Medical Faculty Heidelberg

Molecular insights into the synergism between the HBV/HDV entry inhibitor Myrcludex B and Interferon

…blocking both, intrahepatic spread of HDV through *de novo* entry of virions (MyrcludexB) and mitosis-mediated cell to cell spread of genomes (IFNs)

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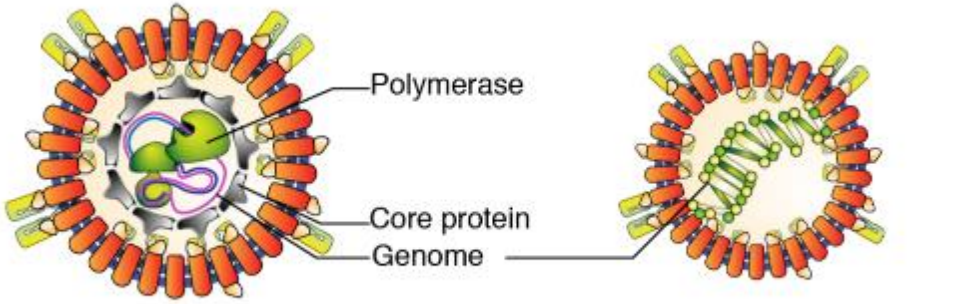
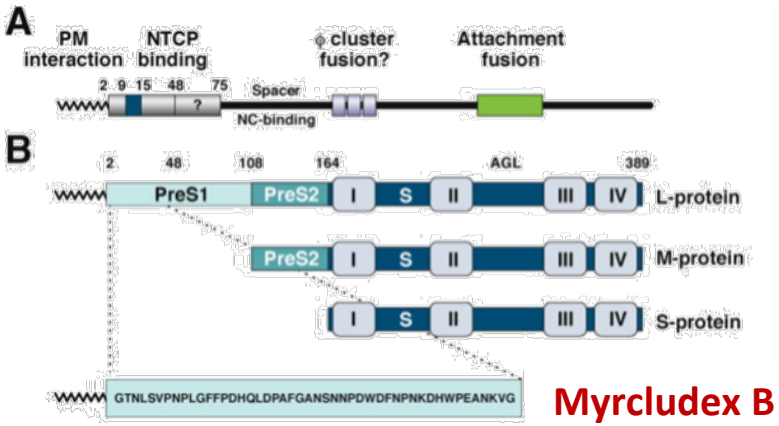
Myrcludex B a specific inhibitor of NTCP



Hepatitis B virion (42 nm) **Hepatitis D virion** (39 nm)



Myrcludex B

Urban et al., Gastroenterology 2014

Myrcludex B specifically binds to sodium taurocholate co-transporting polypeptide (NTCP) at the basolateral membrane of differentiated hepatocytes. ***(Ni et al., Gastroenterology 2014)***

Myrcludex B blocks HBV and HDV infection (IC_{50} 80 pM in PHH). ***(Schulze et al., J. Virology 2010)***

Myrcludex B exclusively hepatocytes in the liver. ***(Schieck et al., Hepatology 2013)***

HDV/HBV persistence of episomes in a chronically infected liver depend on *de novo* entry via NTCP. The Myr201 and Myr 202 study

HDV RNA can be propagated through mitosis of hepatocytes. Giersch et al., Gut, 2017, Ni et al., unpublished

The turnover rates of HBV- and HDV-infected hepatocytes is crucial for efficacy of Myrcludex B.

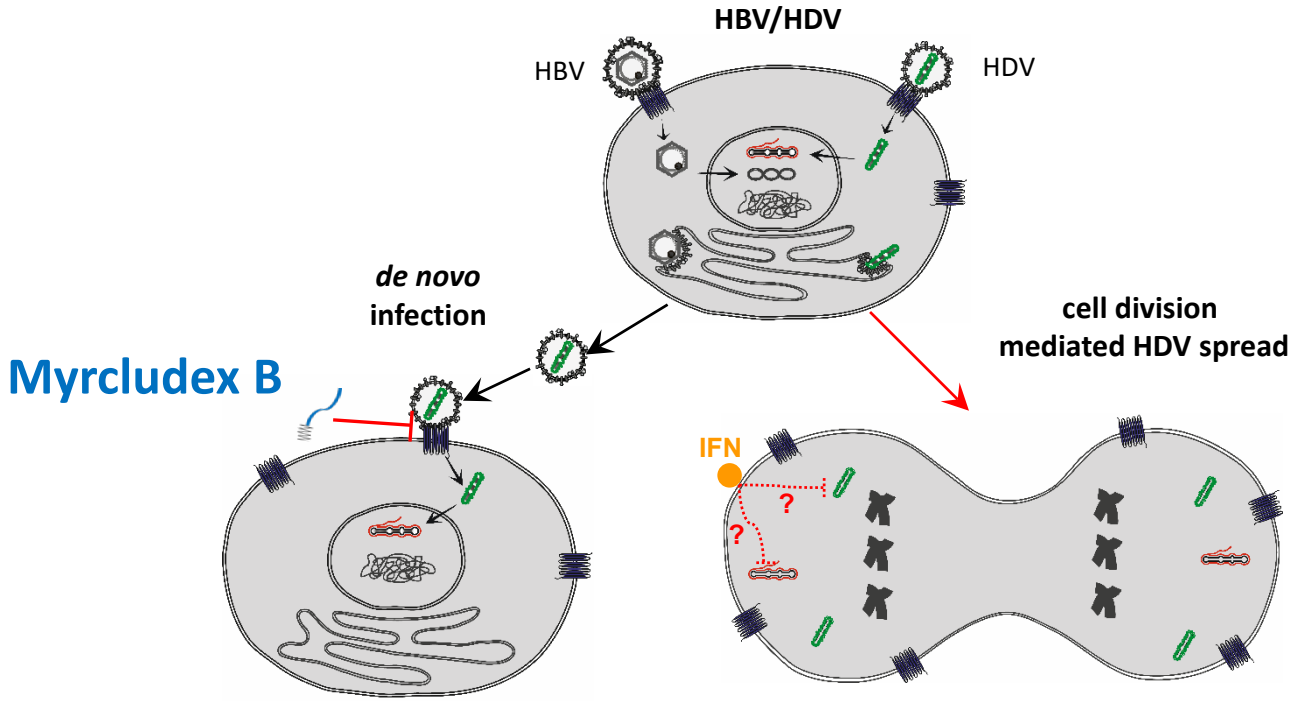
Intrahepatic spread of HDV and persistence

Aim of HDV Therapy: Suppression or elimination of HDV replication in the **liver (!)** of CHD patients

HDV persists…

- …for years in patients in the presence of low levels of HBV replication.
- …for > 24 weeks of Myrcludex B treatment in patients.
- …for at least 6 weeks in mono-infected humanized mice.

