Development of FXR agonists as antivirals against hepatitis viruses

Centre International de Recherche en Infectiologie, **Lyon, France**

This work has been supported by EnyoPharma (Research contract)

HBV cure Task Force

RB-RNA

Endorsed by

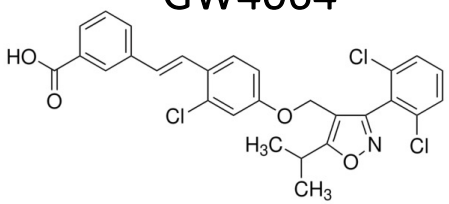
ICE-HBV
International Coalition to Eliminate HBV

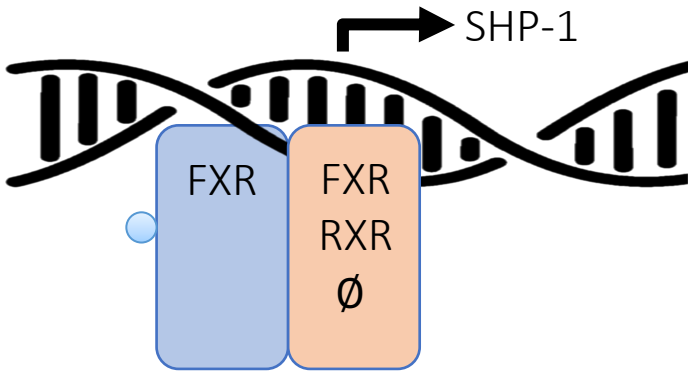
IP-cure-B

Farnesoid X receptor: a master regulator of organism metabolism

- Bile acids / FXR agonists

GW4064

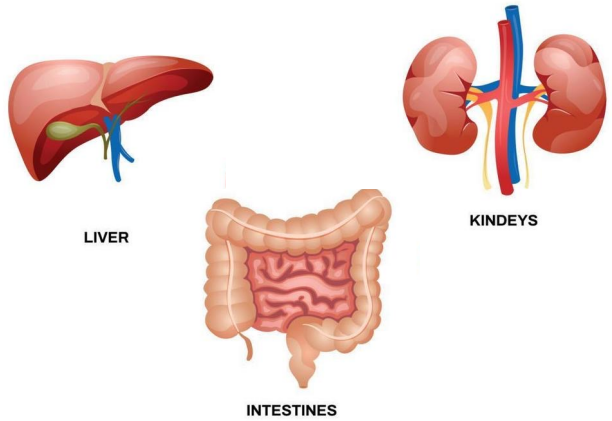




FXR role:

- Bile acids metabolism
- Glucose metabolism
- Lipid metabolism
- Immunomodulation

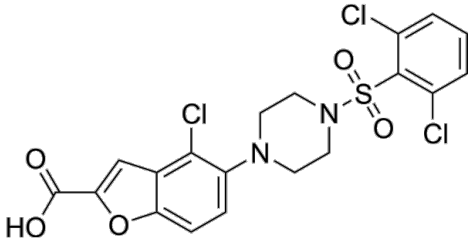
FXR expression:



- Adipose tissue
- Immune cells

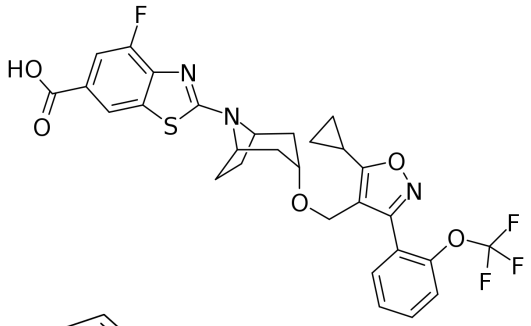
Chiang JYL, Ferrell JM. Discovery of farnesoid X receptor and its role in bile acid metabolism. Mol Cell Endocrinol. 2022
Anderson KM, Gayer CP. The Pathophysiology of Farnesoid X Receptor (FXR) in the GI Tract: Inflammation, Barrier Function and Innate Immunity. Cells. 2021

FXR agonists in clinic: **high repurposing potential**



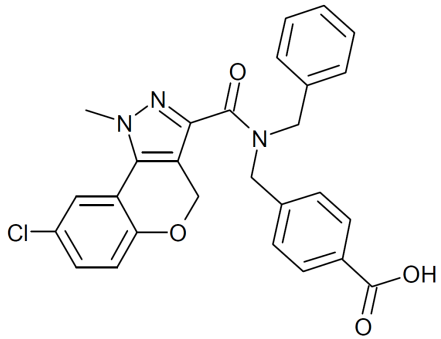
Vonafexor

Alport syndrome (2024, Phase 2)
NASH (2019 phase 2)
Chronic HBV (2017, Phase 1 – 2020, Phase 2)



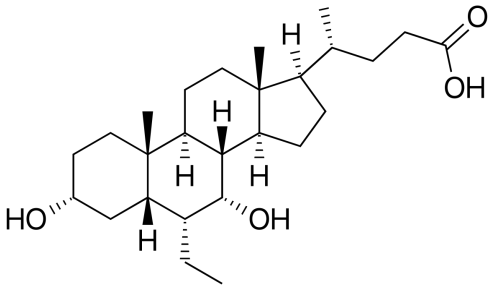
Tropifexor

Primary biliary cholangitis (2015, Phase 2)
NASH and liver fibrosis (2019, Phase 2)



Nidufexor

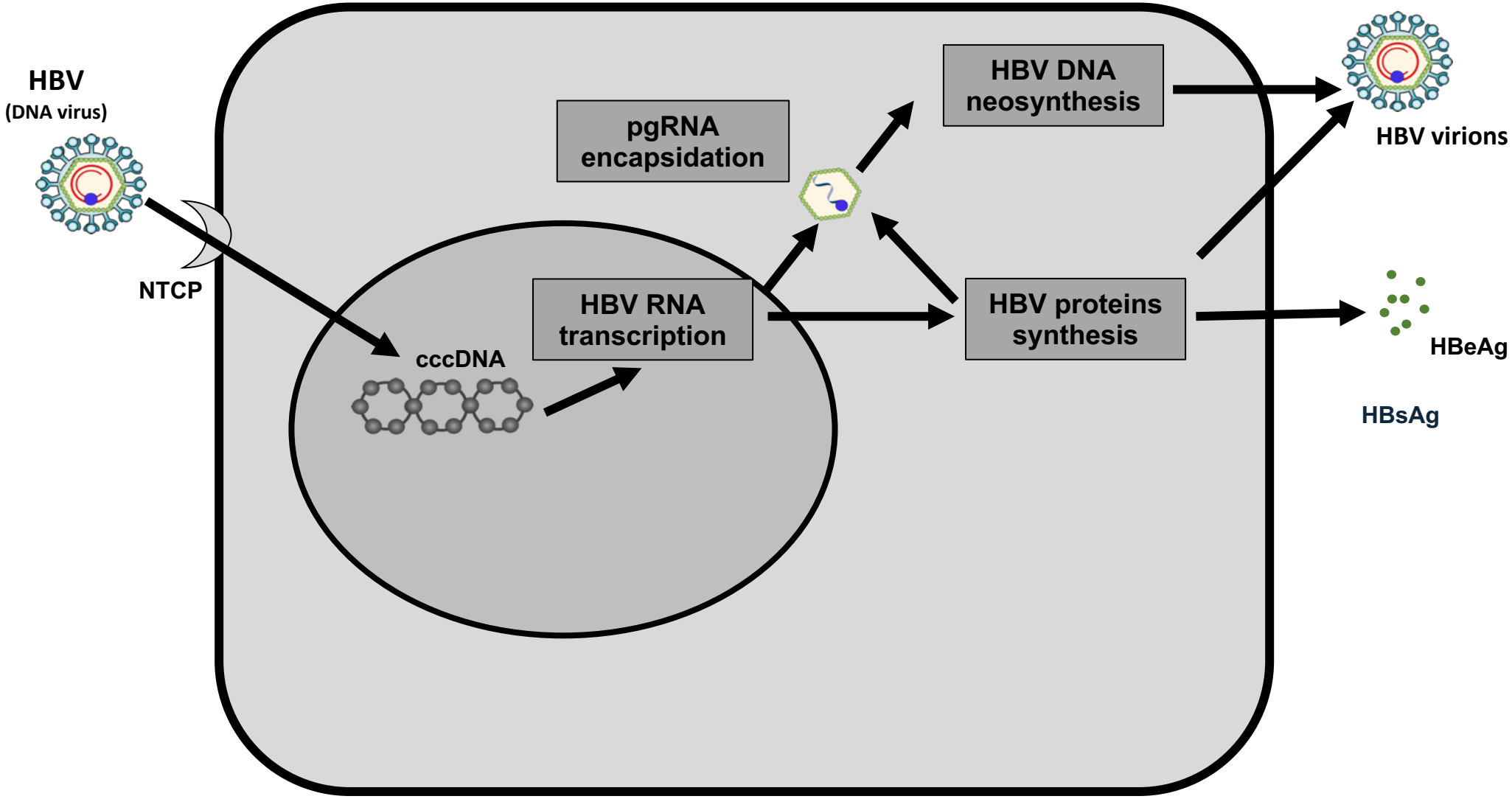
Diabetic nephropathy (2018, Phase 2)



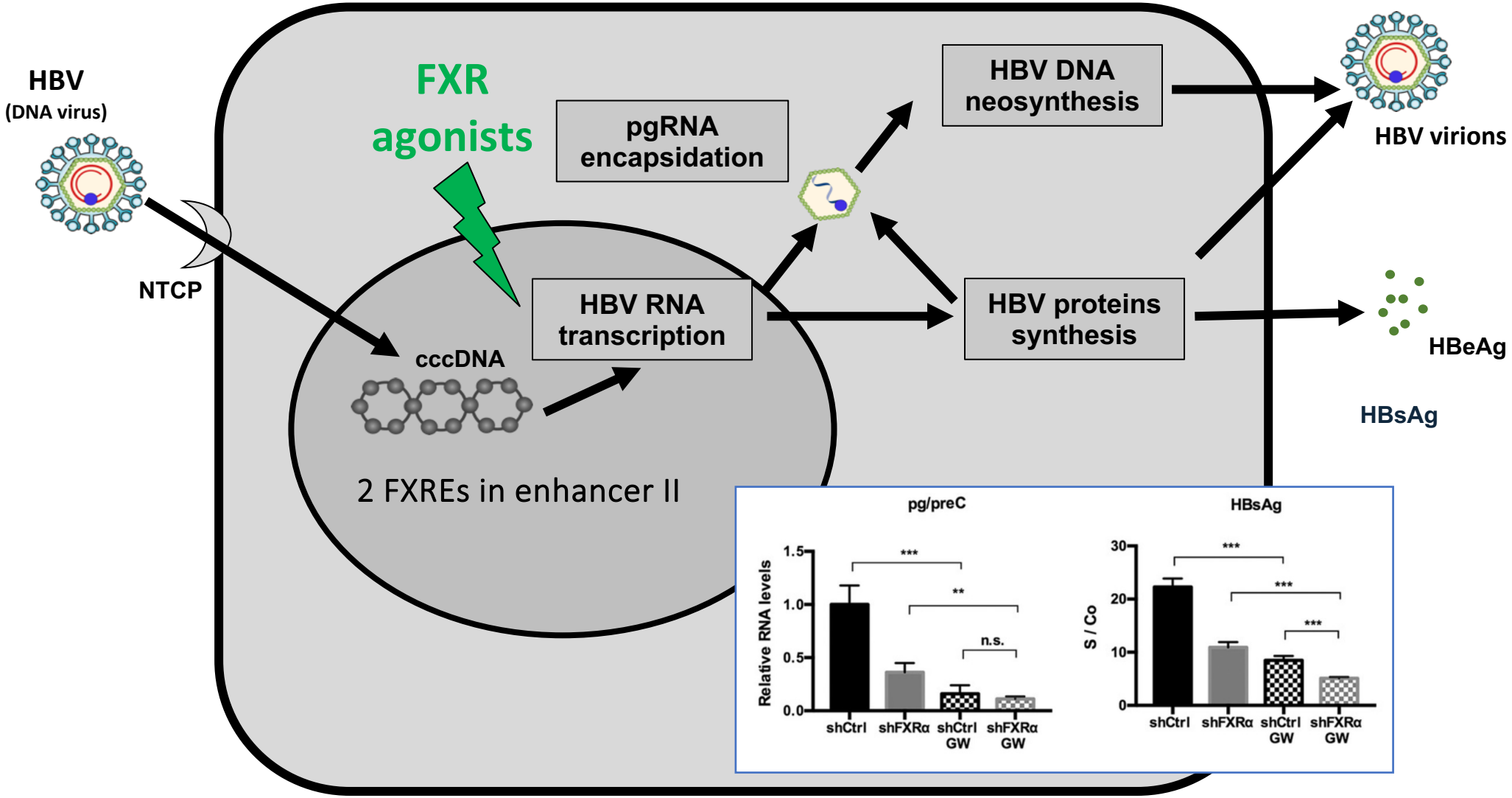
OCA

Primary biliary cholangitis (approval in 2016)
NASH (2017, phase 3 - not approved in 2023)
Biliary Atresia (2024, Phase 2-3)

