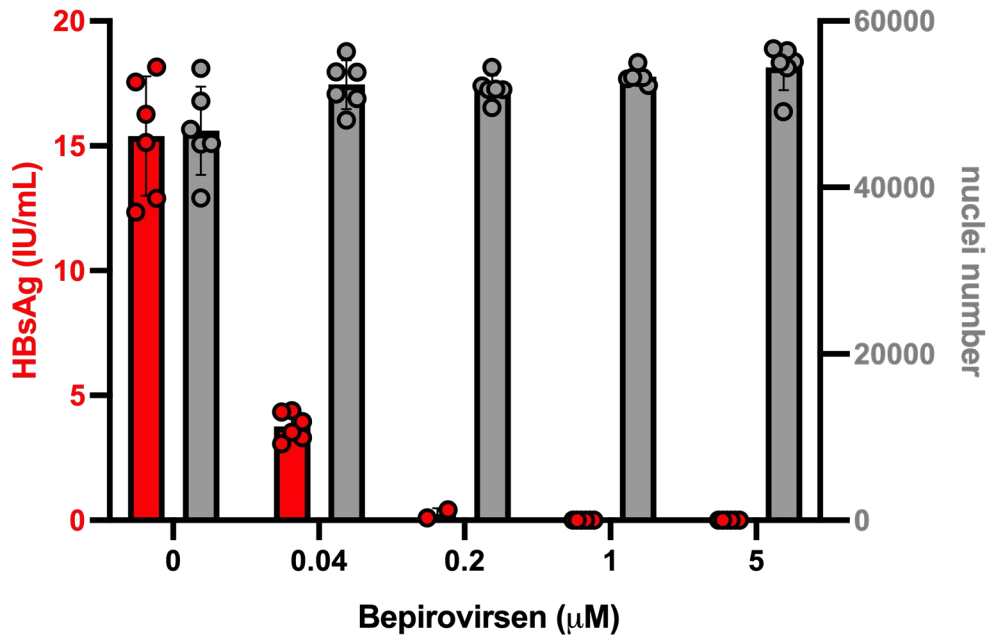
Design of ASOs (gapmer 5-10-5) directed against HDV sequences and screen in HDV mono-infected cells