Pollicino T, *et al*. J Virol 2011.; Schaper M, *et al*. J Hepatol 2010; Mederacke I, *et al*. J Hepatol 2012; Samuel D, *et al*. Hepatology 1995; Giersch K, *et al*. J Hepatol 2014; Allweiss L, *et al*. J Hepatol 2018.



What's the contribution of extracellular and intracellular spread and how can this be counteracted by drugs?

Final results of a multicenter, open-label phase 2b clinical trial to assess safety and efficacy of Myrcludex B in combination with Tenofovir in patients with HBV/HDV coinfection

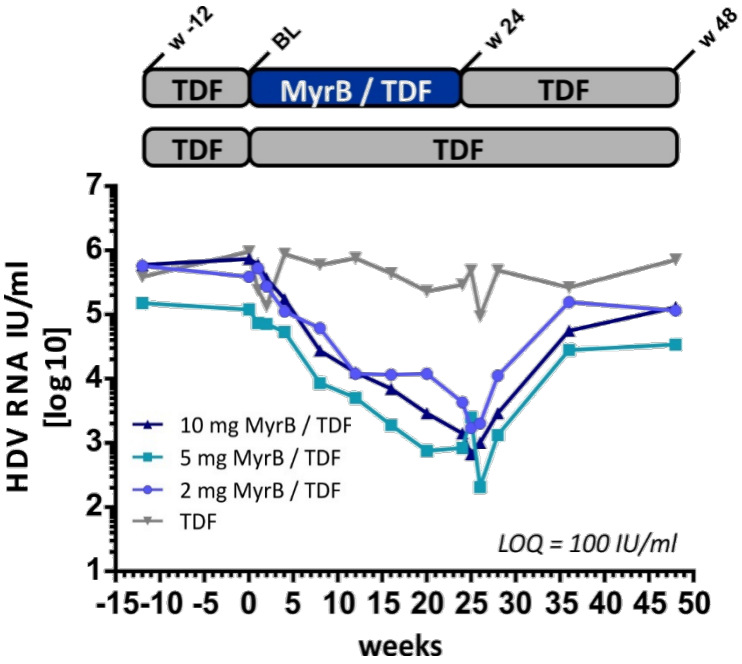
Heiner Wedemeyer[1],
Pavel Bogomolov[2], Antje Blank[3,4], Lena Allweiss[5,6], Maura Dandri-Petersen[5,6], Birgit Bremer[1], Natalia Voronkova[2], Katrin Schöneweis[4,7,11], Anita Pathil[8], Jürgen Burhenne[3,4], Mathias Haag[9,10], Matthias Schwab[9,10], Walter E. Haefeli[3], Julian Schulze zur Wiesch[5,6], Alexander Alexandrov[11], Stephan Urban[4,7]

[1]Hannover Medical School/Essen University Hospital, [2]Moscow Regional Research Clinical Institute,[3]University Hospital Heidelberg, Dept. of Clinical Pharmacology, [4]German Center for Infection Research (DZIF) Heidelberg, [5,]University Medical Center Hamburg-Eppendorf, [6]DZIF Lübeck-Borstel, [7]University Hospital Heidelberg, Dept. of Infectious Diseases,[8]Internal Medicine IV, Heidelberg,[9]Dr. Margarete Fischer-Bosch-Institute, Stuttgart, [10]DZIF Tübingen, [11]MYR GmbH

ILC, Paris, 2018

MyrB monotherapy induces profound reductions of HDV serum and liver RNA and the elimination of HDV infected cells in the liver

The Myr202-trial



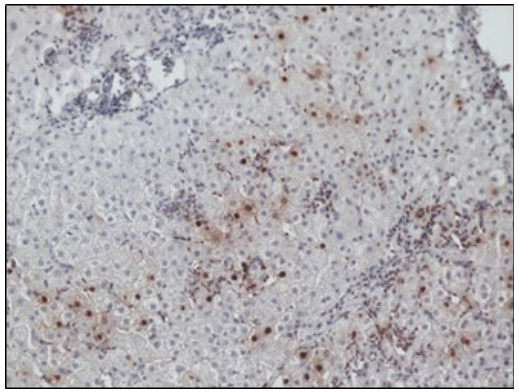
Median RNA log10 change to BL at week 24

MyrB 2mg: -1.75 **MyrB 10mg: -2.70**

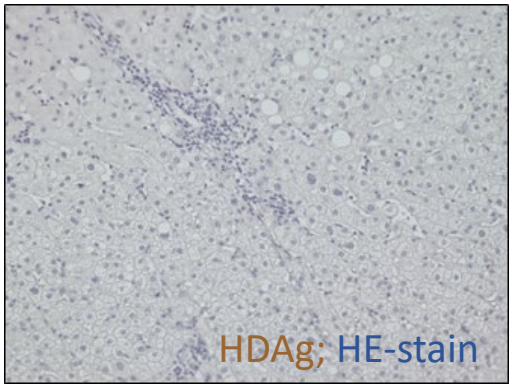
MyrB 5mg: -1.60 TDF: -0.18

Plasma HDV RNA decline correlated with a decrease of intrahepatic HDV RNA *(Allweiss et al., unpublished)*

HDAg at baseline



HDAg at week 24



HDV infected cells are eliminated during Myrcludex B therapy

Blocking only the extracellular route of HDV spread results in 500-fold reduction of HDV RNA within 24 weeks