HBV replication cycle



FXR could bind the cccDNA, its agonisation inhibits cccDNA transcription

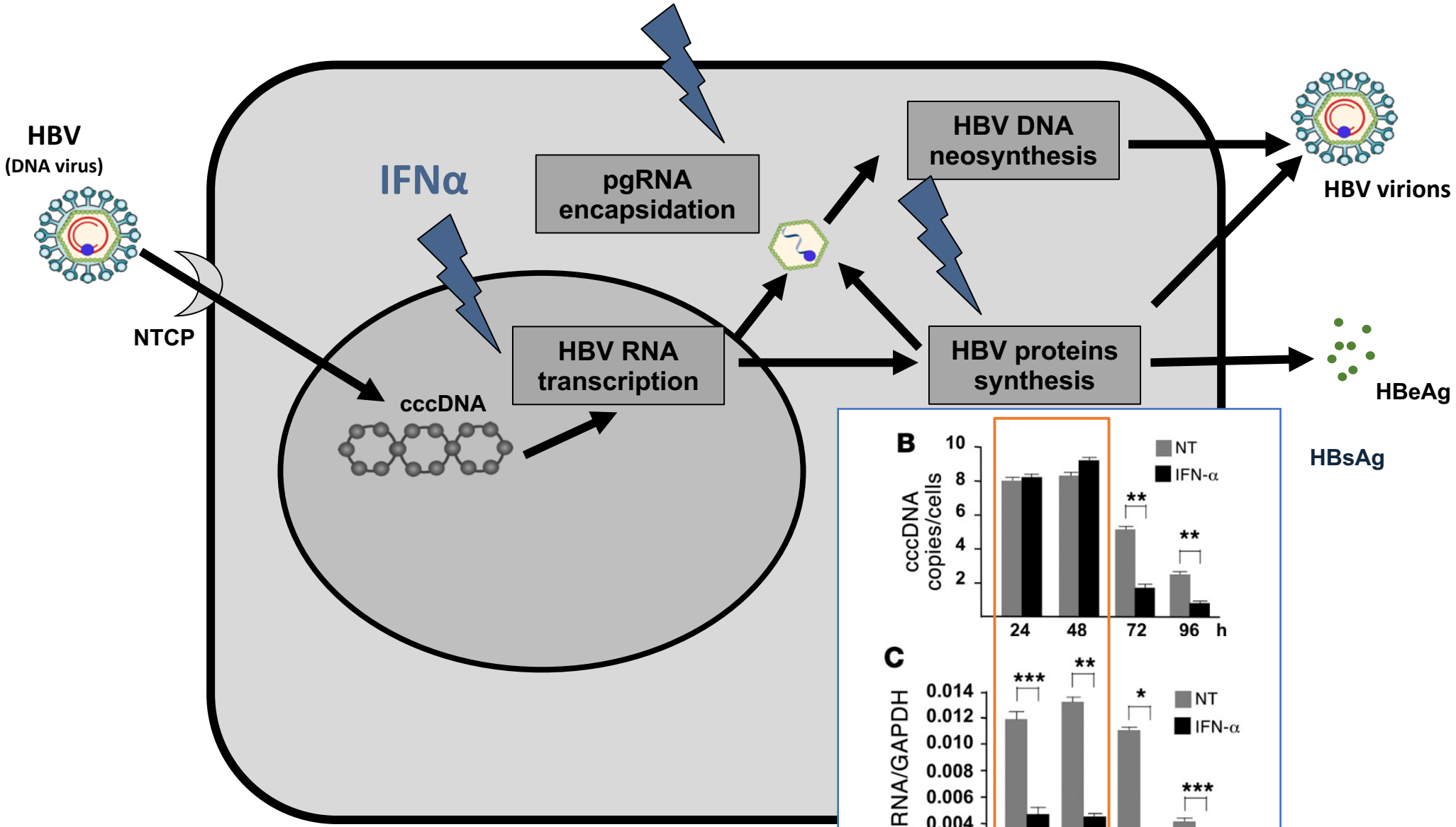


Ramière C, *et al.*, Transactivation of the hepatitis B virus core promoter by the nuclear receptor FXRalpha. J Virol. 2008

Mouzannar K, *et al.*, Farnesoid X receptor-α is a proviral host factor for hepatitis B virus that is inhibited by ligands in vitro and in vivo. FASEB J. 2019 Feb

Barnault R, *et al.*, *in preparation*

IFNα downregulates cccDNA transcription

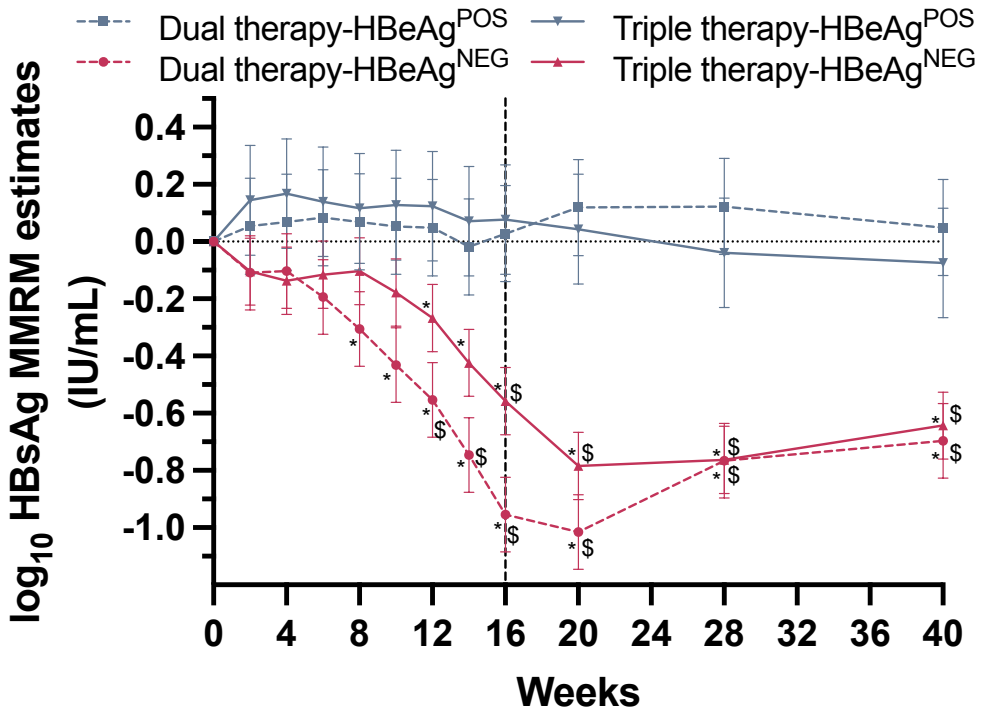


Belloni L, *et al*,. IFN-α inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome. J Clin Invest. 2012 Feb

Barnault R, *et al.,* in preparation

Vonafexor & IFNα combination in an open-label phase 2 clinical trial

Number of patients	HBeAg^{NEG}	HBeAg^{POS}
Dual-therapy	n=6	n=4
Triple therapy	n=7	n=3
Peg-IFNα (external control)	n=18	n=53



* Significance to baseline (W0) ($p<0,05$)

$ Significance to respective HBeAg^{POS} group ($p<0,05$)

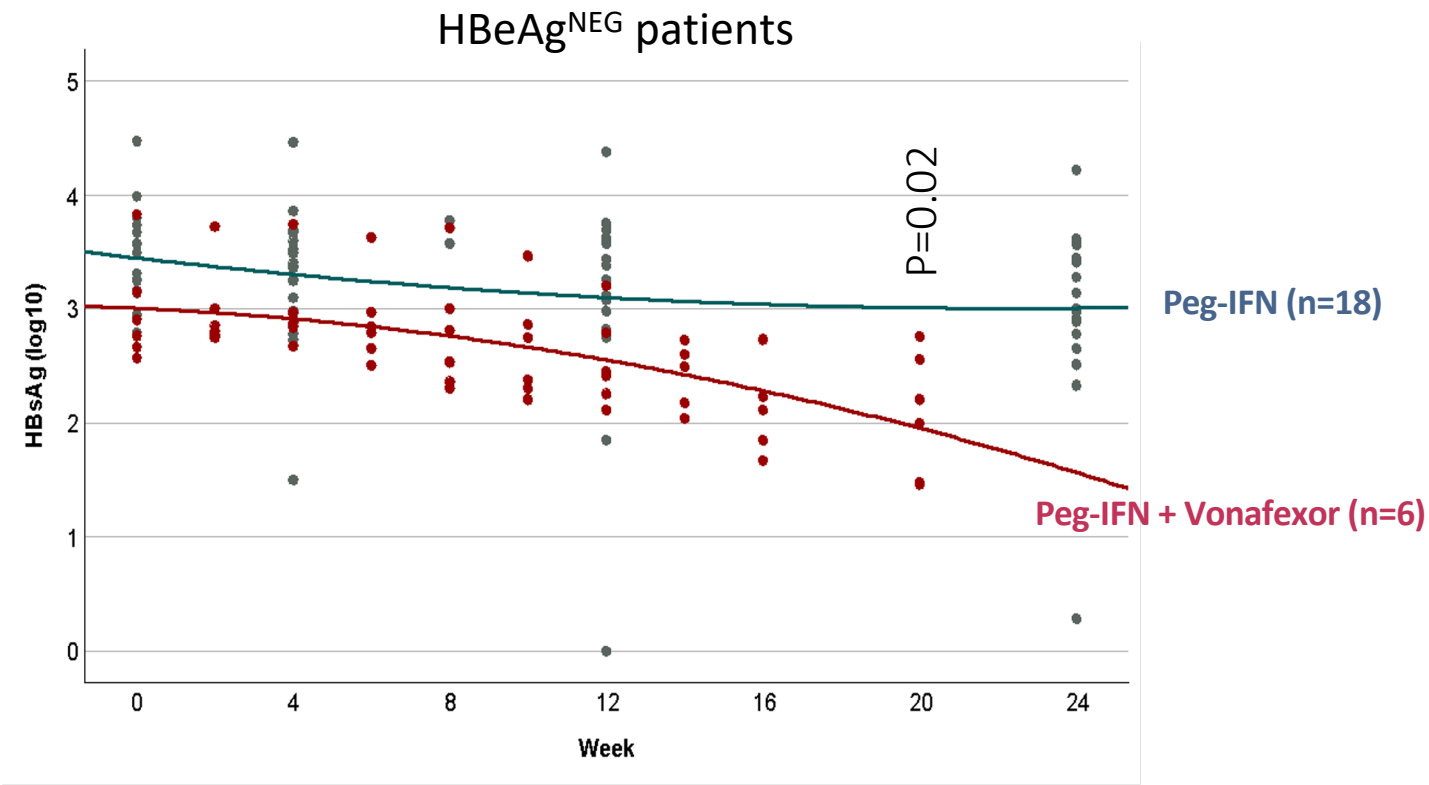
- HBsAg downregulation by 1 Log$_{10}$ in HBeAg^{NEG} patients with dual therapy
- Long lasting effect with no return at baseline
- No toxicity / side effects

Barnault R, et al., in preparation

Hansen Bettina E, *et al.,* Antiviral effects of oral farnesoid X receptor agonist vonafexor combined with pegylated interferon alpha2a in untreated patients with chronic hepatitis B, HBV Cure 2022, Singapore

Vonafexor & IFNα combination in an open-label phase 2 clinical trial

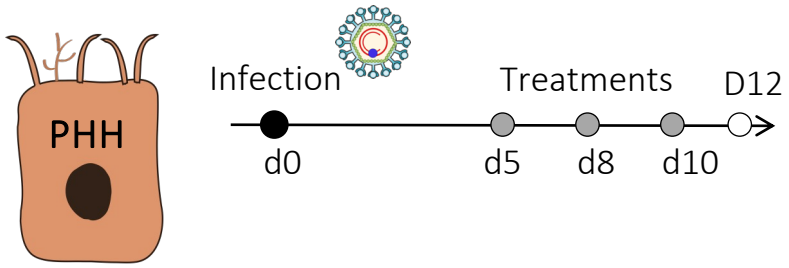
Number of patients	HBeAg^{NEG}	HBeAg^{POS}
Dual-therapy	n=6	n=4
Triple therapy	n=7	n=3
Peg-IFNα (external control)	n=18	n=53