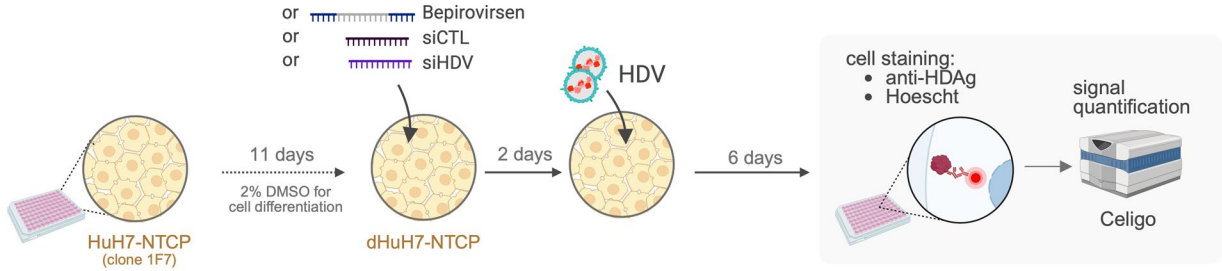


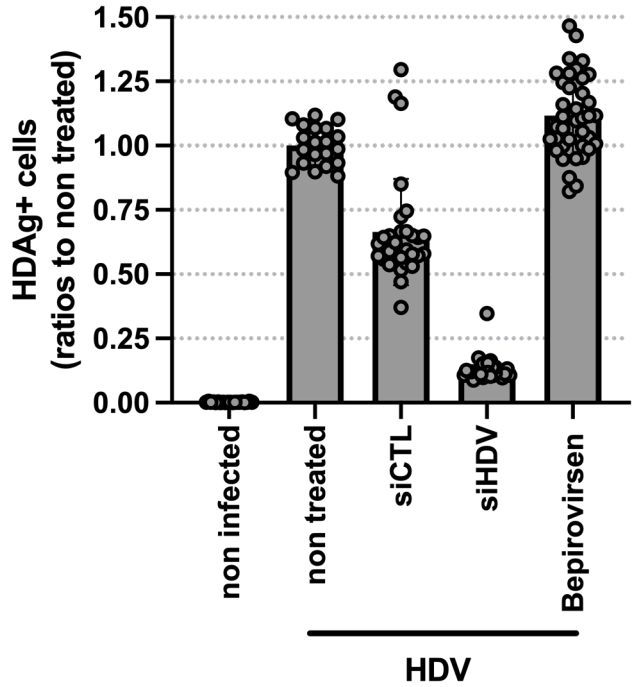


Use of Bepirovirsen as an HDV non-targeting ASO control

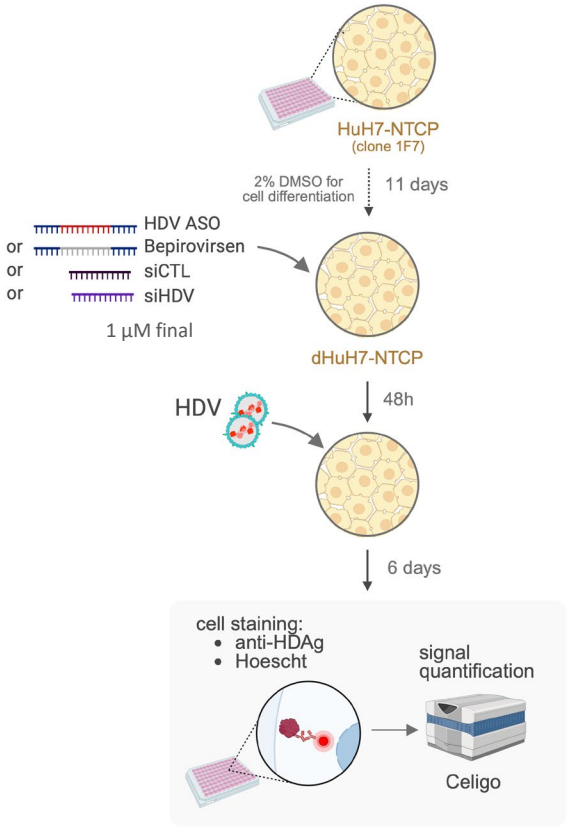




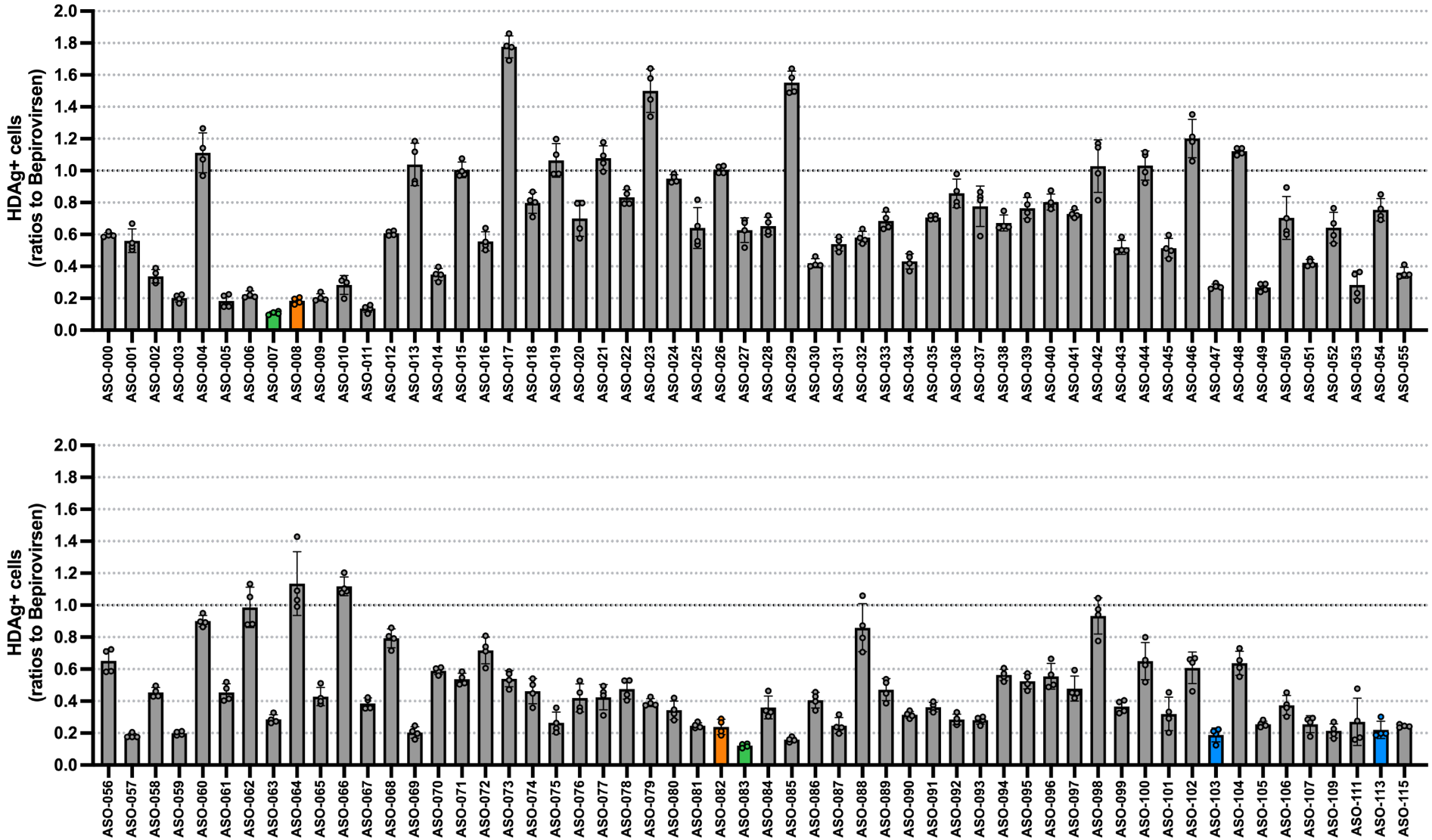




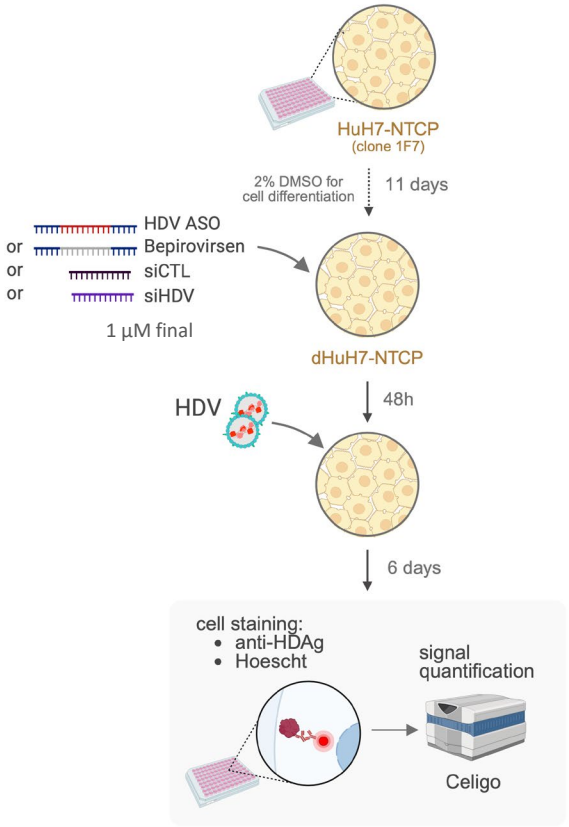
Identification of several effective anti-HDV ASO



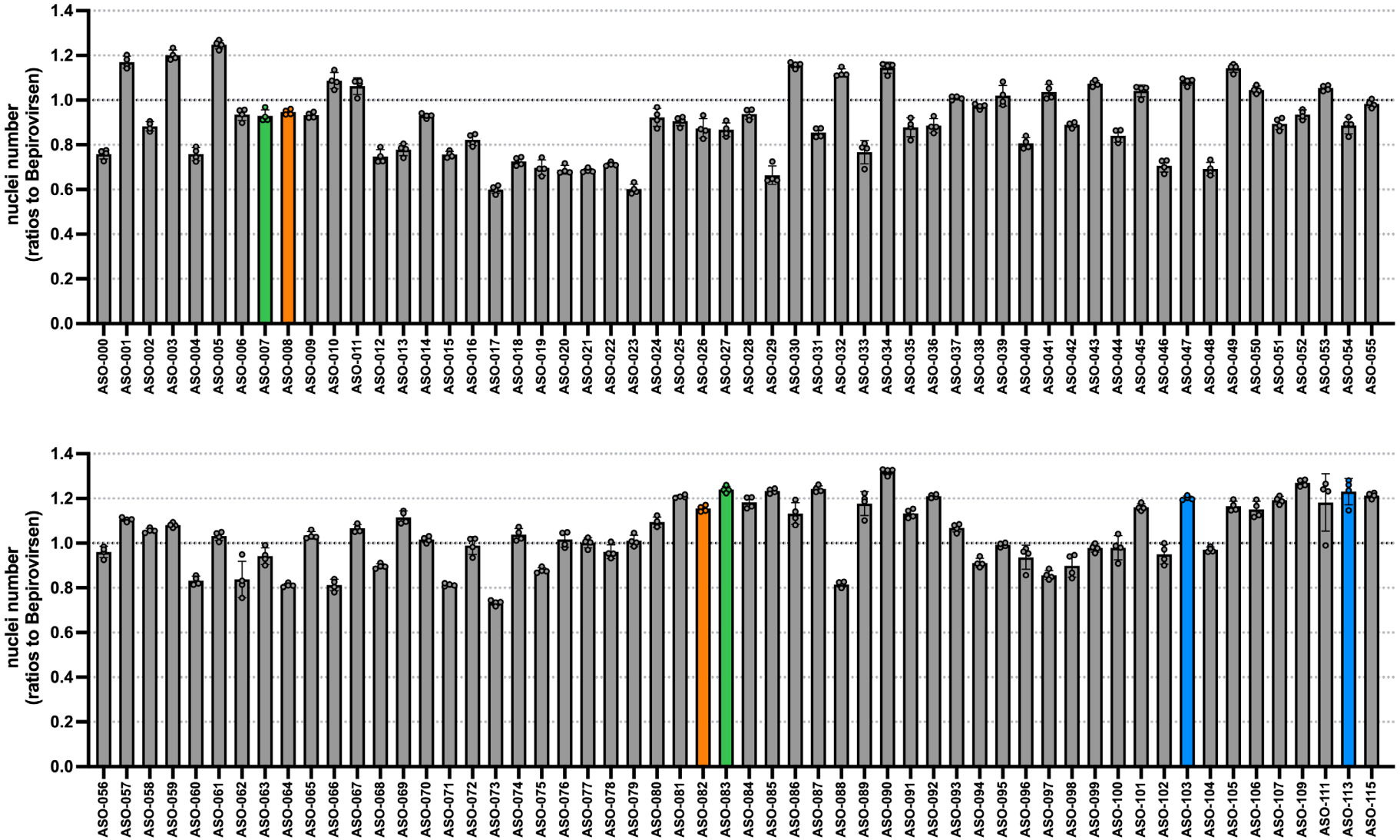
➢ 19 anti-HDV ASO candidates allowed a ≥ 75 % reduction of the levels of HDAg+ cells



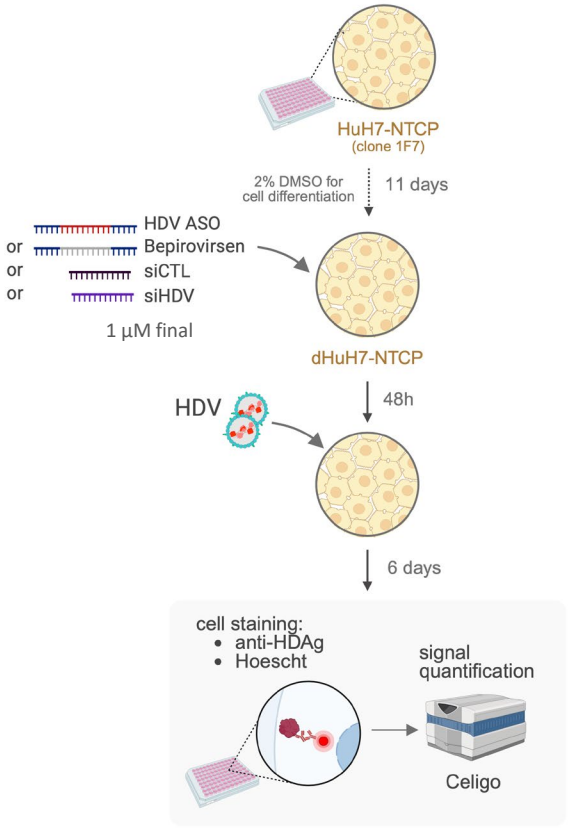
Identification of several effective anti-HDV ASO



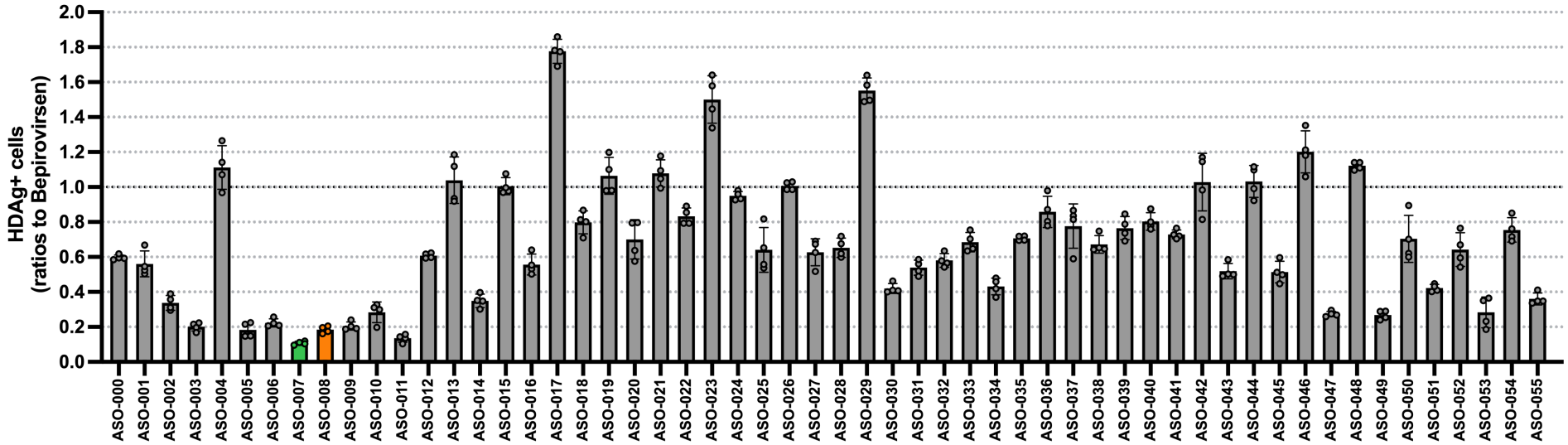
➢ 19 anti-HDV ASO candidates allowed a ≥ 75 % reduction of the levels of HDAg+ cells without toxicity