⇒ Rapid turnover (days, not months) of HDV infected hepatocytes

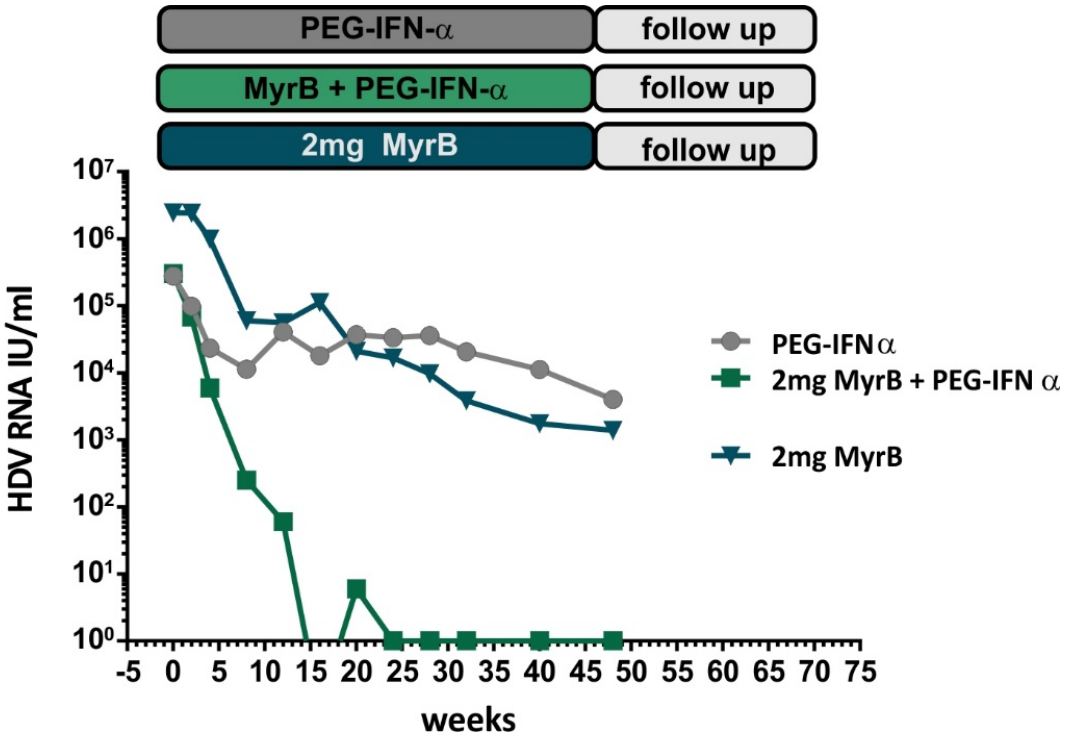
Interim results of a multicenter, open-label phase 2 clinical trial (MYR203) to assess safety and efficacy of Myrcludex B in combination with PEG-IFNα in patients with chronic HBV/HDV co-infection

Heiner Wedemeyer,
Katrin Schöneweis, Pavel Bogomolov, Natalia Voronkova, Vladimir Chulanov,Tatyana Stepanova, Birgit Bremer, Patrick Lehmann, Regina Raupach, Lena Allweiss, Maura Dandri, Sandra Ciesek, Ulf Dittmer, Walter E. Haefeli, Alexander Alexandrov and Stephan Urban

AASLD, San Francisco, 2018

MyrB/IFNa combination show synergistic antiviral effects on HDV

The Myr203-trial

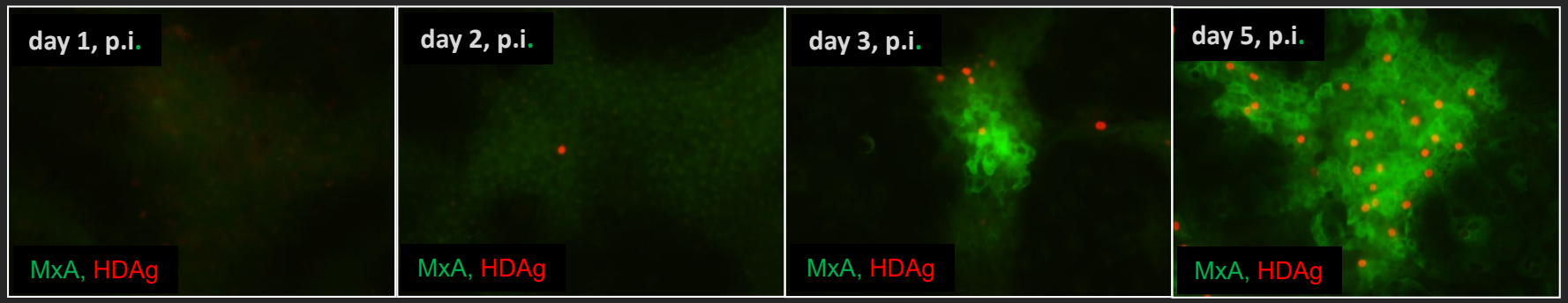


Final results with follow up data in plenary III: GS-013

How can the strong synergistic effect between Myrcludex B and IFNα on HDV RNA be explained ?

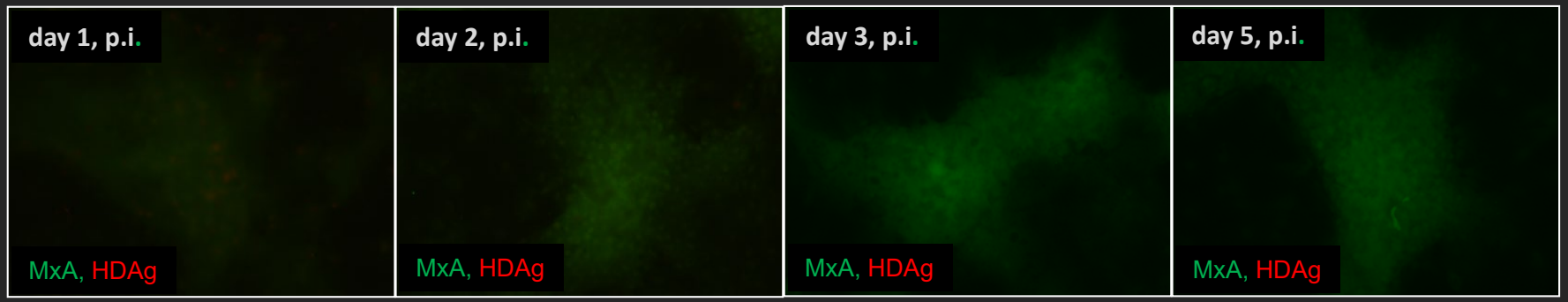
HDV infection induces an IFN response in HepaRG cells

Time course of HDV infection and expression of IFN-induced MxA in the absence …..