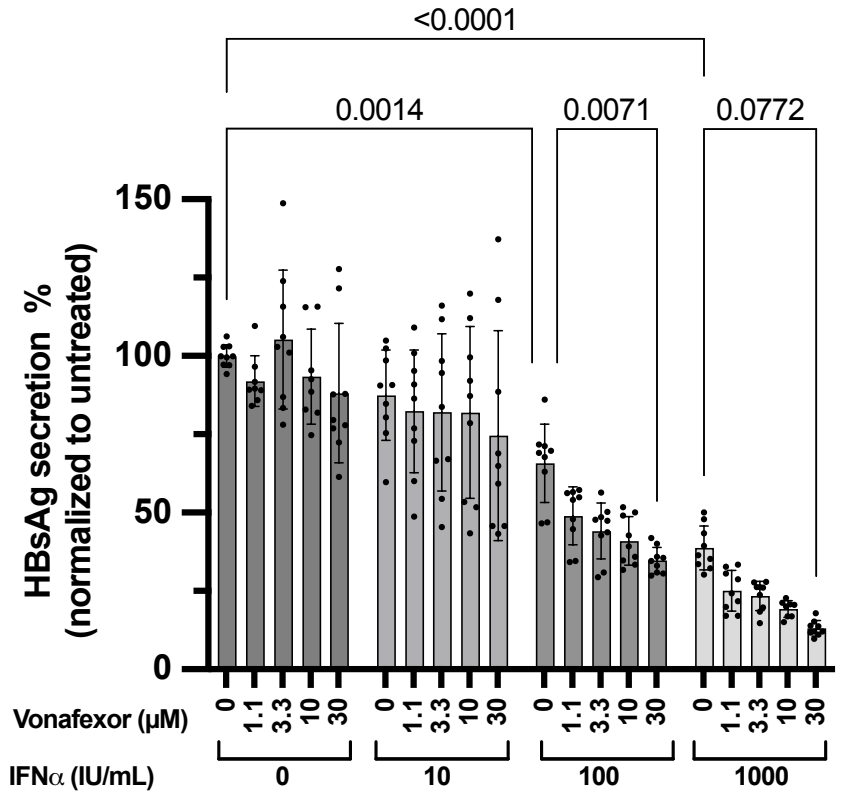


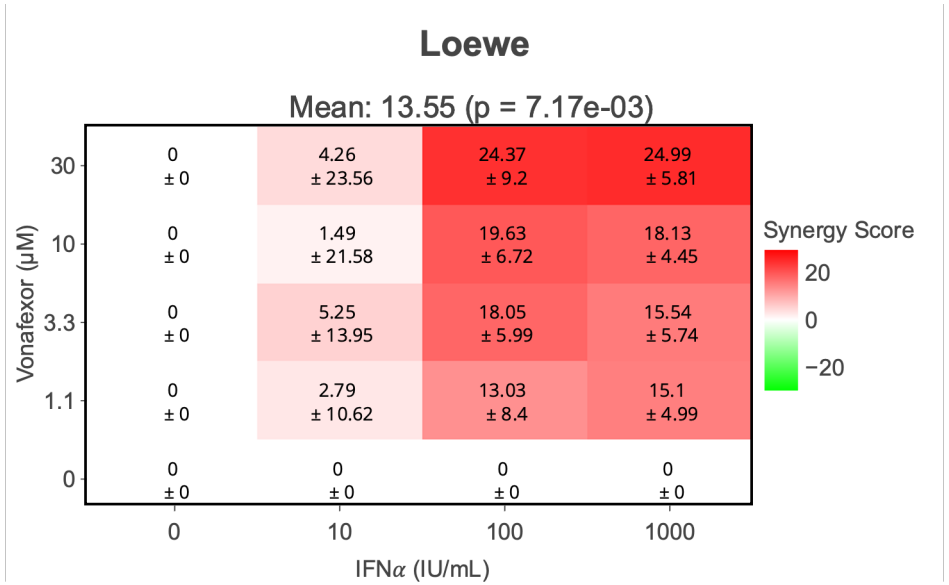
- At week 20, the -1 Log_{10} difference between cohorts is significant, favoring IFN+Vonafexor

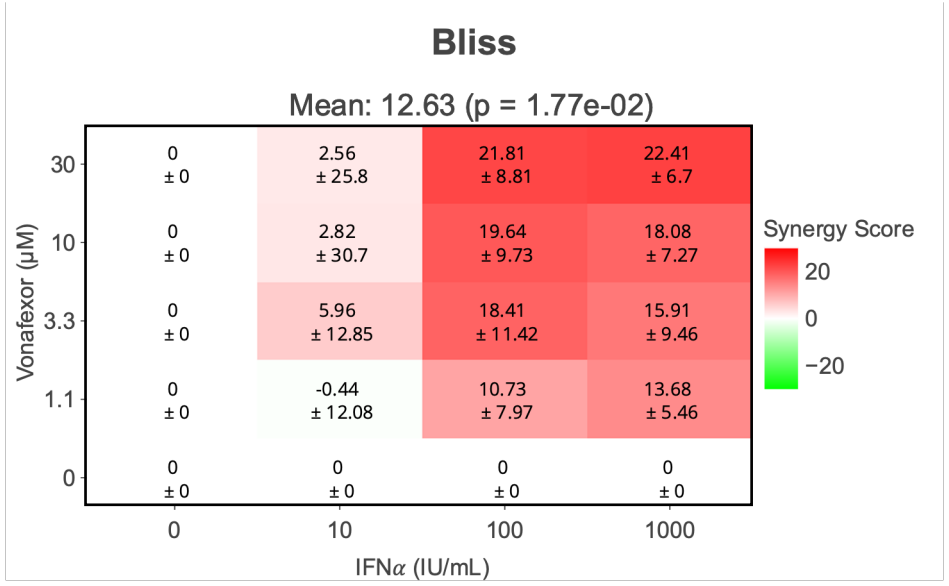
Barnault R, et al., in preparation
Hansen Bettina E, *et al.,* Antiviral effects of oral farnesoid X receptor agonist vonafexor combined with pegylated interferon alpha2a in untreated patients with chronic hepatitis B, HBV Cure 2022, Singapore