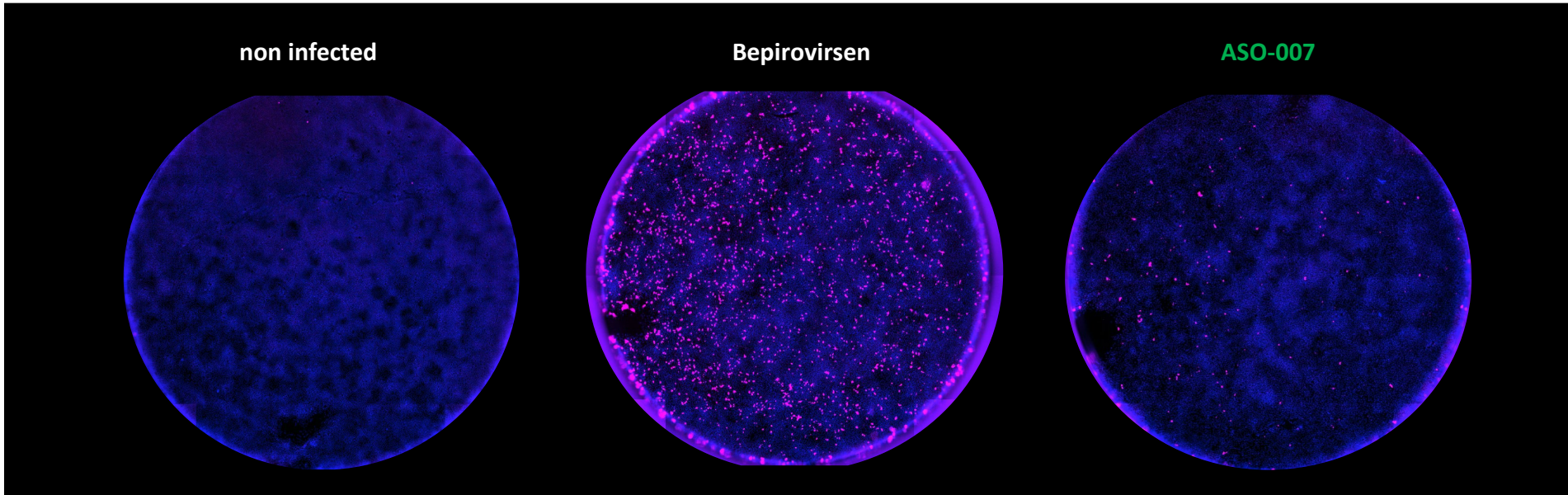


Identification of several effective anti-HDV ASO

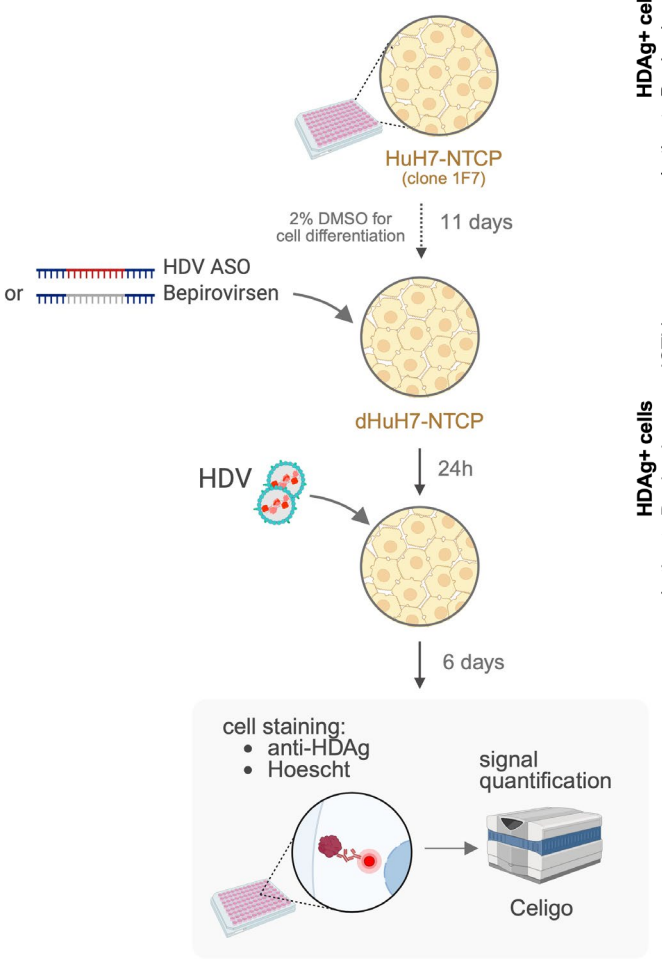


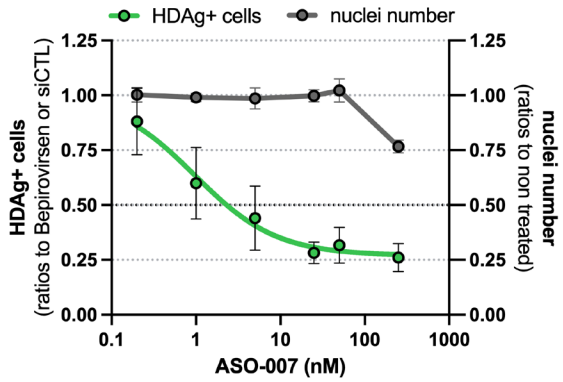
➢ 19 anti-HDV ASO candidates allowed a ≥ 75 % reduction of the levels of HDAg+ cells

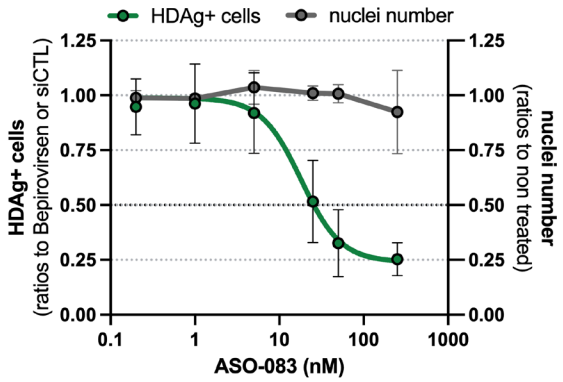


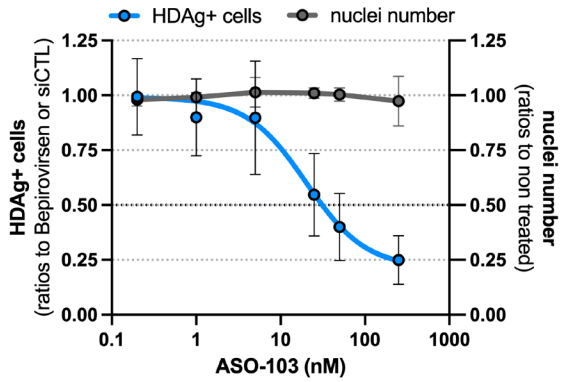


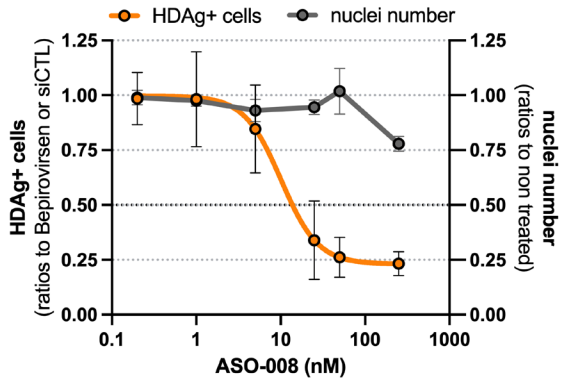
Dose dependent antiviral effect of anti-HDV ASO