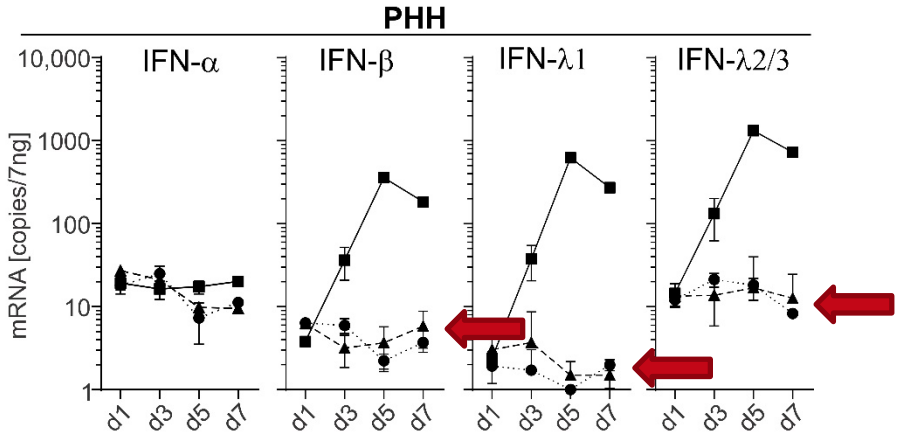
Zhang, et al. J. Hepatology, 2018

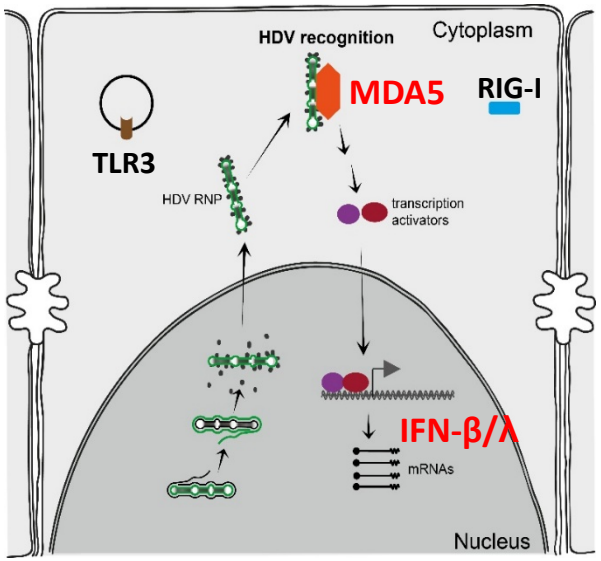
……and in the presence of the entry inhibitor Myrcludex B



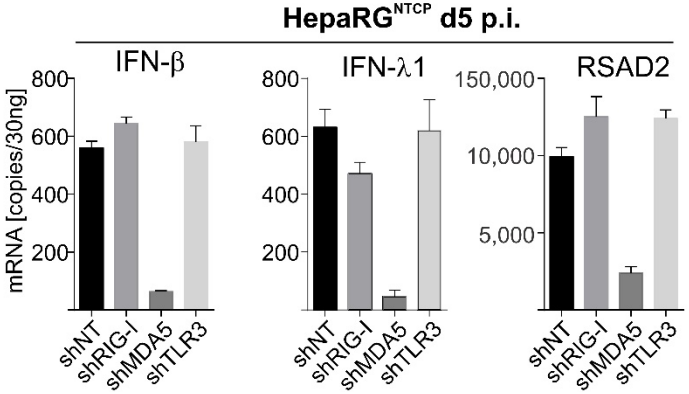
- HDV infection of HepaRG cells induces ISGs responses following HDV infection
- Myrcludex B inhibits de novo induced HDV IFN responses

The PRR MDA5 selectively senses HDV replication and mediates induction of IFN-ß and λ



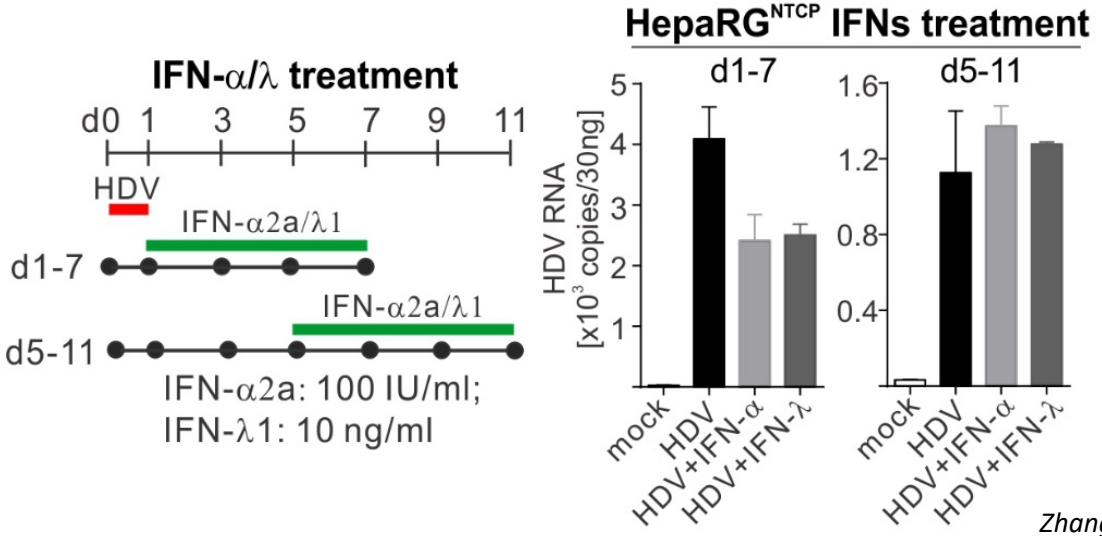


- HDV infection activates profound IFN-β/λ responses in primary human hepatocytes
- Myrcludex B suppresses IFN responses induced by de novo infection
- MDA5 (not TLR3 or RIG-I) is the key PRR sensing HDV replication