Vonafexor-IFNα combination synergistically inhibits HBsAg secretion in PHH



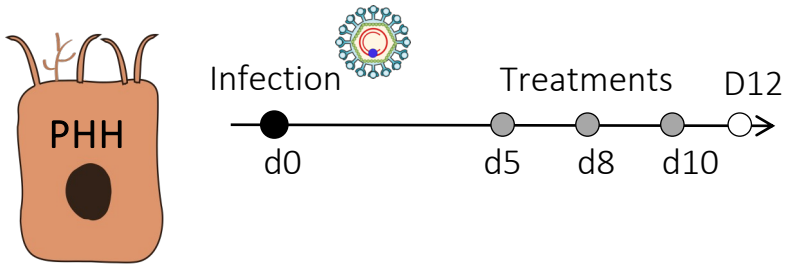


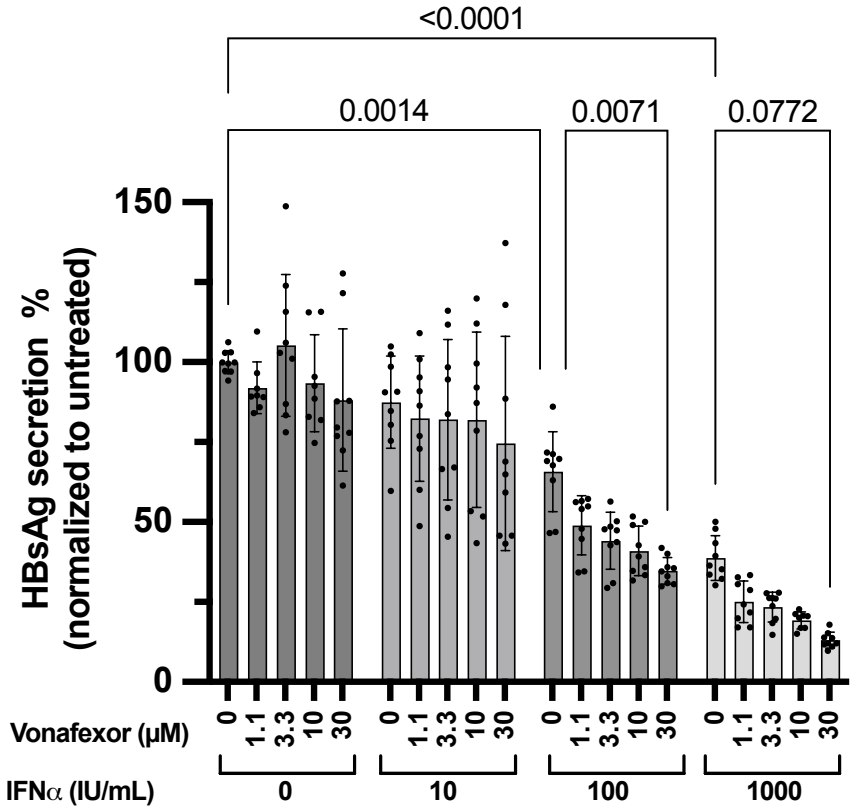


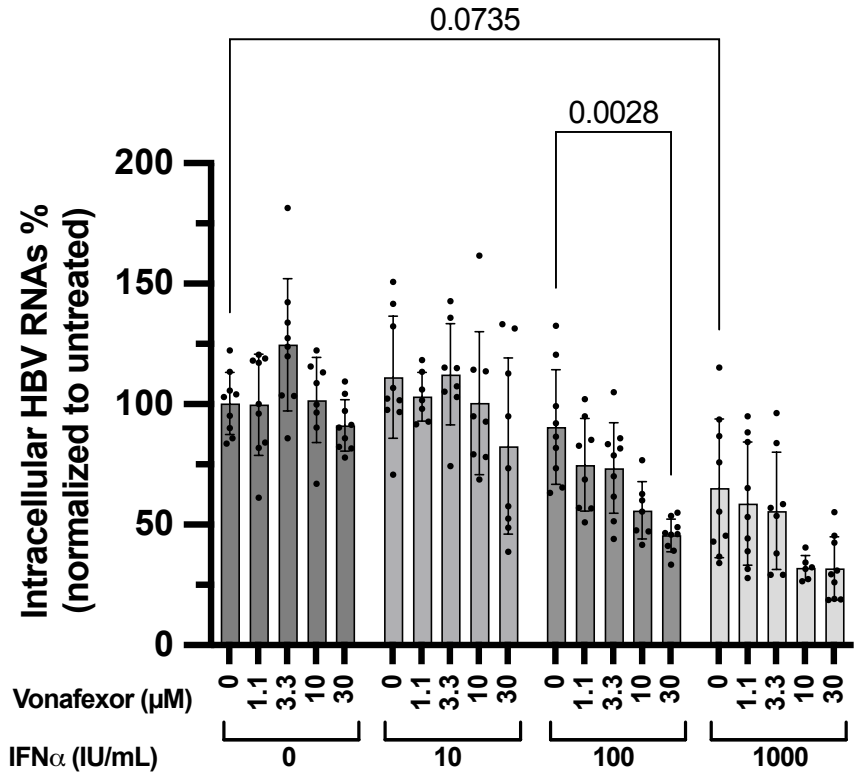


Barnault R, *et al., in preparation*

HBsAg secretion downregulation is correlated with HBV RNA downregulation

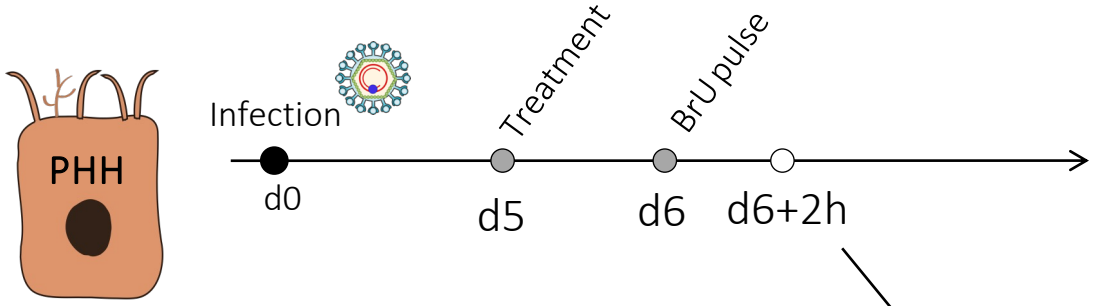






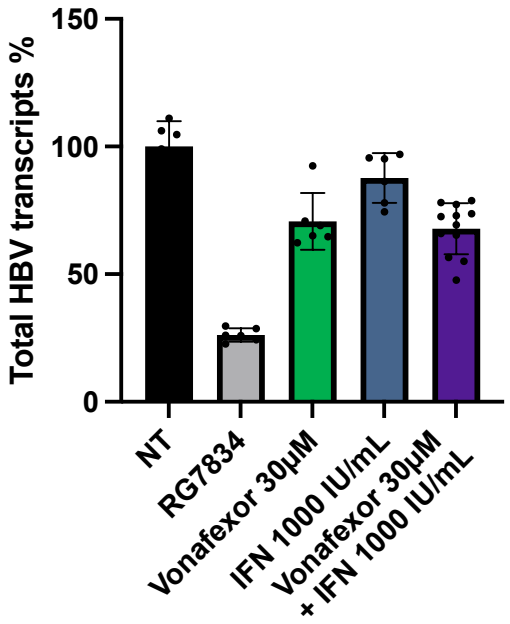
Barnault R, *et al.*, in preparation

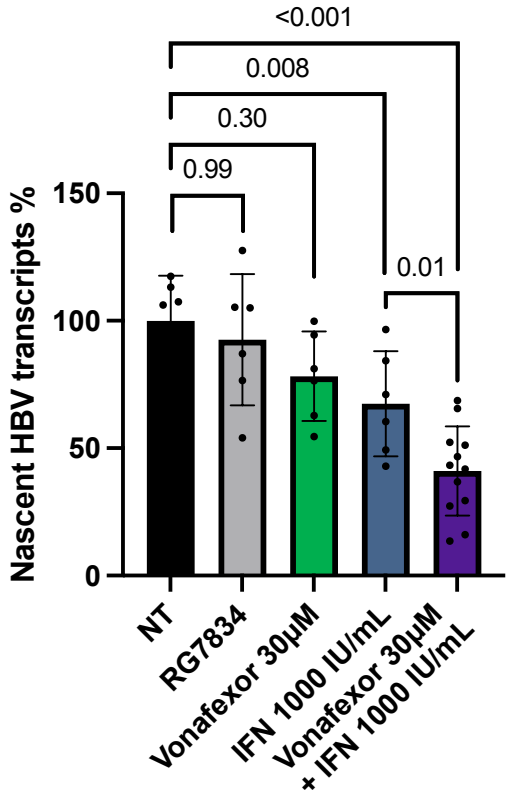
Vonafexor-IFNα combination inhibits cccDNA transcription



Total RNA extraction

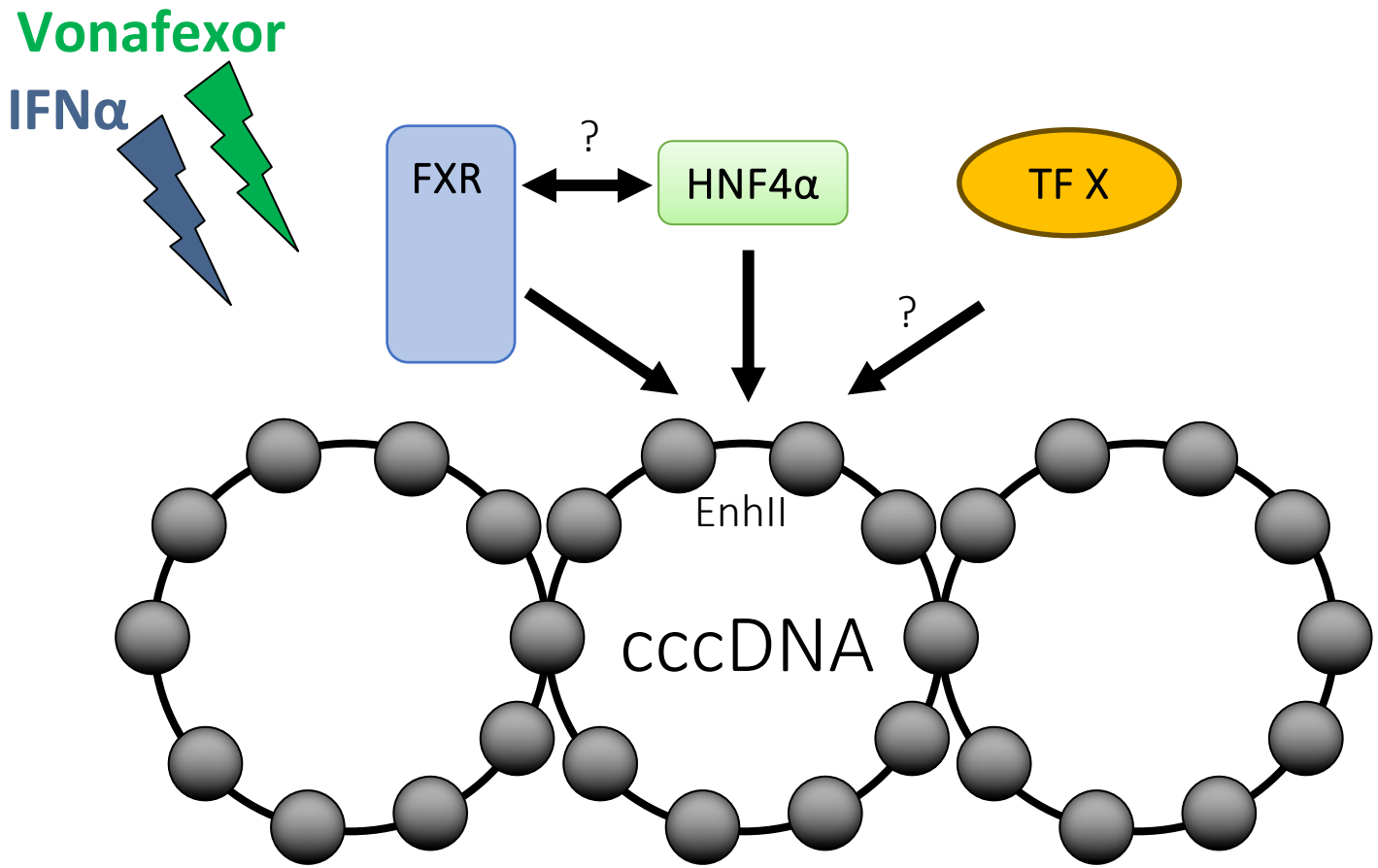
Nascent BrU-stained RNA purification

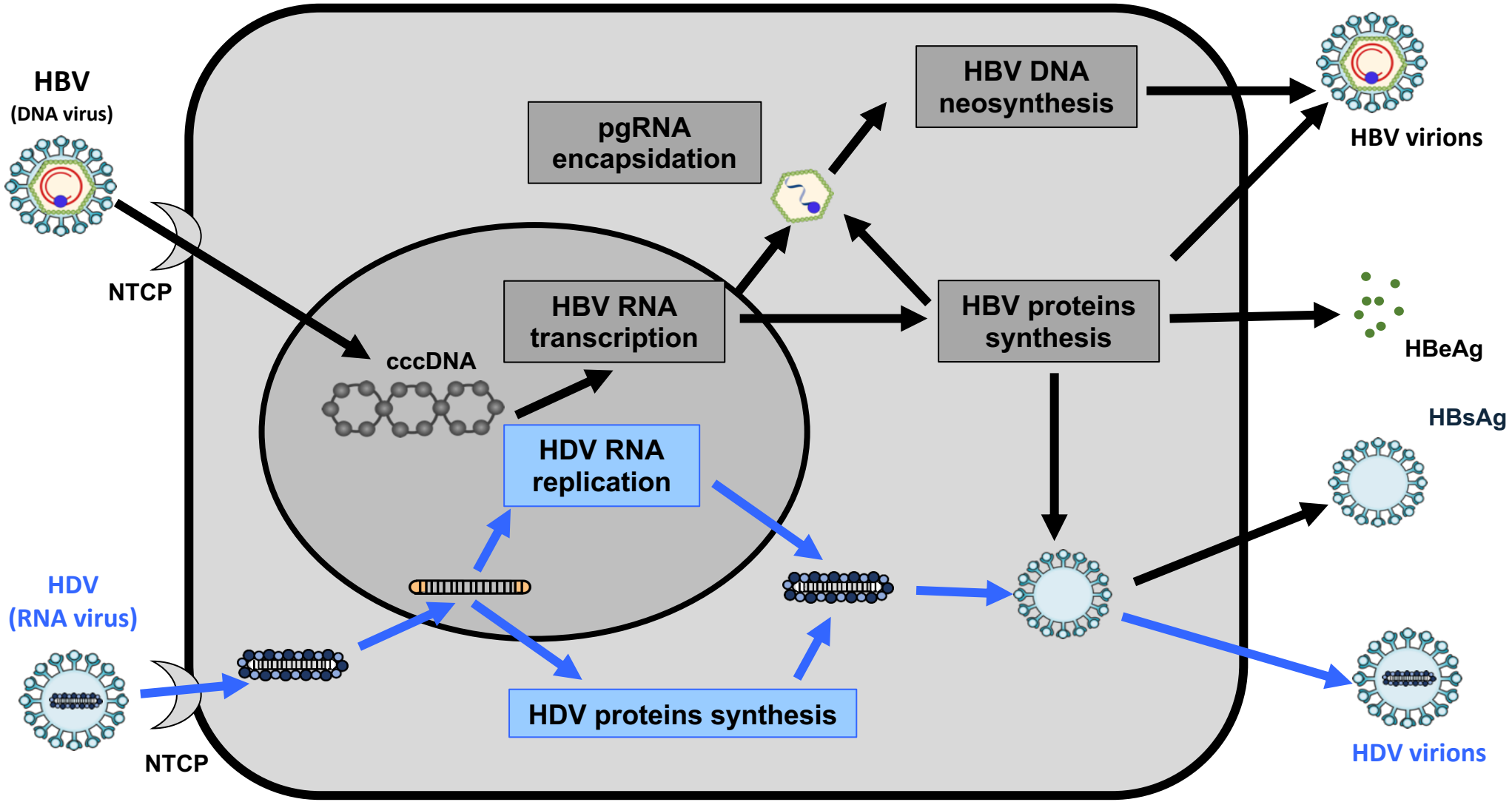




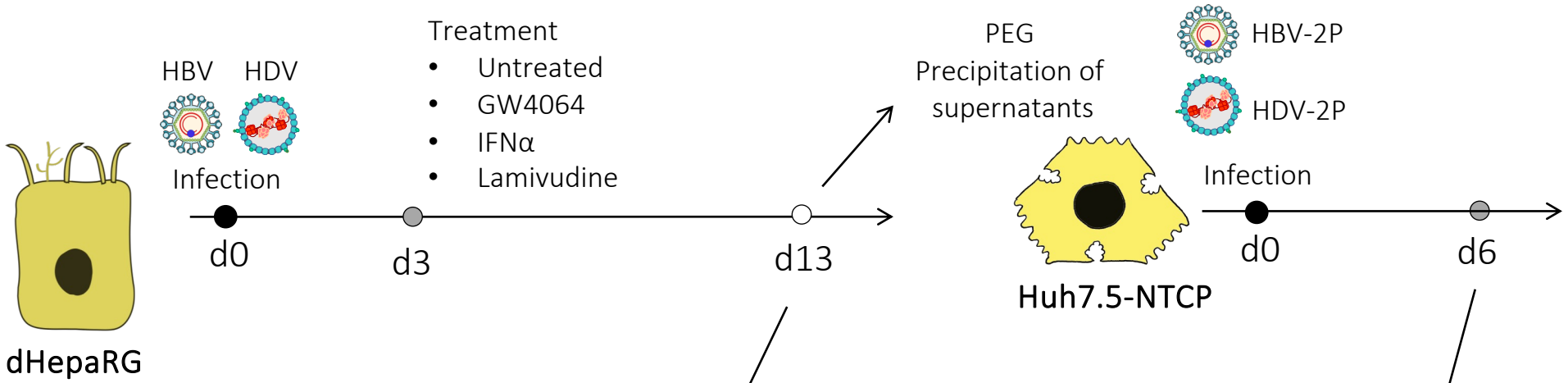
Barnault R, *et al., in preparation*

Competition between different transcription factors could explain transcription inhibition