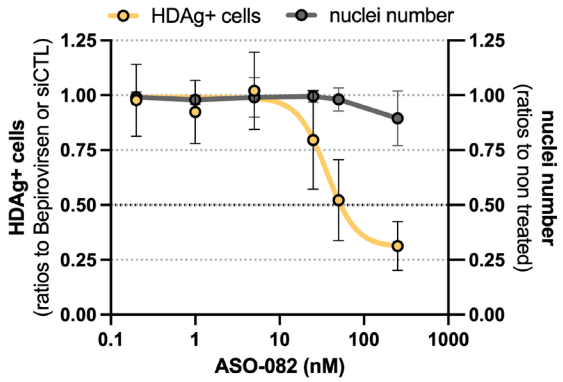


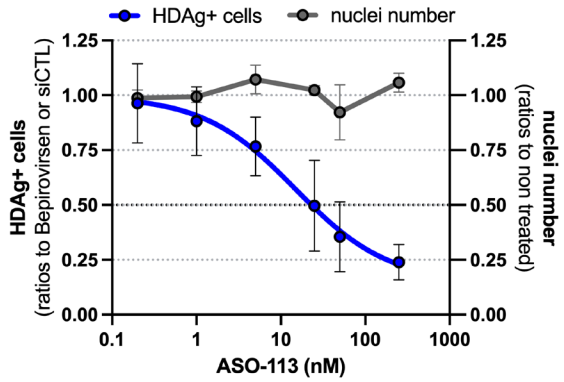






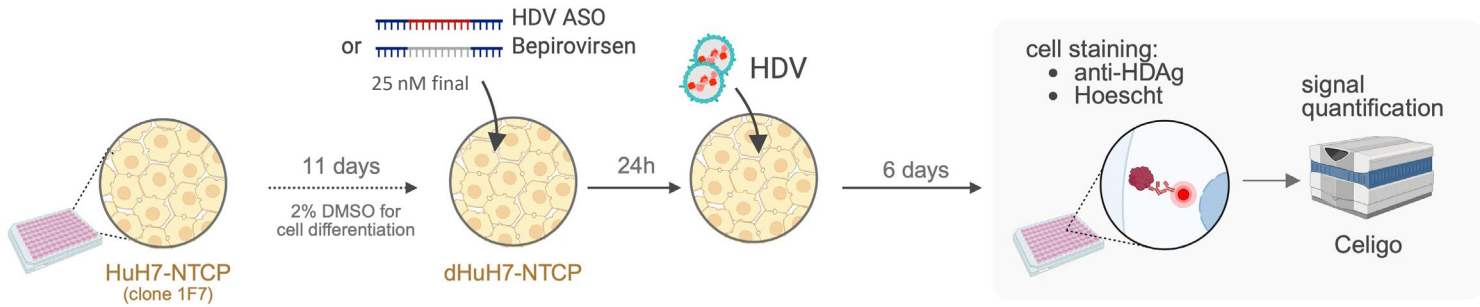






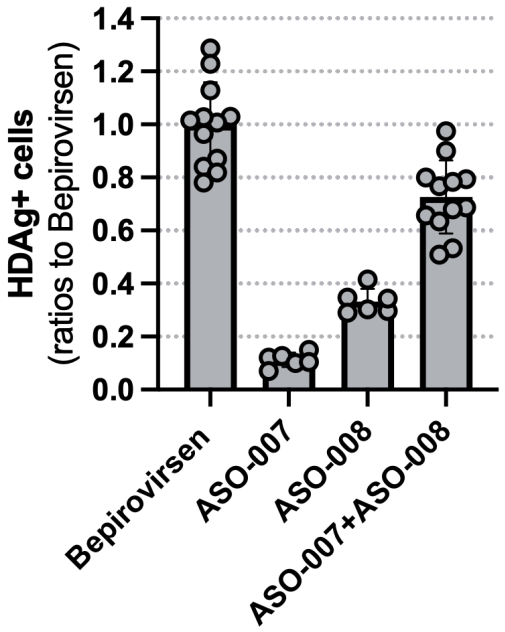
	IC_{50} (nM)
ASO-007	2.3
ASO-008	14
ASO-082	53
ASO-083	26
ASO-103	31
ASO-113	23

Complementary anti-HDV ASO are antagonists

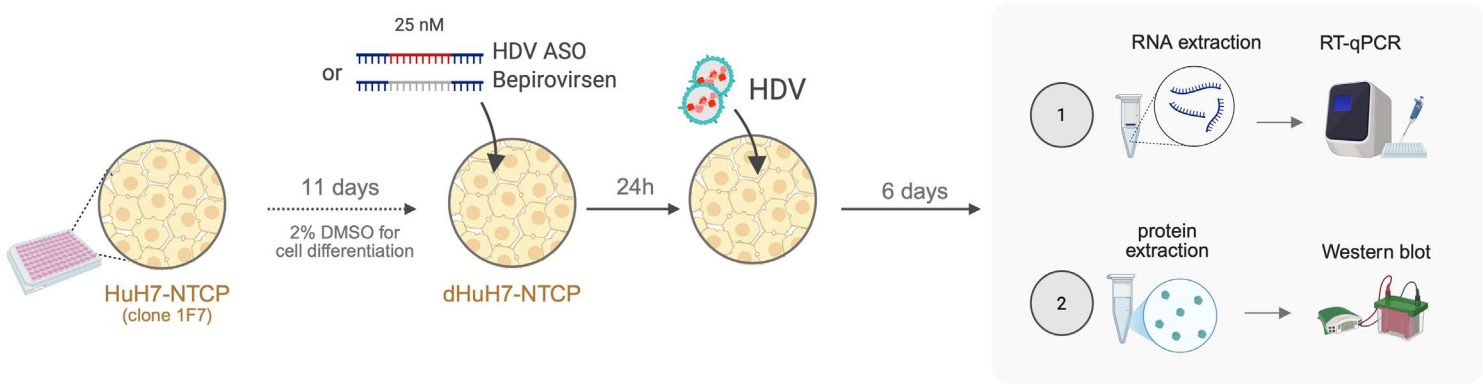


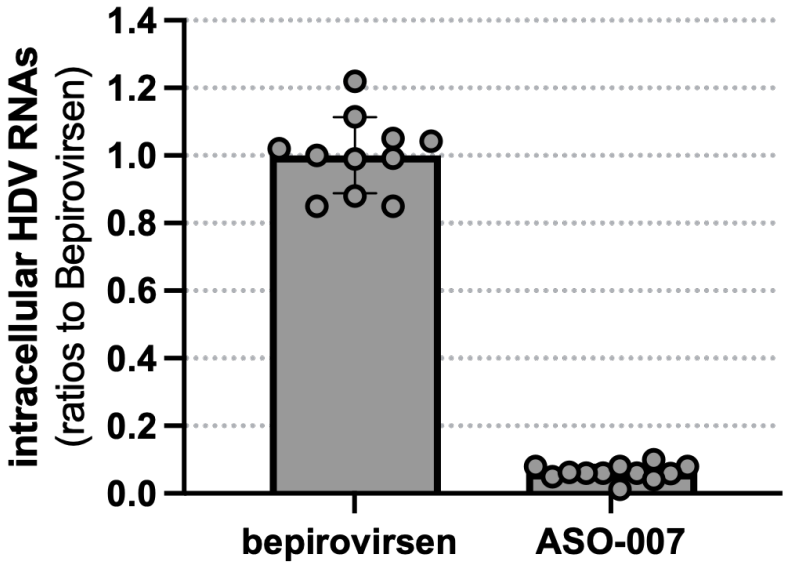
ASO-007

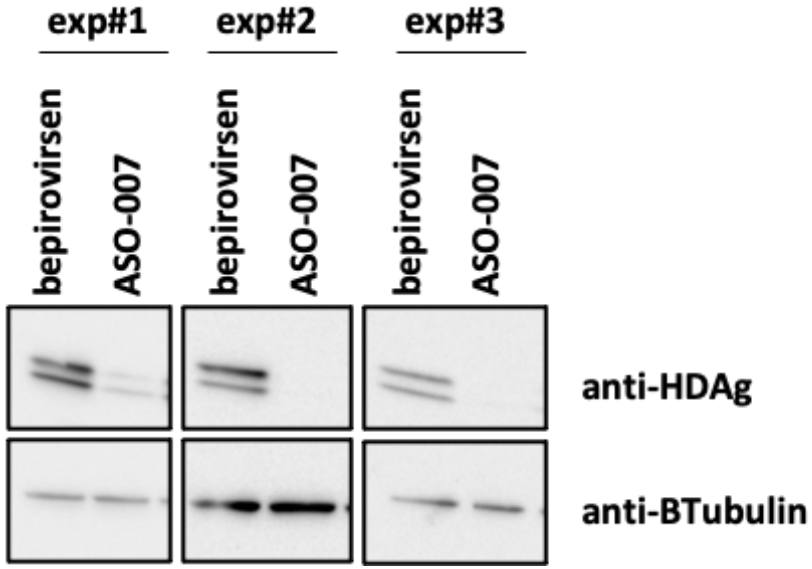
ASO-008



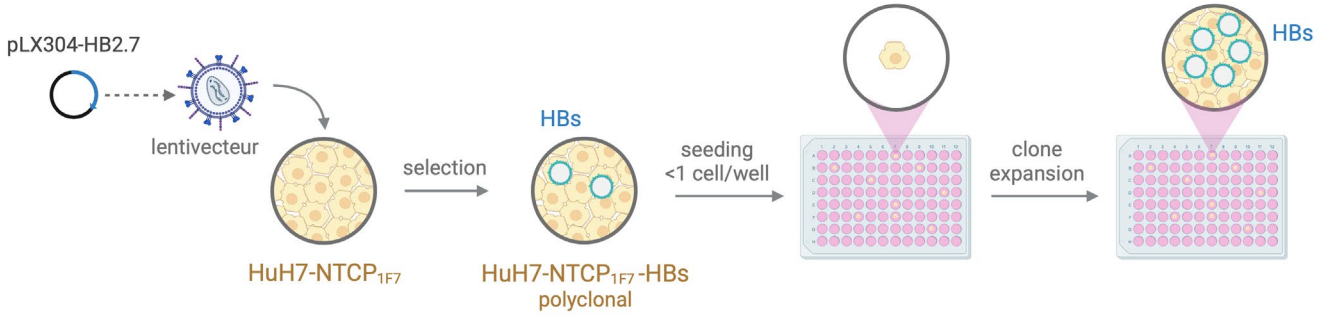
Anti-HDV ASOs reduced levels of intracellular HDV RNAs and proteins