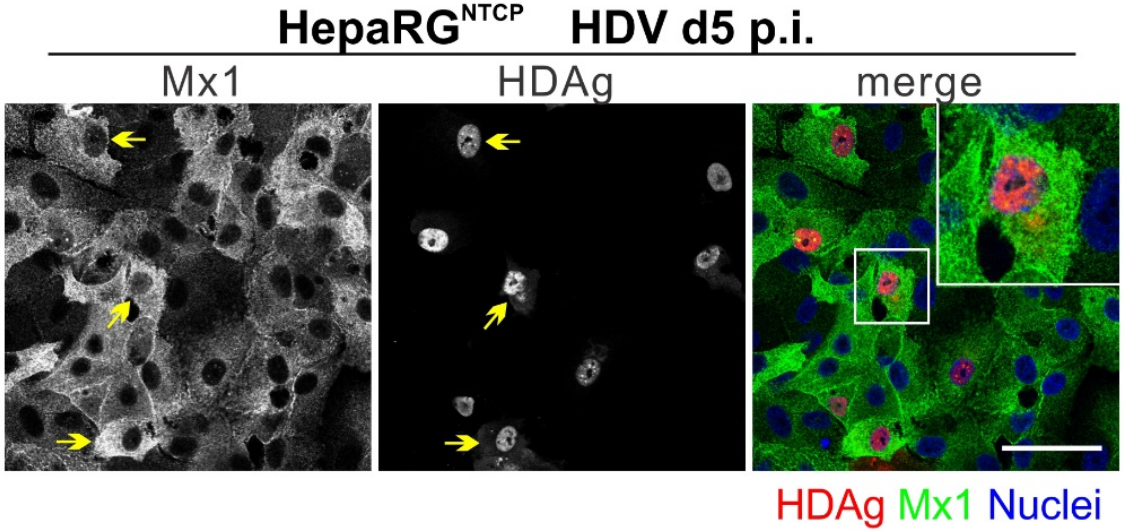


Zhang, *et al*. J Hepatol. 2018.

Exogenous IFN cannot abrogate HDV replication in hepatocytes



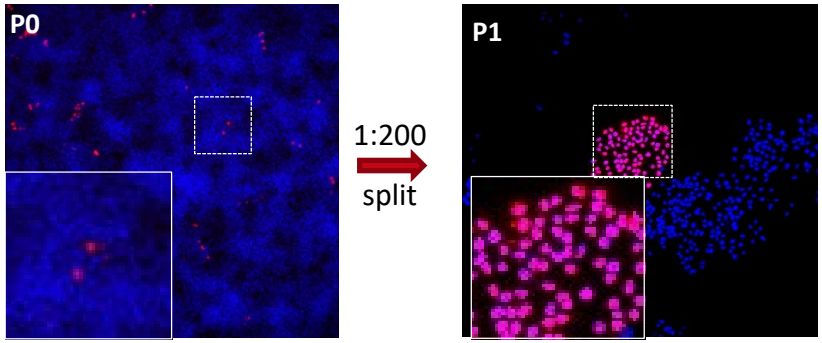
Zhang, et al. J. Hepatology, 2018

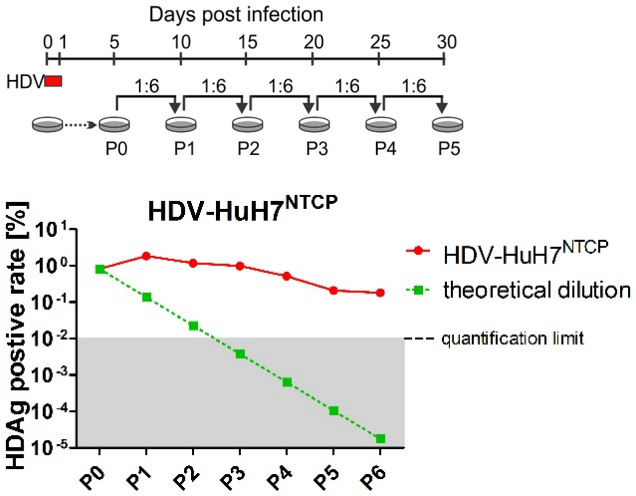


HDV replication is insensitive to IFN-alpha and IFN-lambda treatment in resting hepatocytes

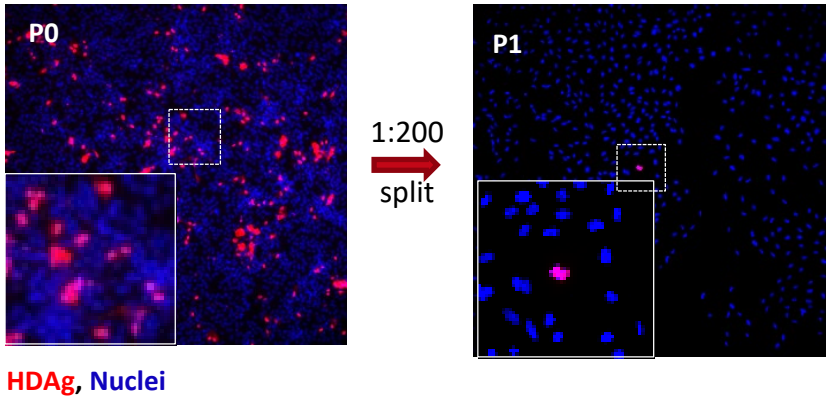
HDV spreads through cell division: spread is controlled by endogenous innate immune responses