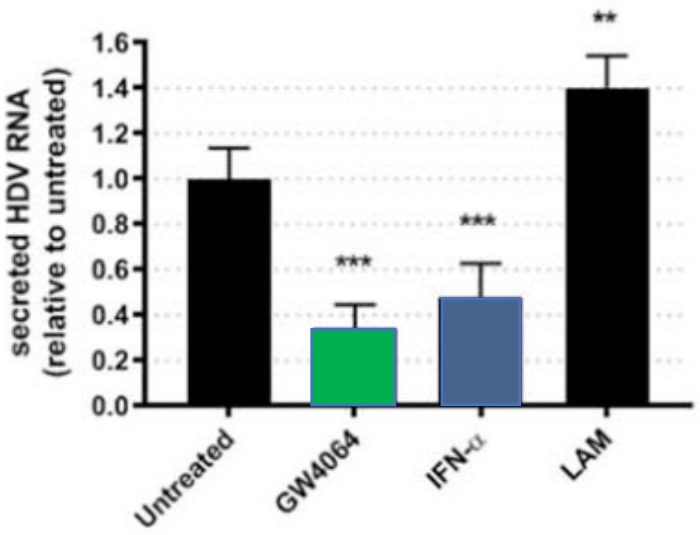



HBV
(DNA virus)
NTCP
cccDNA
pgRNA
encapsidation
HBV DNA
neosynthesis
HBV virions
HBV RNA
transcription
HBV proteins
synthesis
HBeAg
HBsAg
HDV RNA
replication
HDV
(RNA virus)
HDV proteins synthesis
NTCP
HDV virions

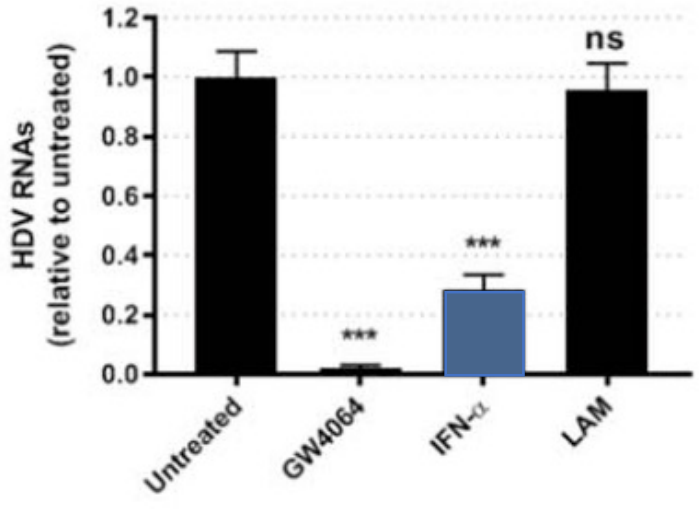
HDV particles specific-infectivity inhibited by FXR ligand GW4064



Secreted HDV RNA

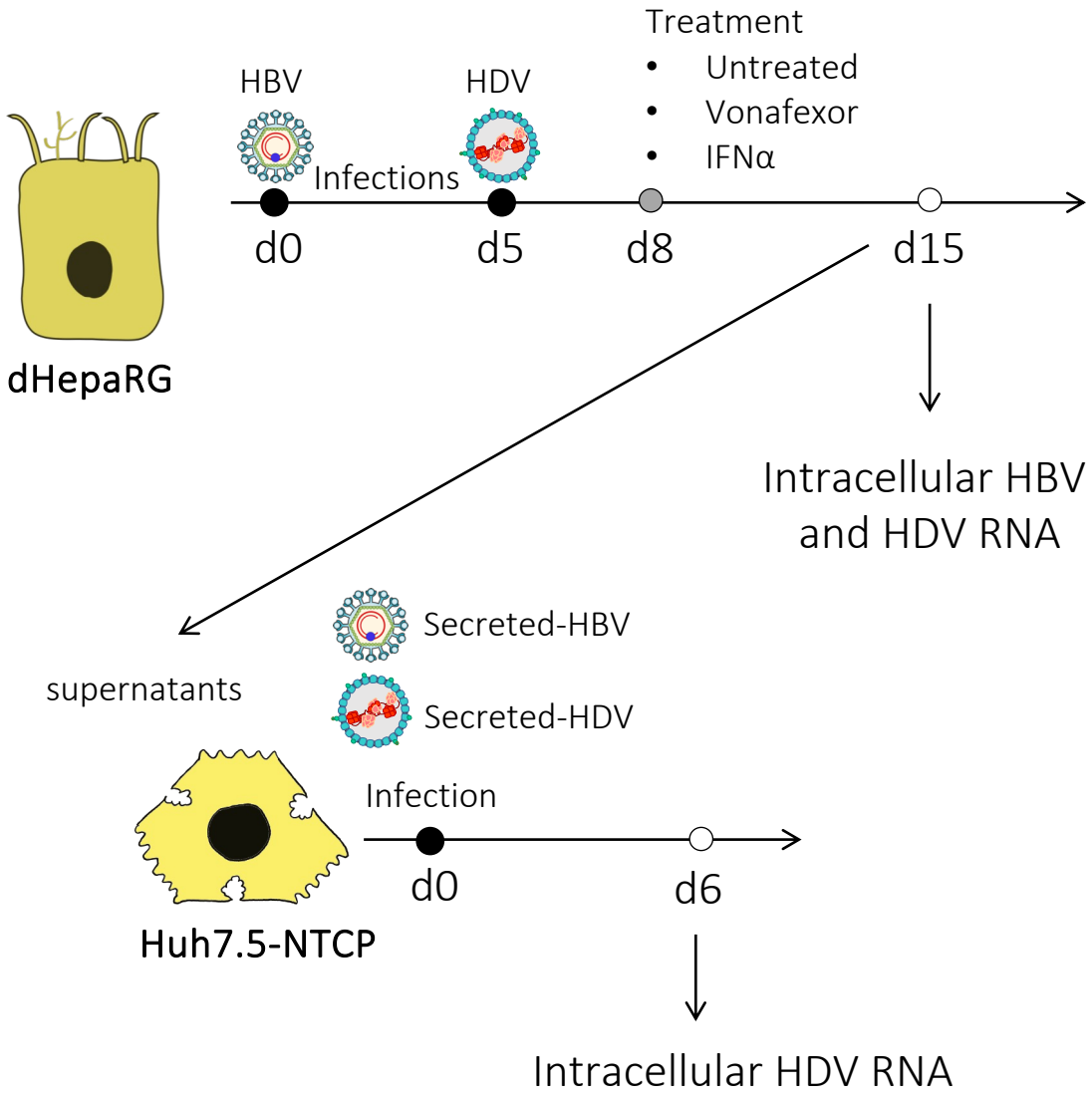


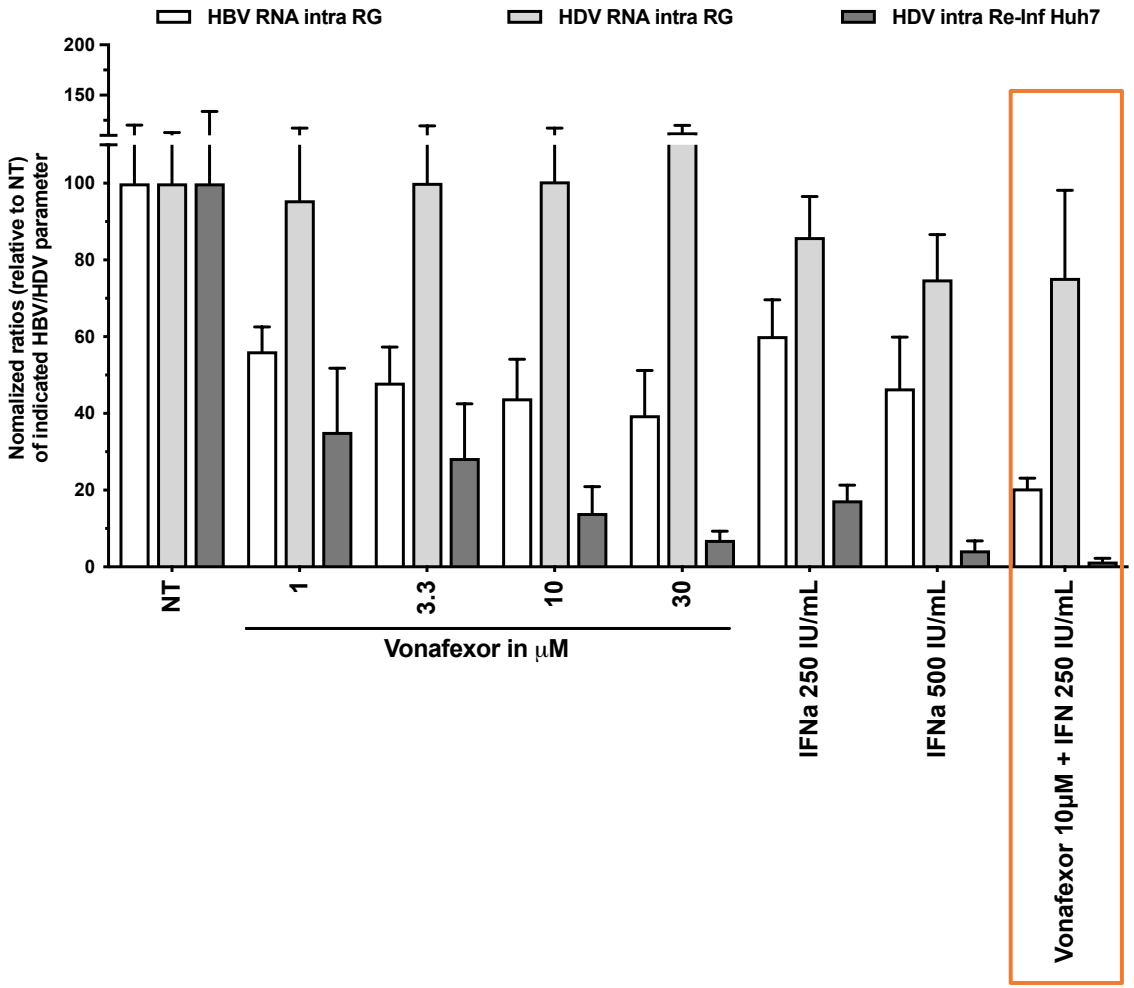
Intracellular HDV RNA



Legrand AF, et al.,Farnesoid X receptor alpha ligands inhibit HDV in vitro replication and virion infectivity. Hepatol Commun. 2023 Apr

Vonafexor combined with IFNα impairs HDV reinfection

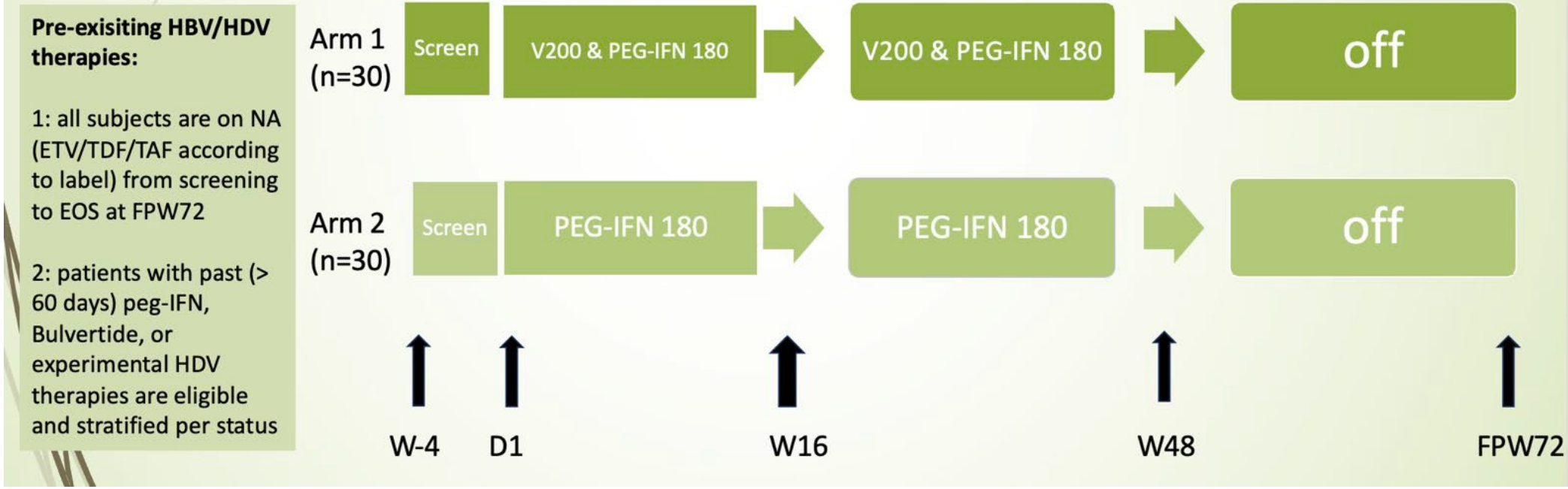




Barnault et al., in preparation
PCT/EP2021/050625
EP23305289

Clinical trial in chronic hepatitis D patients to start?