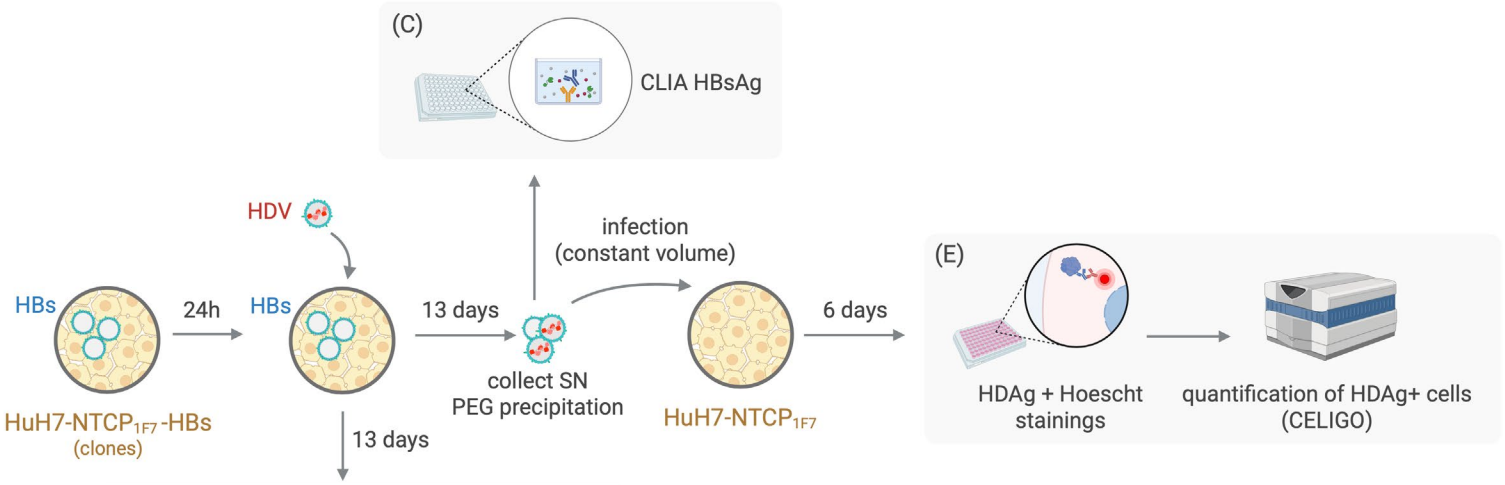


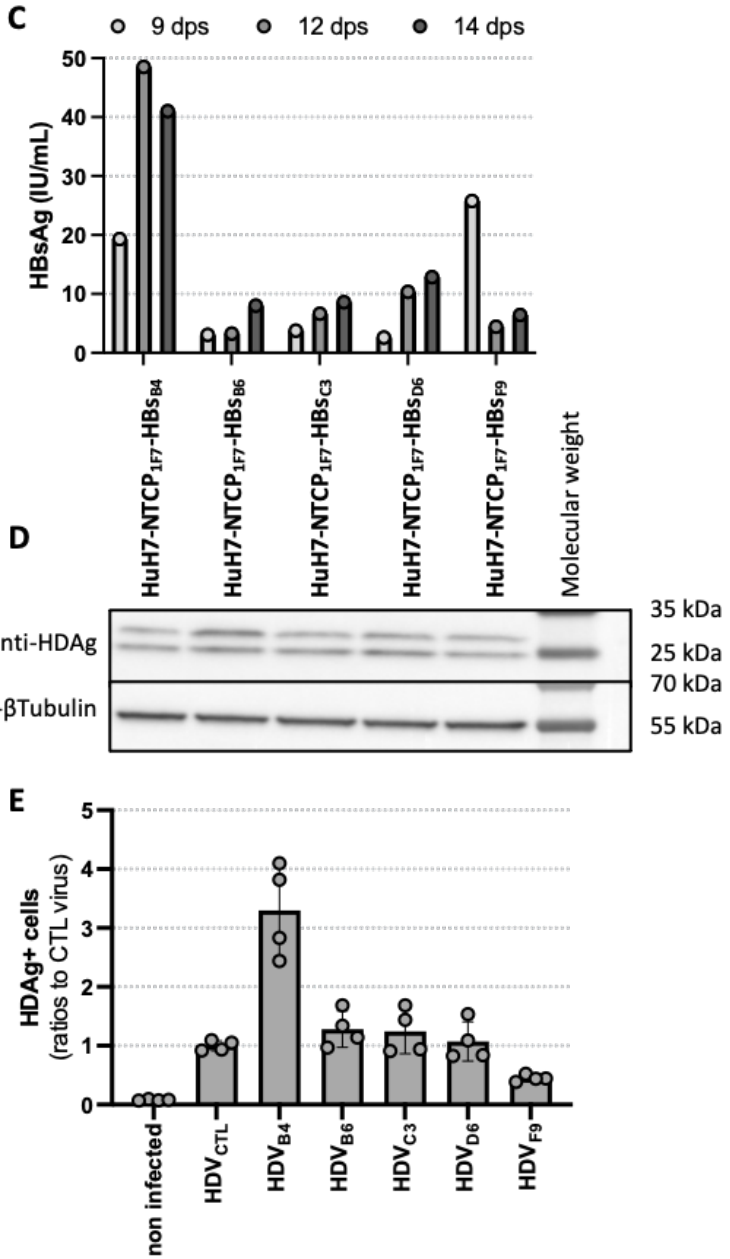




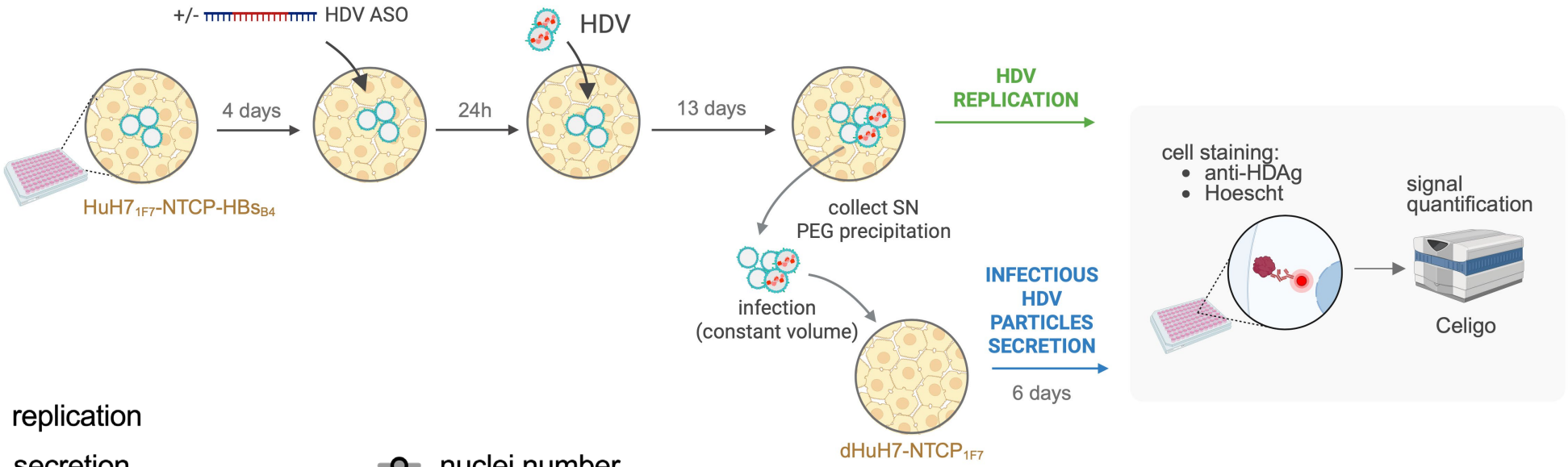
Establishment of the HuH7-NTCP-HBs cell line allowing production of HDV infectious particles