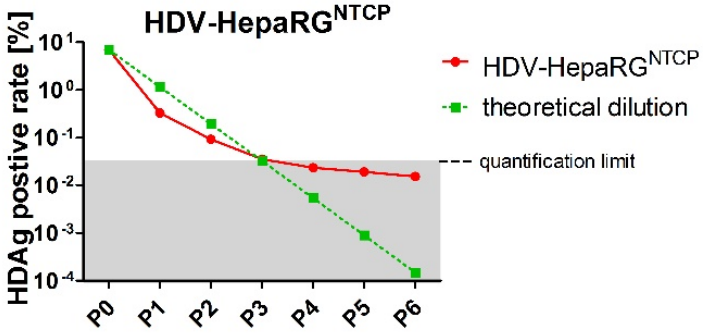
HDV infection of HuH7^{NTCP} (**deficient** for **IFN** activation)





HDV infection of HepaRG^{NTCP} (**IFN competent**)

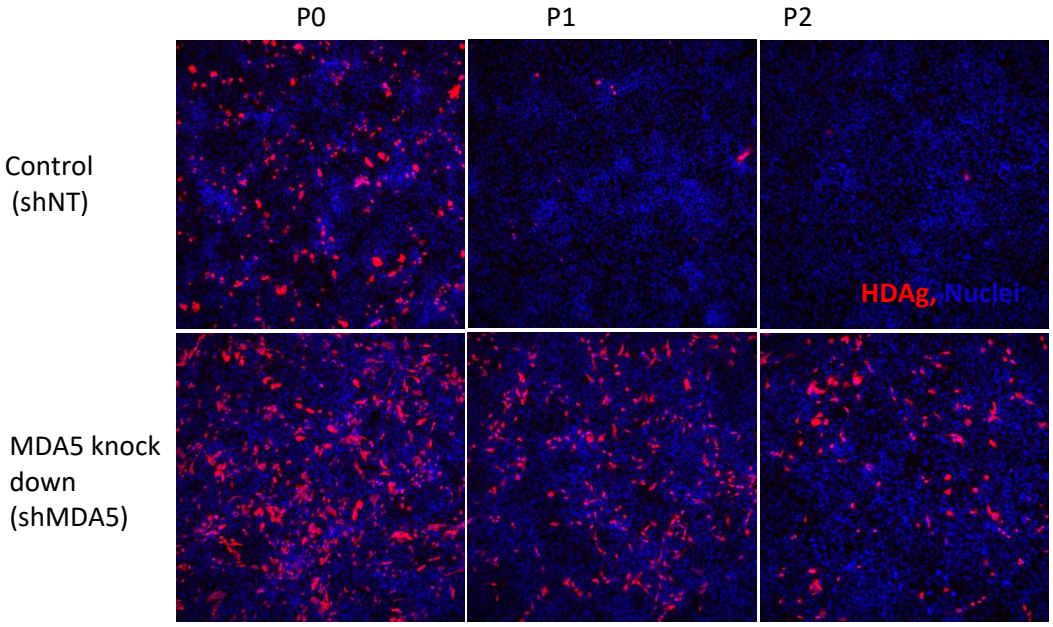


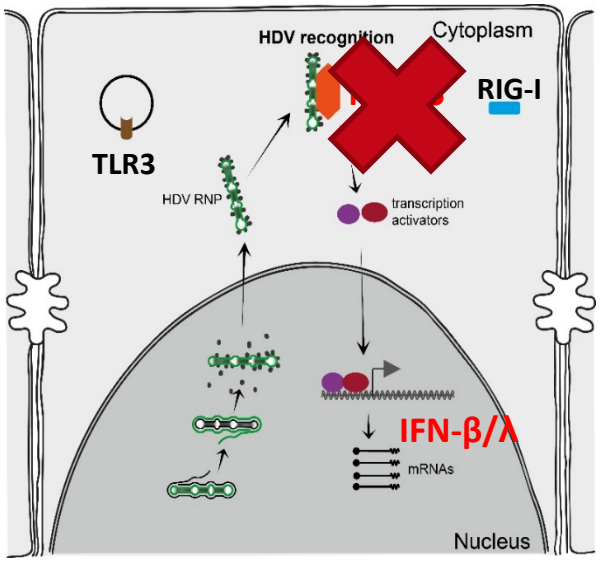


HDV cell to cell spread (no extracellular route) is restricted in IFN-competent cells (PHH)

Knock down of MDA5 in IFN competent cells allows unrestricted HDV spread

Passaging of HDV-infected HepaRG^{NTCP} cells



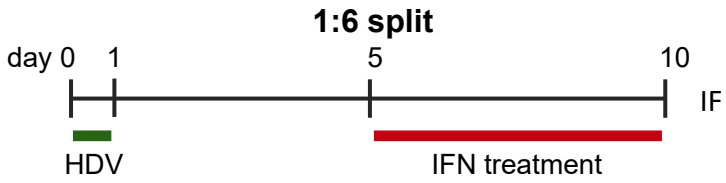


Clinical implications

MDA5 is an important host factor controlling intrahepatic HDV replication (a role for MDA5 polymorphisms in fulminat HDV ?)

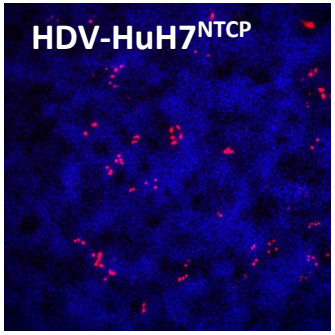
The strength of the endogenously HDV-induced IFN response influences virus spread and probably the responsiveness to IFN-therapy

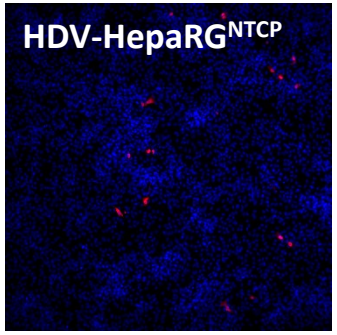
Exogenous IFN treatment suppresses HDV spread during cell division