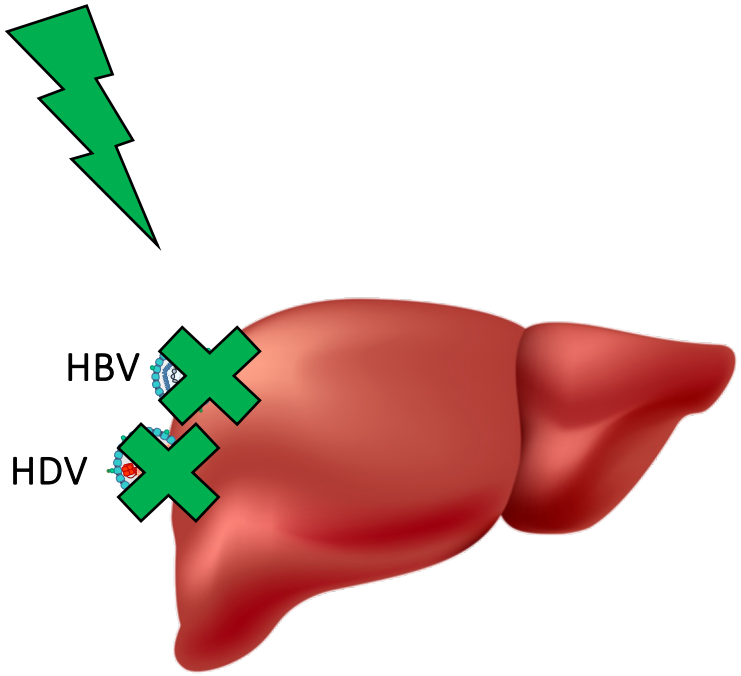
Open label, single country, multisite, two arm, randomised (1:1) phase 2 trial in CHD patients, stratified per past HDV therapy. The bi-therapy of Vonafexor on top of SOC peg-IFN compares to SOC peg-IFN alone. The study is powered at 90% to detect a 40% responder rate difference on the primary endpoint: HDV viral load decline > 2log10 from baseline at W48.



- ✓ 60 CHD patients to be enrolled and treated with Vonafexor +/- Peg-IFNa
- ✓ Grant application at ANRS AO-2 2024 CSS13 submitted in March 2024
- ✓ In kind contribution by Enyo
- ✓ Coordination of the trial: Pr Tarik Asselah (Beaujon Hospital, Paris)

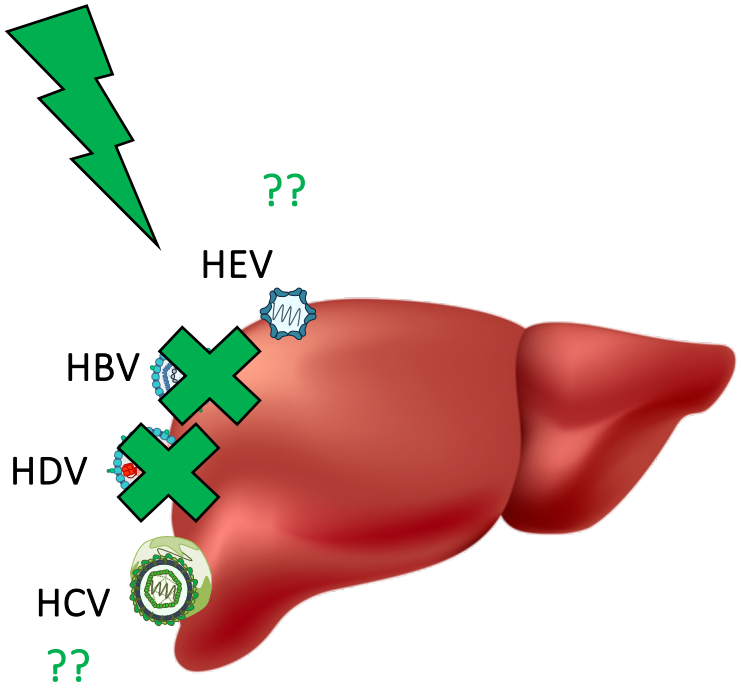
FXR ligands are efficient to limit HBV and HDV infections

FRX Ligands



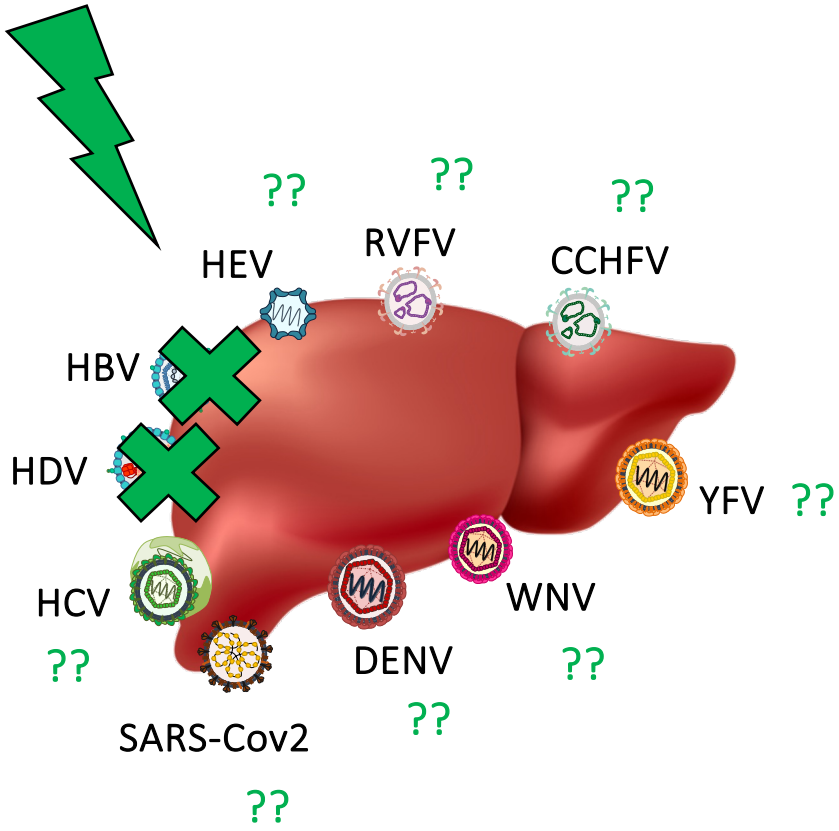
Broad spectrum antiviral activity of FXR-ligand against hepatotropic viruses?

FRX Ligands



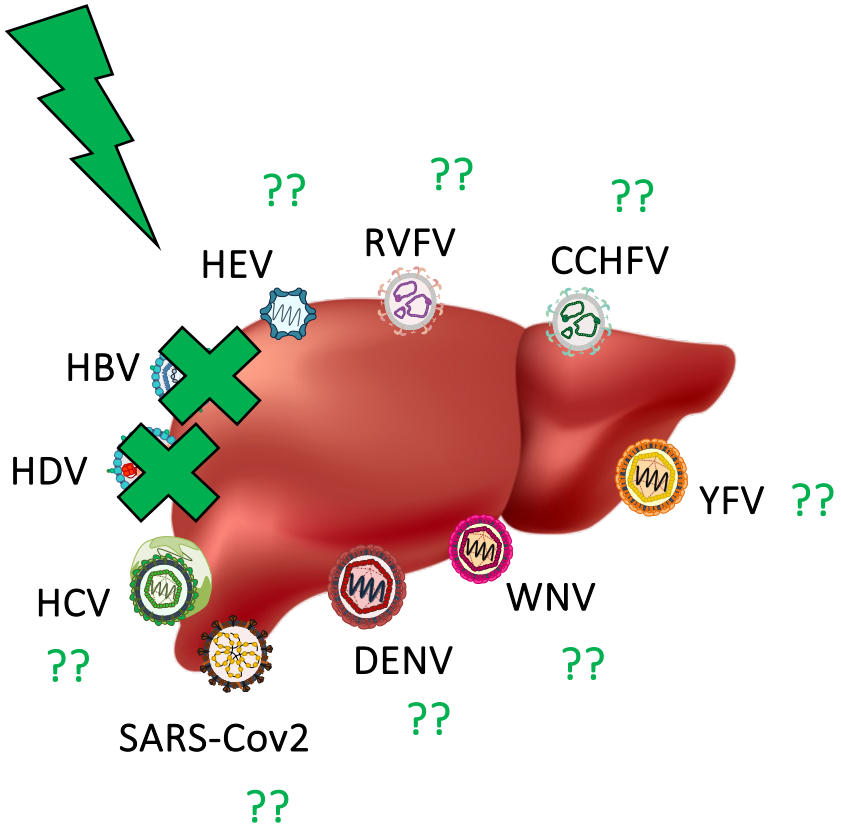
Broad spectrum antiviral activity of FXR-ligand against hepatotropic viruses?

FRX Ligands

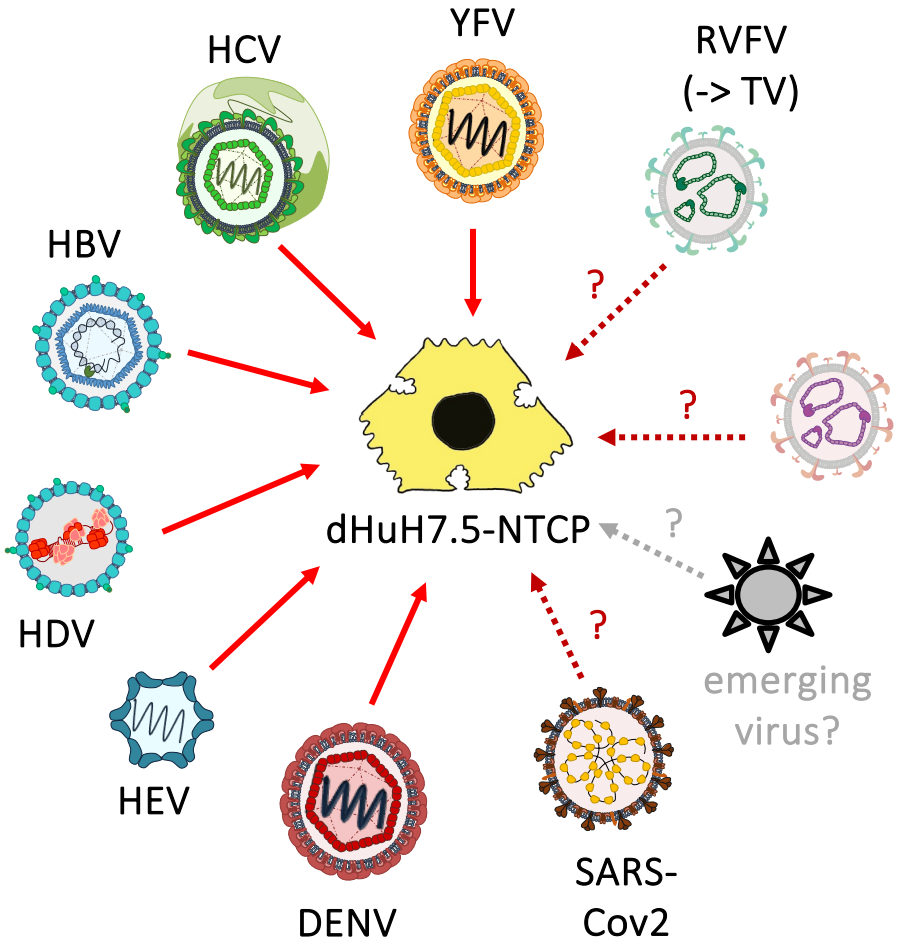


Broad spectrum antiviral activity of FXR-ligand against hepatotropic viruses?