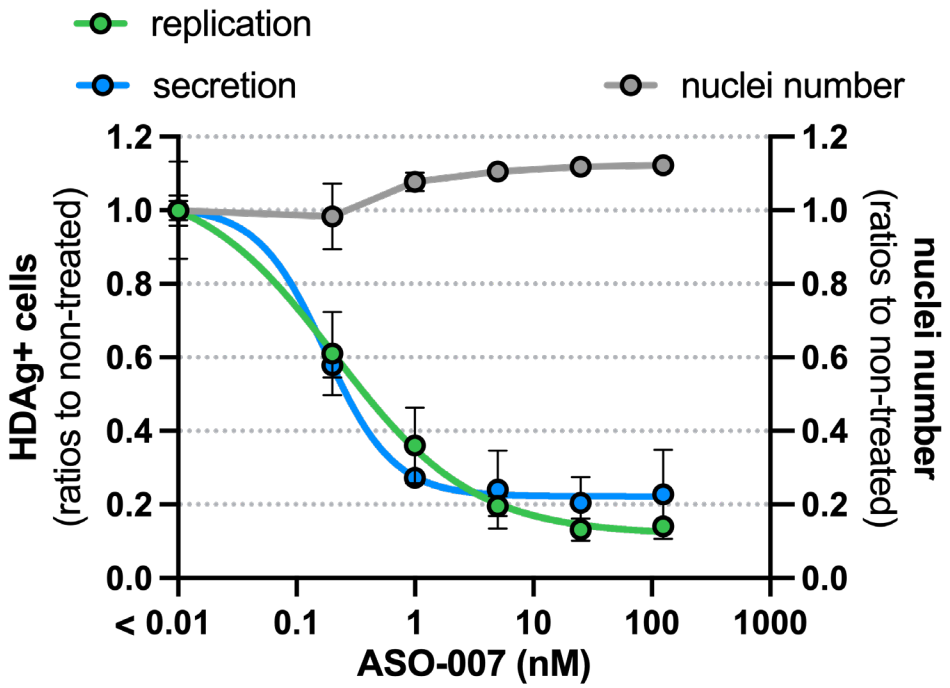




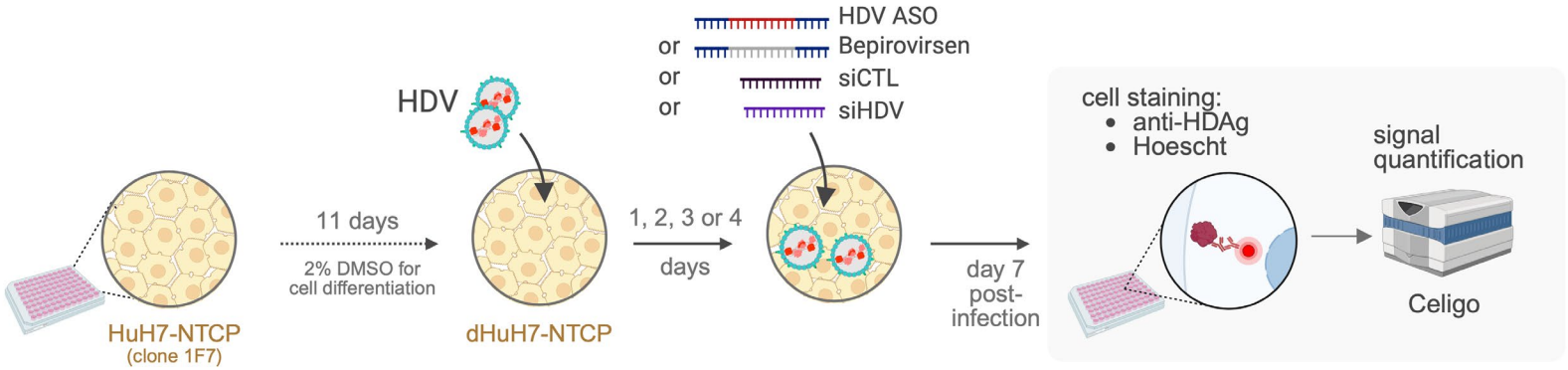


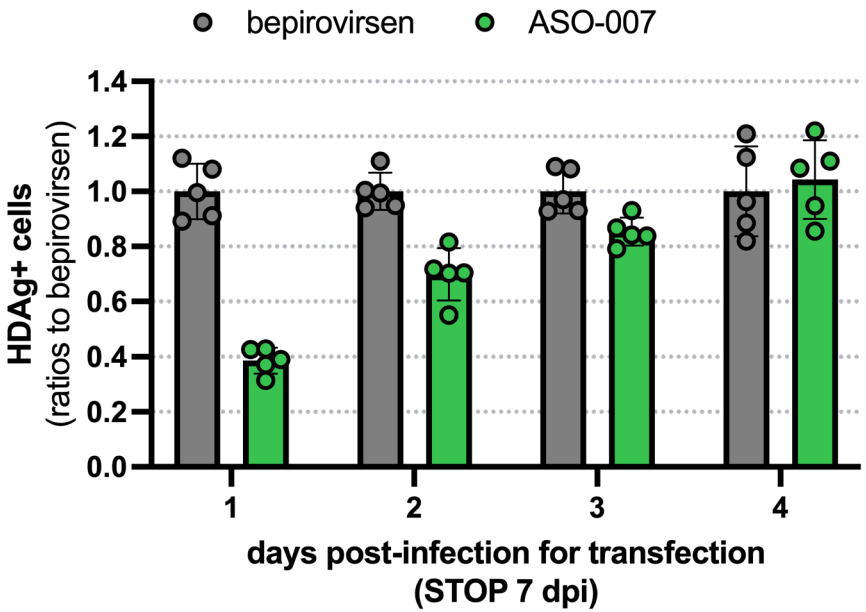
Anti-HDV ASOs impair HDV replication and secretion of infectious HDV particles

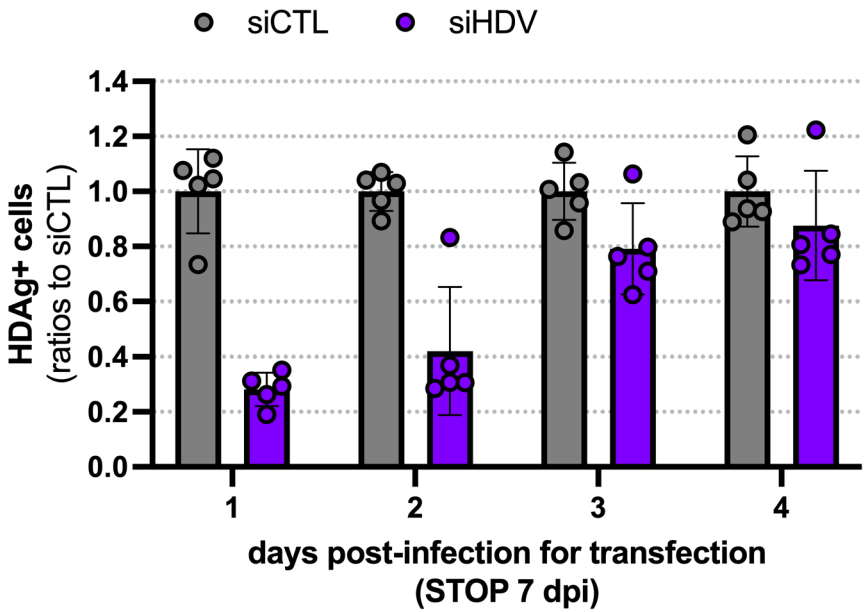




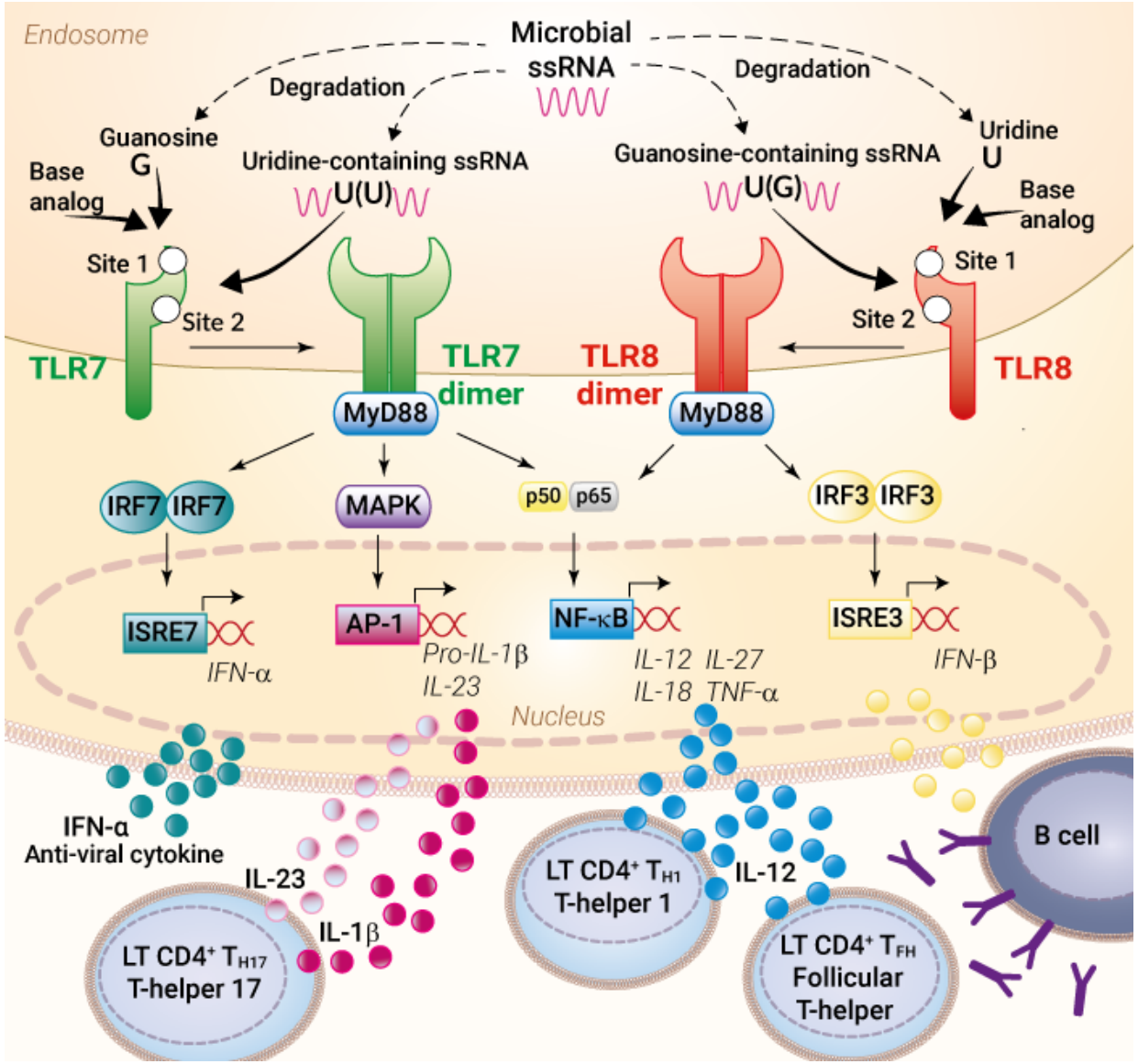
Anti-HDV ASOs and siRNA reduced the levels of HDAg+ cells when transfected early after HDV infection