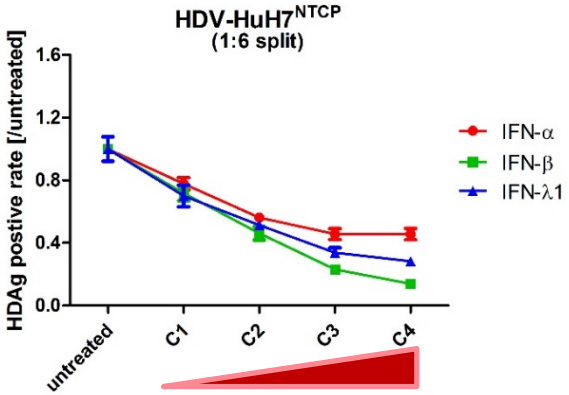
	IFN-α (IU/ml)	IFN-β (ng/ml)	IFN-λ1 (ng/ml)
C1	4	0.01	0.4
C2	20	0.05	2
C3	100	0.25	10
C4	500	1.25	50

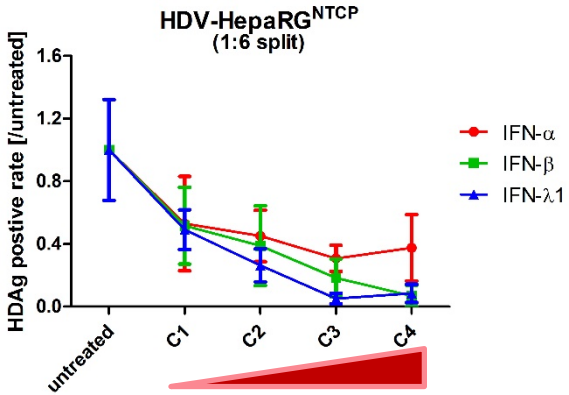
Initial infection
day 5 p.i





HDAg, Nuclei





IFN-α, IFN-β, and IFN-λ profoundly suppress HDV cell to cell spread

Poster: SAT-202

Endogenous and exogenous IFN responses suppress HDV persistence during proliferation of hepatocytes *in vitro*

Z. Zhang[1], T. Walther[1], FA. Lempp[1], Y. Ni[1,2] and S. Urban[1,2]

1 Department of Infectious Diseases, Molecular Virology, University Hospital Heidelberg, Germany;
2 German Center for Infection Research (DZIF) - Heidelberg Partner Site, Heidelberg, Germany.

1 INTRODUCTION

1.1 HBV/HDV infection and IFN response

- Chronic HBV and HDV co-infections cause the most severe form of viral hepatitis.
- The long-term persistence of HBV/HDV makes it challenging to develop curative therapies.
- HDV needs HBV envelope proteins for assembly and secretion.
- HDV genome replicates in the nucleus of hepatocytes.
- HDV RNA replication is sensed by innate immune sensor MDA5 and induces profound IFN-β/λ response [1].

1.2 HDV spread pathways

- Extracellular HDV spread:
  HBV envelope proteins are needed for progeny virus.
  Sensitive to the entry inhibitor Myrcludex B (MyrB) [2].
- Cell division mediated HDV spread [3]:
  Independent of HBV envelope proteins.
- Both pathways are supposed to contribute to HDV persistence in patients.

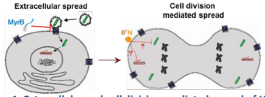


Figure 1. Extracellular and cell division mediated spread of HDV

2 AIM

- Characterize cell division mediated HDV spread in cell culture models.
- Evaluate the role of IFN responses in cell division mediated HDV spread.
- Measure possible synergism of investigational drugs (MyrB, Lonafarnib and IFNs) against HDV spread.

3 METHOD

- Susceptible cells were infected with HDV and split (1:6) at day 5 post infection and further split every 5 days.
- Blockade of IFN responses was done by shRNA mediated depletion of MDA5 or inhibitor targeting JAK1/2.
- Exogenous IFN-α/-β/-λ were applied HDV infected cells.
- Immunofluorescence and ImageJ were used for HDV antigen (HDAg) positive quantification and RT-qPCR for HDV RNA.
- HuH7^{NTCP}-HB2.7 cells expressing viral receptor NTCP and HBV envelope proteins were infected with HDV and seeded at low density after infection to support both extracellular and cell division mediated HDV spread.

4 RESULTS

Cell division mediated HDV spread is restricted in innate immune competent HepaRG^{NTCP} cells

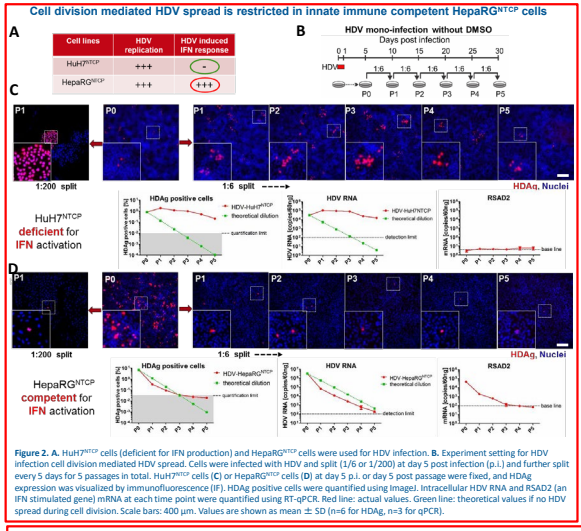


Figure 2. A. HuH7^{NTCP} cells (deficient for IFN production) and HepaRG^{NTCP} cells were used for HDV infection. **B.** Experiment setting for HDV infection cell division mediated HDV spread. Cells were infected with HDV and split (1/6 or 1/200) at day 5 post infection (p.i.) and further split every 5 days for 5 passages in total. HuH7^{NTCP} cells (**C**) or HepaRG^{NTCP} cells (**D**) at day 5 p.i. or day 5 post passage were fixed, and HDAg expression was visualized by immunofluorescence (IF). HDAg positive cells were quantified using ImageJ. Intracellular HDV RNA and RSAD2 (an IFN stimulated gene) mRNA at each time point were quantified using RT-qPCR. Red line: actual values. Green line: theoretical values if no HDV spread during cell division. Scale bars: 400 µm. Values are shown as mean ± SD (n=6 for HDAg, n=3 for qPCR).