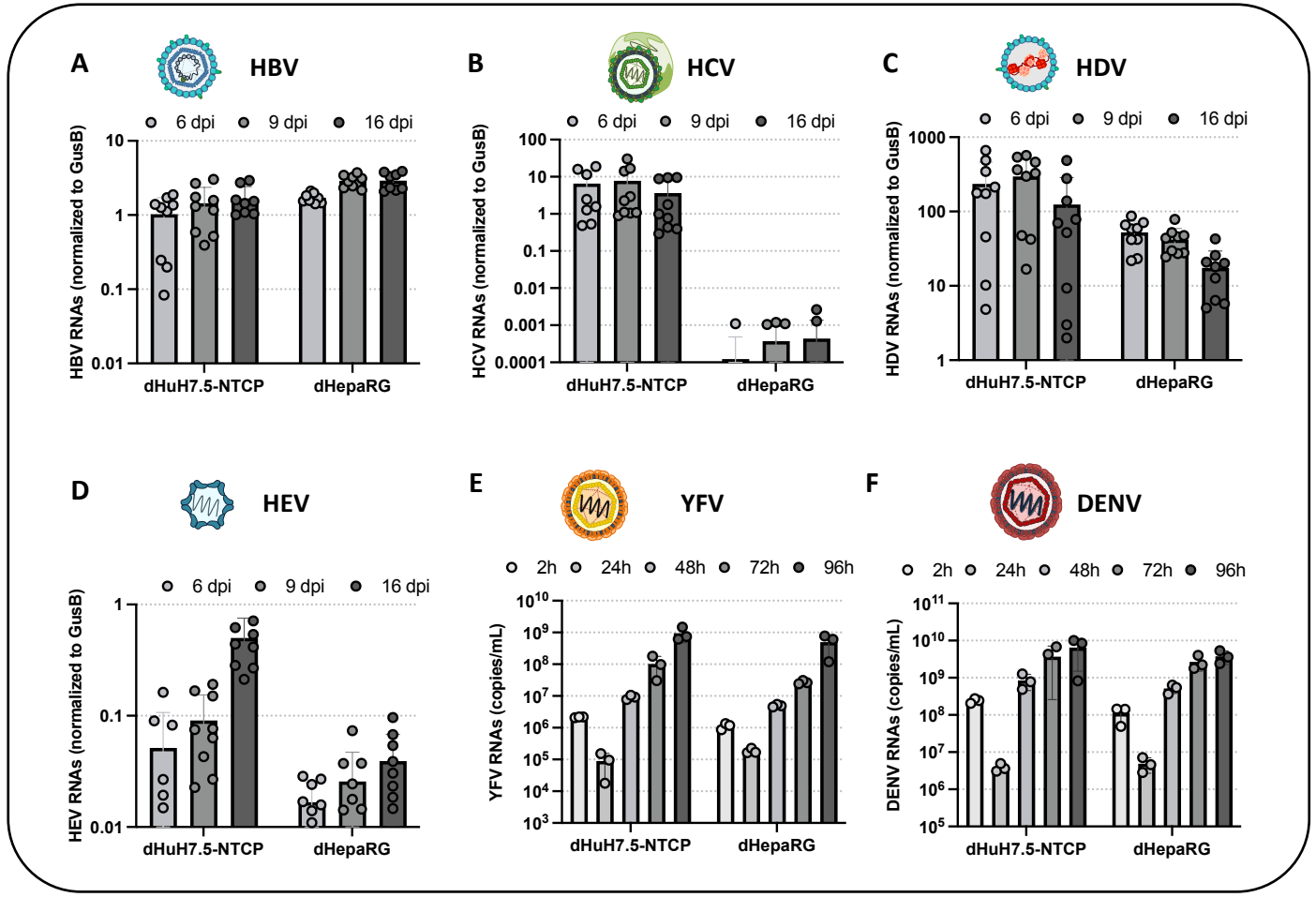
FRX Ligands

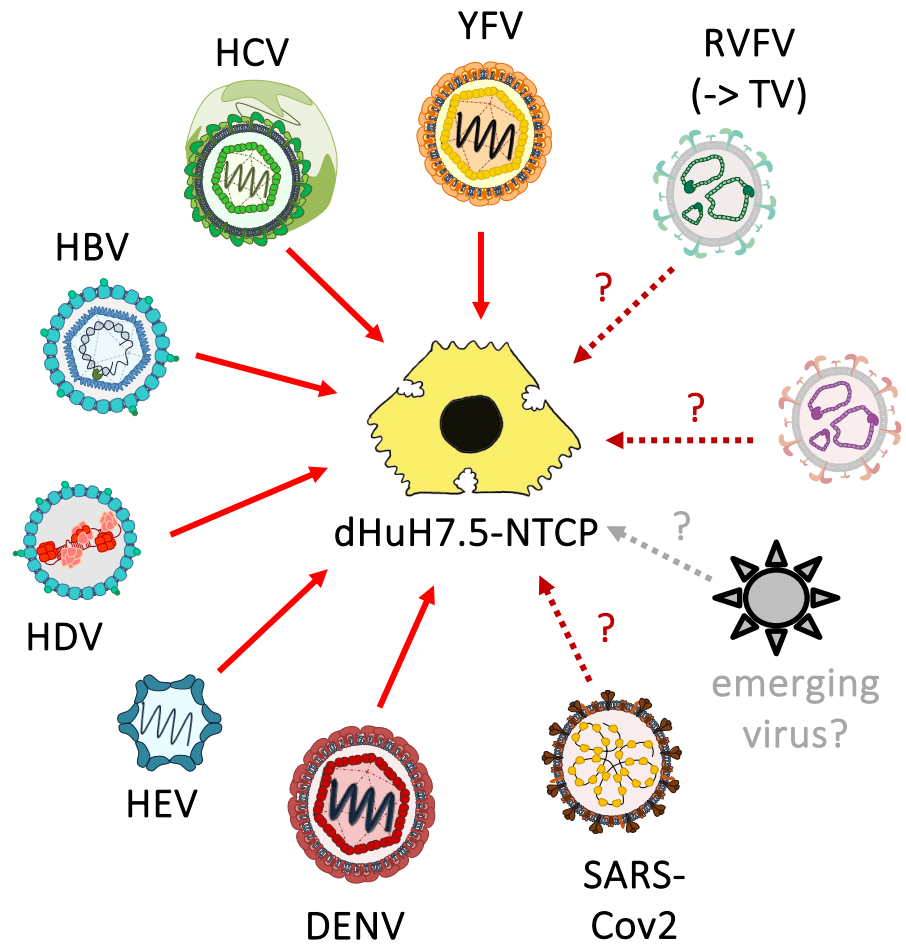


Development of a unique cell line allowing infection with all hepatotropic viruses



Development of a unique cell line allowing infection with all hepatotropic viruses

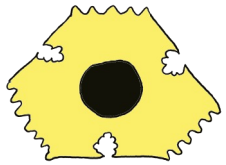




Collaboration with

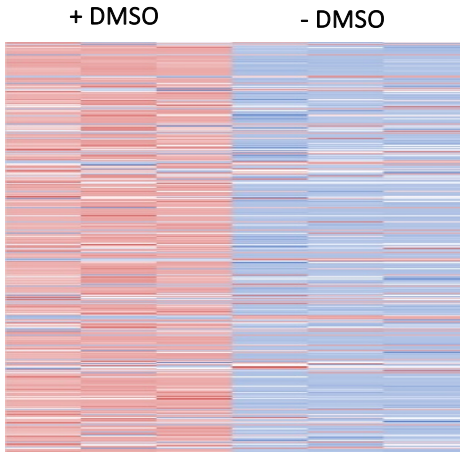
Fouillé *et al.*, in preparation

dHuH7.5-NTCP cells display key features of primary human hepatocytes

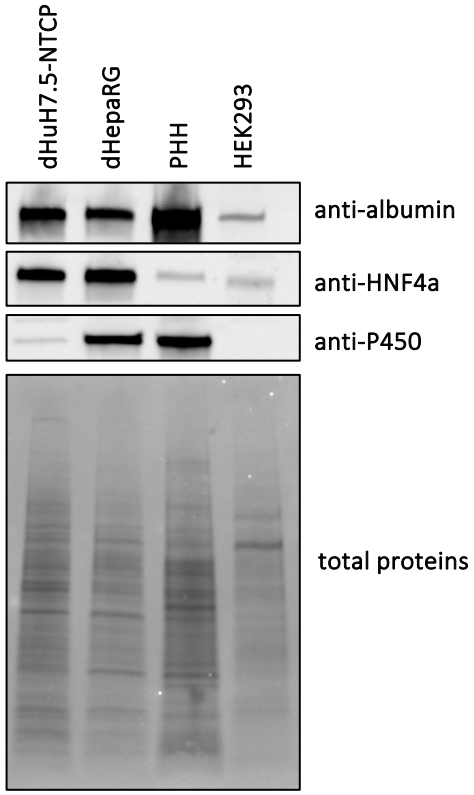


dHuH7.5-NTCP

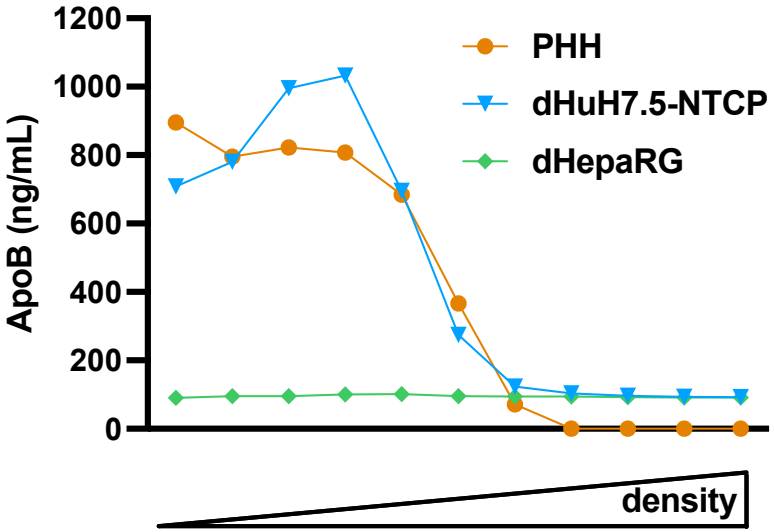
Liver specific genes expression



Liver specific protein expression

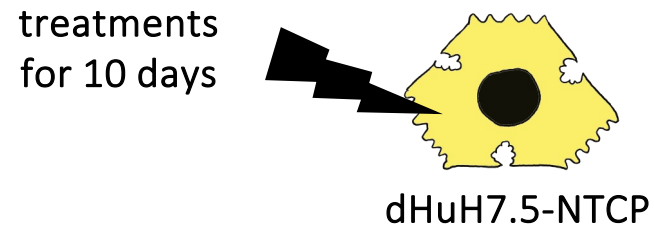


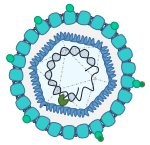
VLDL production



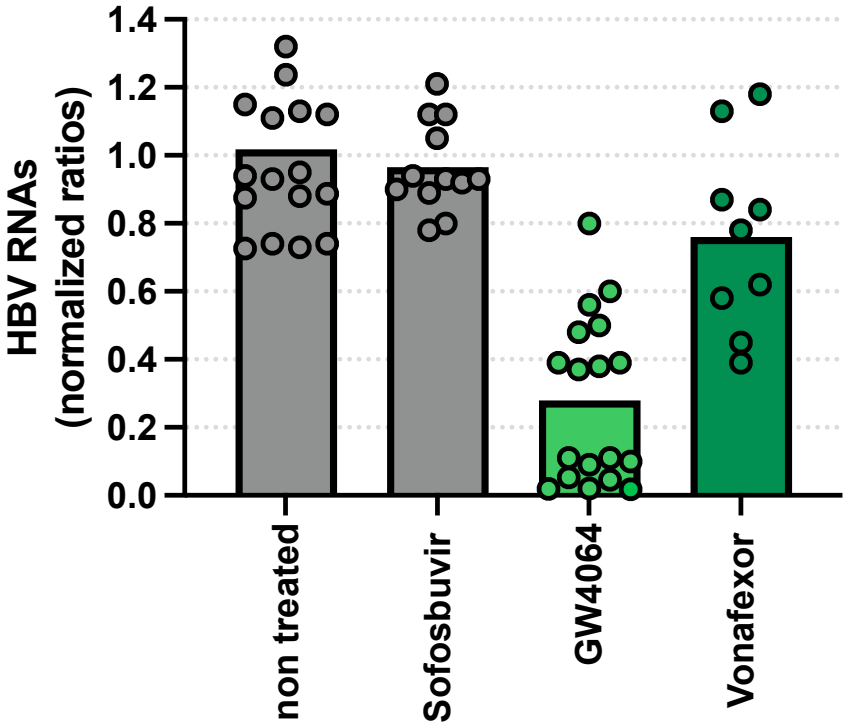
Fouillé *et al.*, in preparation

Confirmation of the antiviral effect of FXR ligands in HBV infected dHuH7.5-NTCP cells