ASOs might be recognized by Toll Like Receptors (TLR) such as TLR7/8



Invivogen

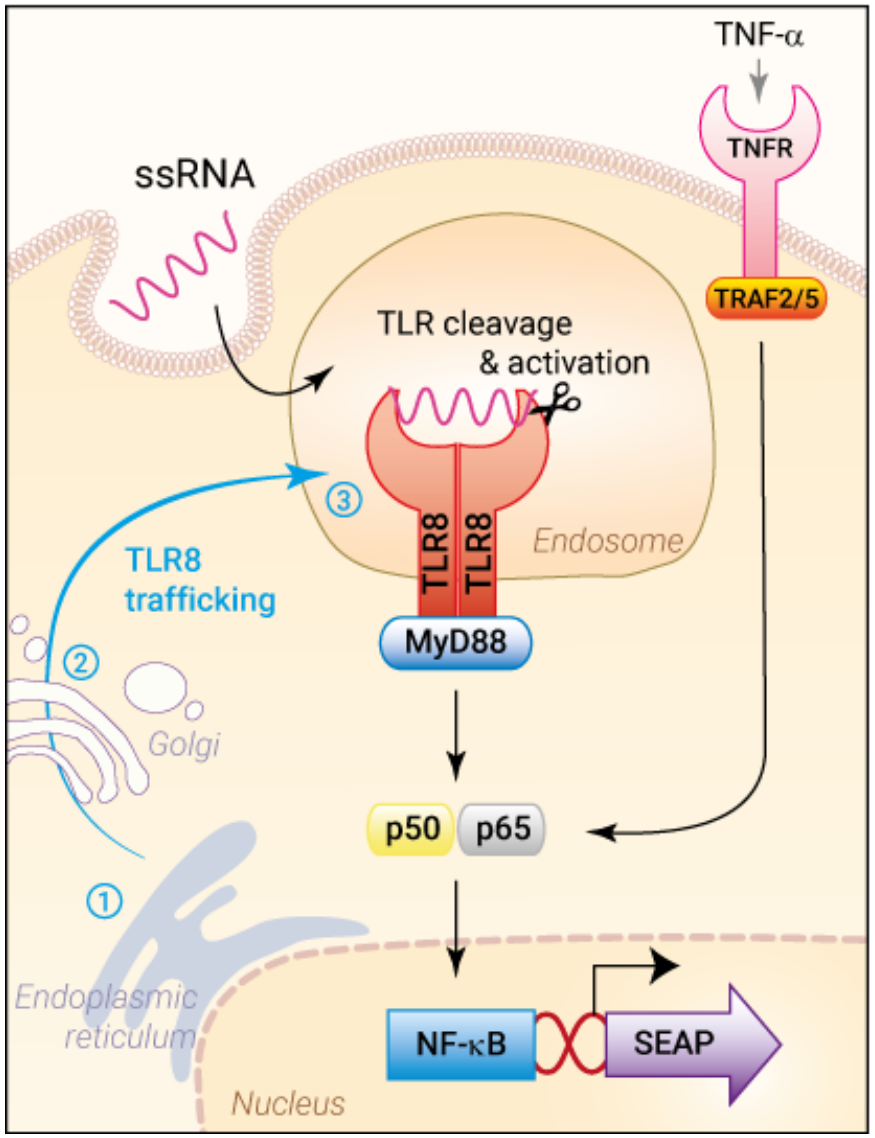
Bepirovirsen induced TLR8 activation

Figure 4. Modest and specific activation of TLR8 observed with transfected BPV in PRR-overexpressed reporter cell lines

Test Article	Dose	hkb-htlr2	hkb-htlr3	hkb-htlr4	hkb-htlr5	hkb-htlr7	hkb-htlr8	hkb-htlr9
Control (-)	0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
DOTAP Blank	1:10	0.9	1.1	1.0	1.3	1.1	1.1	1.1
	1:5	0.9	1.1	1.0	1.5	1.1	1.3	1.2
	3:10	0.9	1.1	1.0	1.7	1.2	1.6	1.5
BPV/DOTAP (ng/well)	100	1.0	1.0	1.0	1.2	1.1	1.7	1.1
	200	1.0	1.0	1.0	1.2	1.2	1.8	1.1
	300	1.0	1.0	1.0	1.3	1.2	1.8	1.1
scrASO/DOTAP (ng/well)	100	1.4	1.2	1.2	3.1	1.6	2.6	1.7
	200	1.5	1.2	1.3	2.8	2.0	2.6	1.6
	300	1.3	1.1	1.1	2.0	1.6	2.1	1.3
ssPoly(U)/DOTAP (ng/well)	100						4.3	
FLA-ST (ng/mL)	100				39.9			
ODN 2006 (ug/mL)	10							17.5

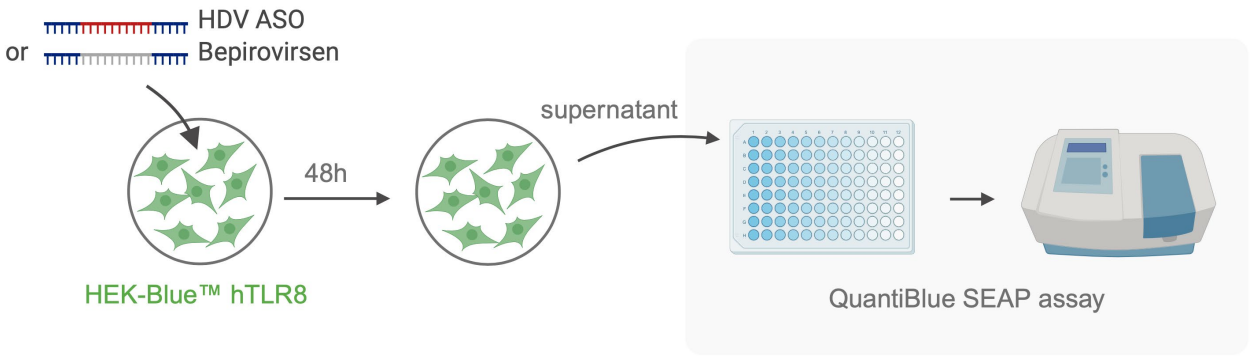
10 20

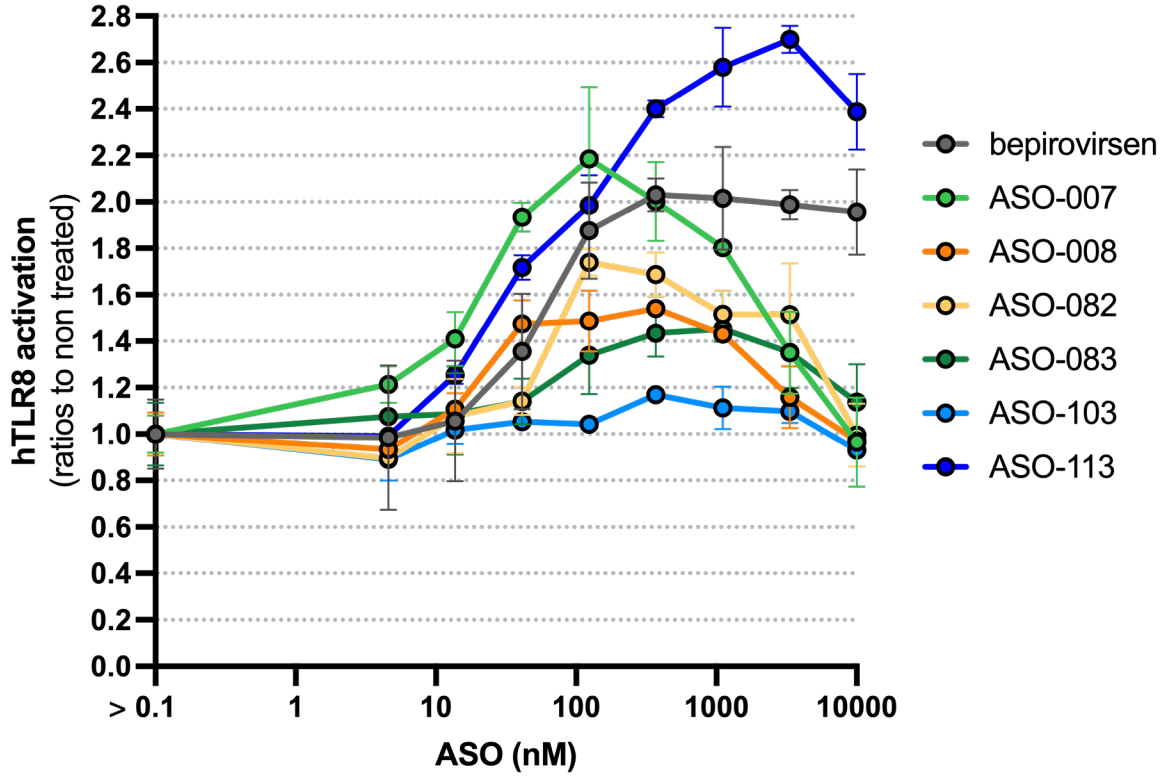
You *et al.*, ILC 2022, abstract N° SAT439

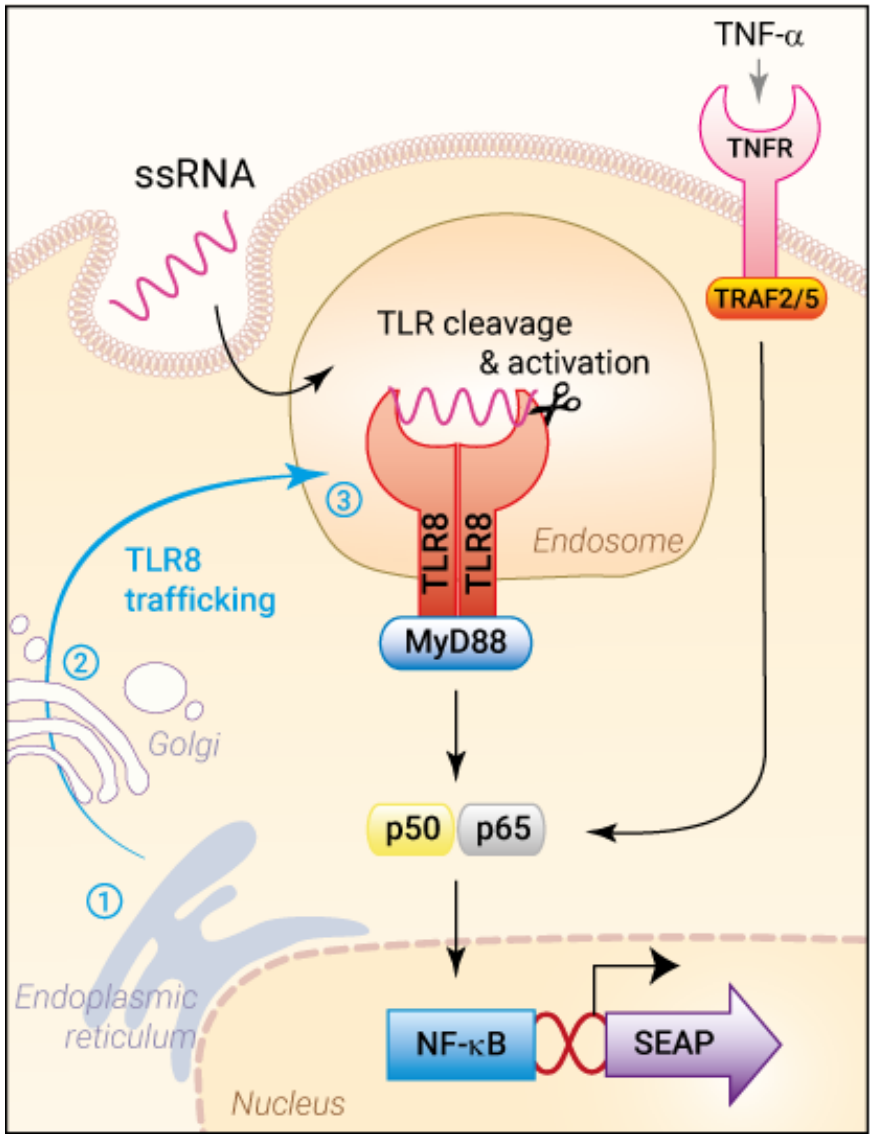


HEK-Blue™ hTLR8 (Invivogen)

Slight activation of TLR8 by some anti-HDV ASOs

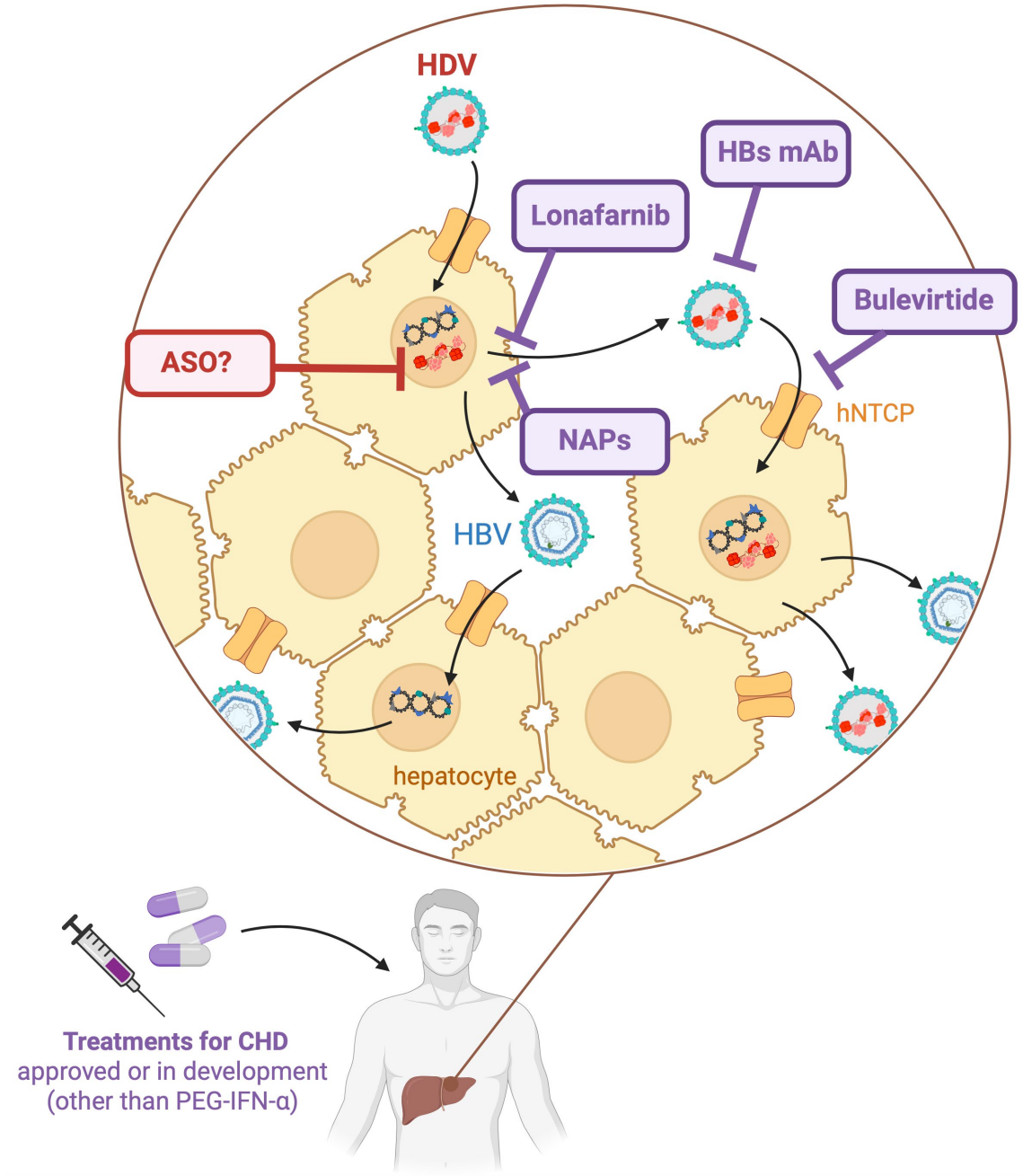






HEK-Blue™ hTLR8 (Invivogen)

Conclusions / Perspectives



To be addressed for further development of anti-HDV ASOs:

- ✓ **Test of anti-HDV ASOs against all HDV genotypes**
- ✓ **Anti-HDV ASOs mode of action**
  - ➢ Degradation of HDV RNAs?
  - ➢ Steric blocking?
  - ➢ Role of their immunostimulatory properties
- ✓ **Combination of anti-HDV ASOs with bepirovirsen**
- ✓ **Anti-HDV ASOs antiviral effect *in vivo***

Acknowledgments

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