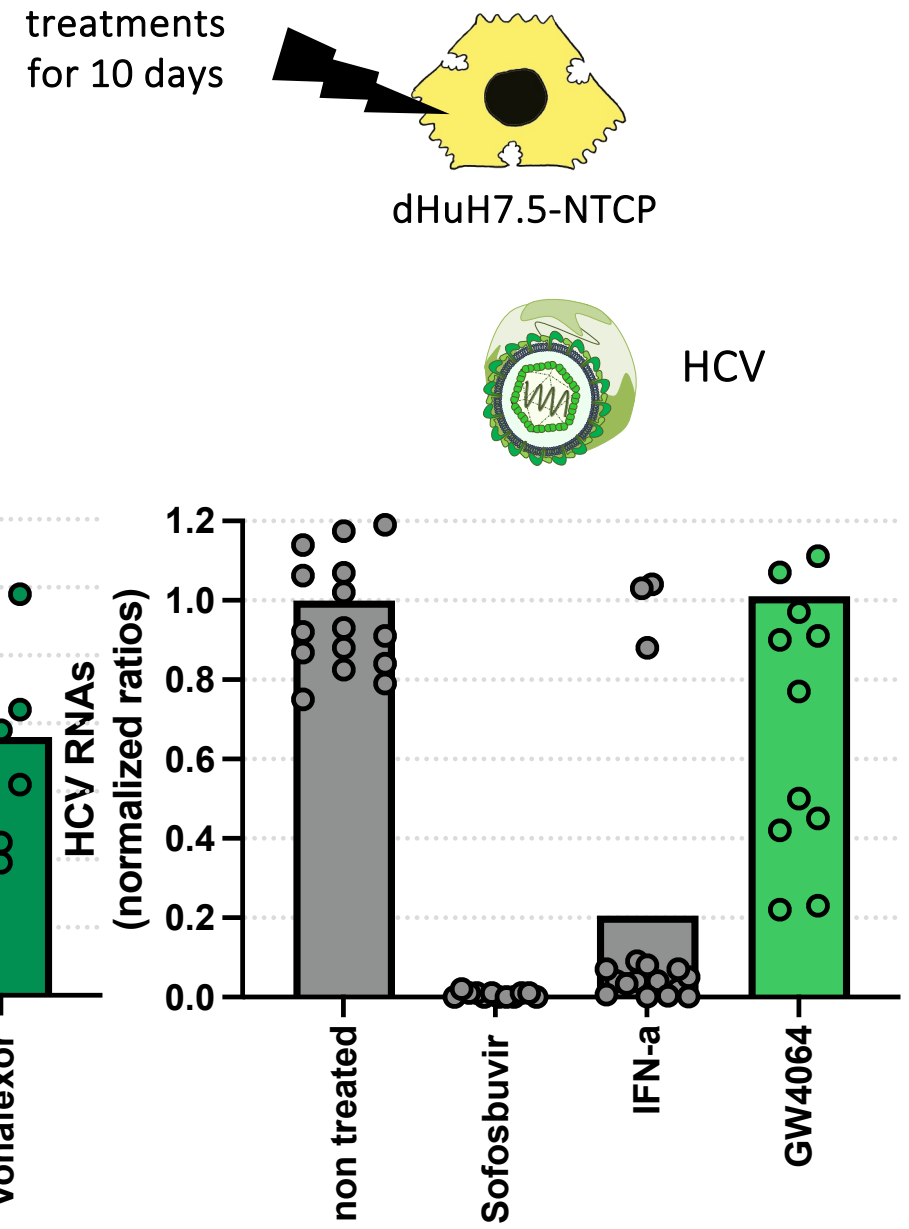


HBV



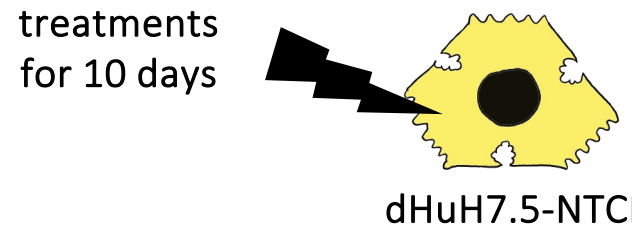
Fouillé *et al.*, in preparation

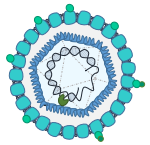
FXR ligands do not affect HCV infection in dHuH7.5-NTCP cells



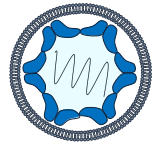
Fouillé *et al.*, in preparation

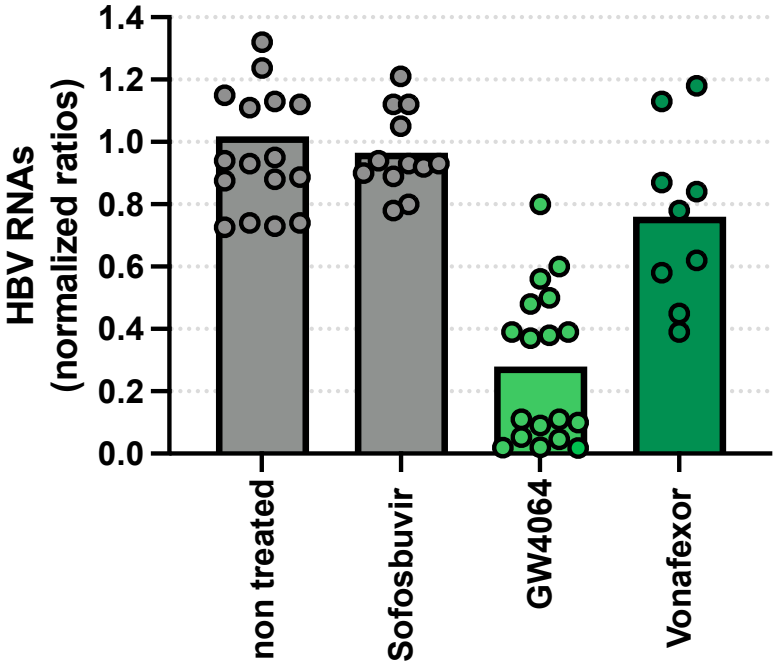
FXR ligands strongly inhibit HEV-3 infection in infected dHuH7.5-NTCP cells

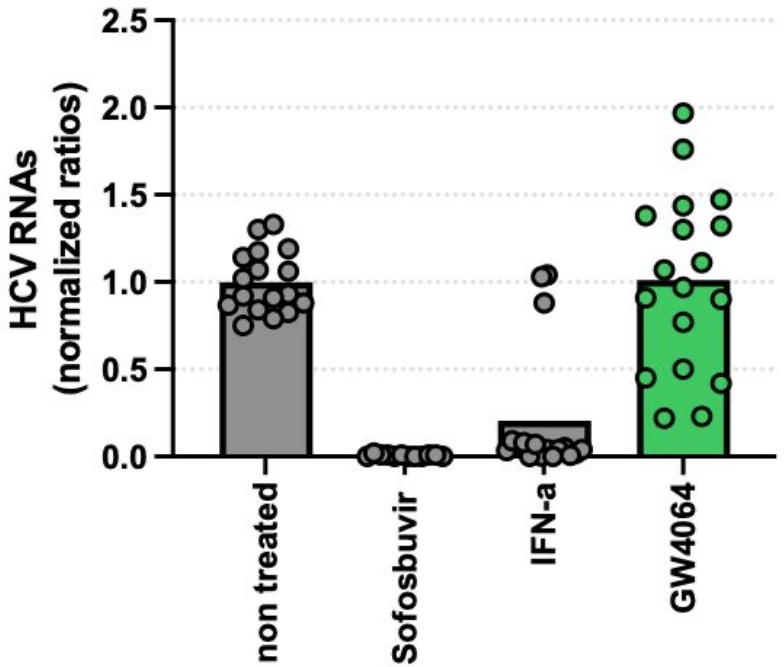


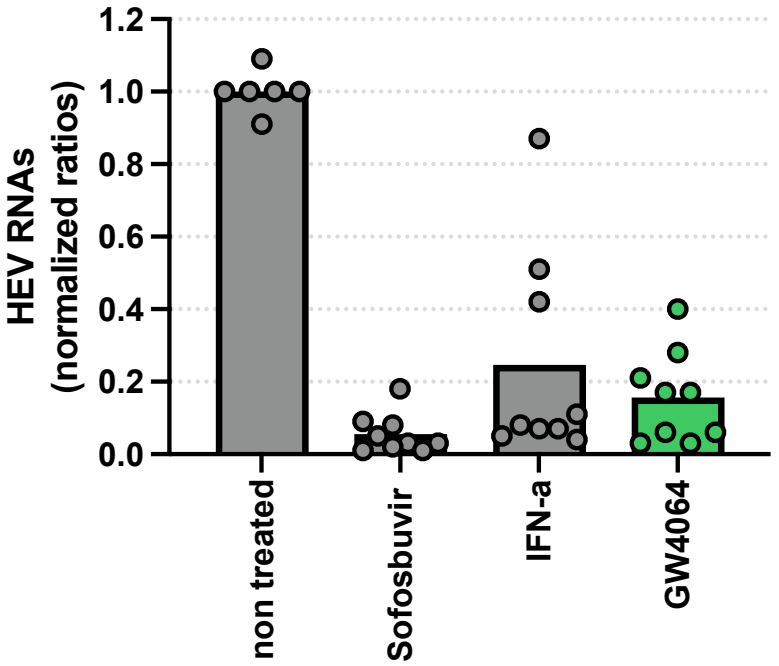

HBV


HCV


HEV-3



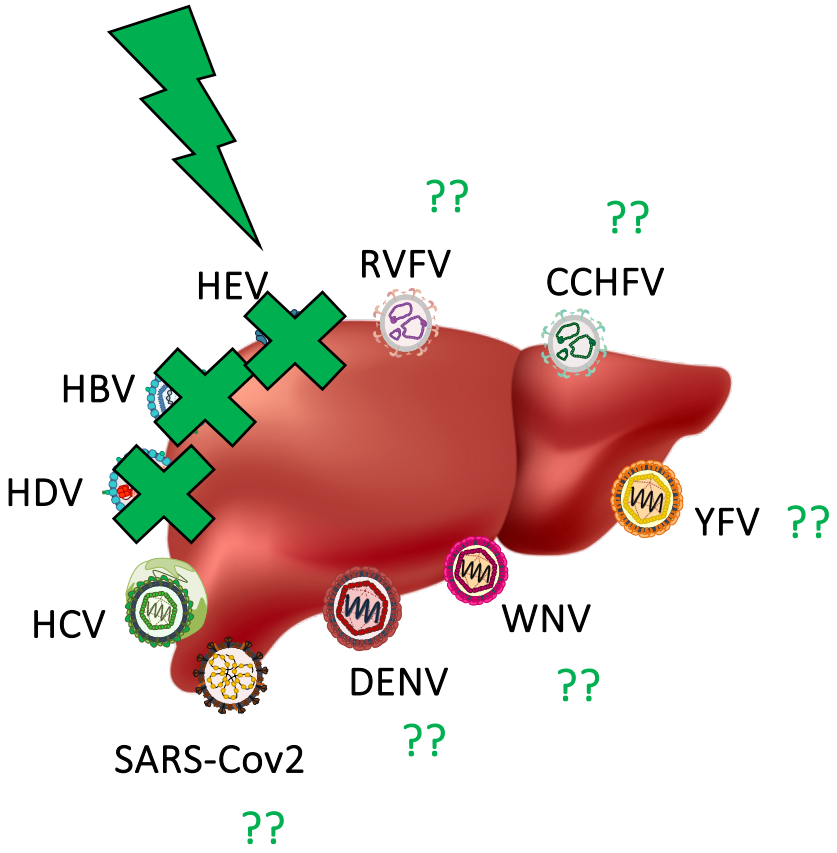




Fouillé *et al.*, in preparation

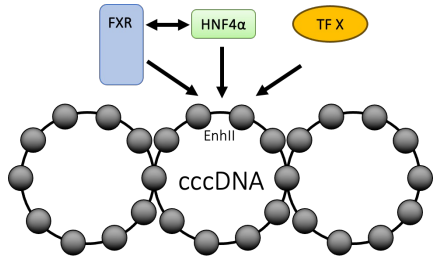
Conclusions: broad spectrum antiviral activity of FXR-ligand against hepatotropic viruses ?

FXR Ligands



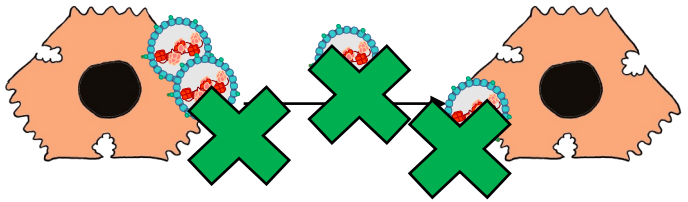
Main Mode of Action

HBV



- FXR-L combined to IFN-α inhibit HBV RNA synthesis through epigenetic modulation

HDV



- FXR-L strongly reduce HDV propagation

HEV

- to be determined

Acknowledgment

David Durantel
Julie Lucifora
Lorane Durimel
Inés Vincente-Navarro
Roxanne Fouillé

Raphaël Darteil
Jacky Vonderscher
Benoît De Chassey
Pietro Scalfaro

Philip Meuleman

Maud Michelet

PHH Platform
Fabien Zoulim
Michel Rivoire
Guillaume Passot
Anaëlle Dubois
Jennifer Molle
& all the team

Bettina Hansen