depletion of MDA5 or inhibition of JAK1/2 signalling enable efficient cell division mediated HDV spread in HepaRG^{NTCP} cells

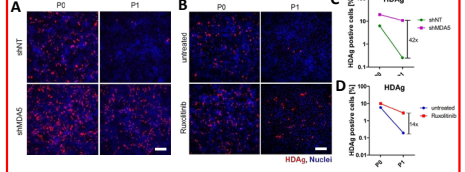


Figure 3. A. HepaRG^{NTCP}-derived cell lines expressing shRNA against MDA5 (shMDA5) and an non-targeting control (shNT) were infected with HDV and split (1/6) at day 5 p.i. HDAg expression at day 5 p.i. (P0) and day 5 post passage (P1) was visualized by IF, and the percentage of HDAg positive cells is shown in **C** (n=6). **B.** HDV infected HepaRG^{NTCP} cells were split (1/6) at day 5 p.i. Ruxolitinib (JAK1/2 inhibitor) was added into the medium from day 1 p.i. on. The percentage of HDAg positive cells is shown in **D** (mean, n=6). Scale bars: 400 µm.

IFN-α, IFN-β and IFN-λ1 suppress cell division mediated HDV spread

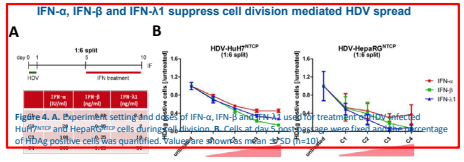


Figure 4. A. Experiment setting and doses of IFN-α, IFN-β and IFN-λ1 used for treatment of HDV infected HuH7^{NTCP} and HepaRG^{NTCP} cells during cell division. **B.** Cells at day 5 post passage were fixed, and the percentage of HDAg positive cells was quantified. Values are shown as mean ± SD (n=10).

Effect of IFN-α and investigational drugs (MyrB, Lonafarnib and IFN-λ1) in a cell culture system supporting both extracellular and cell division mediated HDV spread

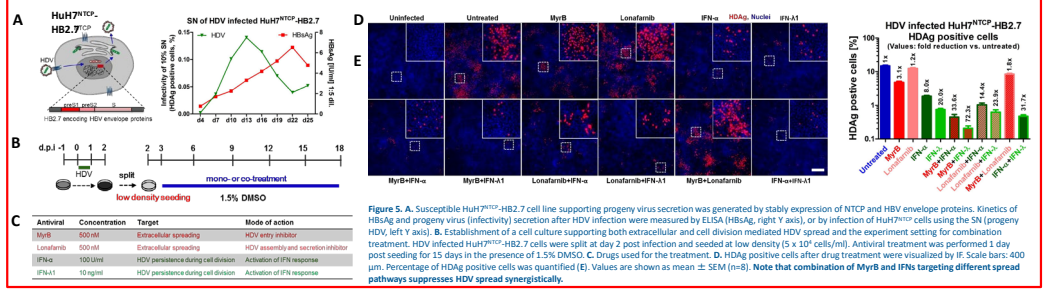


Figure 5. A. Susceptible HuH7^{NTCP}-HB2.7 cell line supporting progeny virus secretion was generated by stably expression of NTCP and HBV envelope proteins. Kinetics of HBsAg and progeny virus (infectivity) secretion after HDV infection were measured by ELISA (HBsAg, right Y axis), or by infection of HuH7^{NTCP} cells using the SN (progeny HDV, left Y axis). **B.** Establishment of a cell culture supporting both extracellular and cell division mediated HDV spread and the experiment setting for combination treatment. HDV infected HuH7^{NTCP}-HB2.7 cells were split at day 2 post infection and seeded at low density (5 x 10^5 cells/ml). Antiviral treatment was performed 1 day post seeding for 15 days in the presence of 1.5% DMSO. **C.** Drugs used for the treatment. **D.** HDAg positive cells after drug treatment were visualized by IF. Scale bars: 400 µm. Percentage of HDAg positive cells was quantified (**E**). Values are shown as mean ± SEM (n=8). **Note that combination of MyrB and IFNs targeting different spread pathways suppresses HDV spread synergistically.**

5 CONCLUSIONS

Conclusions:

- IFN responses profoundly suppress cell division mediated HDV spread.
- Combination treatment with MyrB and IFN-α/-λ1 blocking both extracellular and cell division mediated spread pathways suppresses HDV spread synergistically *in vitro*.

Clinical implication:

- This study helps to understand the clinical observation of the Myr-203 study demonstrating a strong synergism of combining IFN-α and the entry inhibitor MyrB [4] and Wedemeyer H, GS-13.
- The system provides a cell culture model for the identification of novel synergistically acting immune modulators for future clinical combination therapies.

6 ACKNOWLEDGEMENTS

- This work received funding by DZIF, TTU Hepatitis (Project 5.807 and 5.704) and the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Projektnummer 272983813 – TRR 179 and Projektnummer 240245660 – SFB 1129
- We are grateful to Dr. Camille Sureau for providing material. We thank the members of Urban's lab for assistance, Prof. Ralf Bartenschlager for continuous support and Drs. Volker Lohmann, Alessia Ruggieri, Macro Binder and other members in the department of infectious diseases in University Hospital Heidelberg for useful discussions.

7 REFERENCES

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2. **Lütgehetmann M, et al.** Humanized chimeric uPA mouse model for the study of hepatitis B and D virus interactions and preclinical drug evaluation. *Hepatology. 2012 Mar;55(3):685-94.*
3. **Giersch K, Bhadra OD, *et al.*** Hepatitis delta virus persists during liver regeneration and is amplified through cell division both in vitro and in vivo. *Gut. 2019; 68(1):150-157.*
4. **Wedemeyer H, *et al.*** Interim Results of a Multicentre, Open-Label Phase 2 Clinical Trial (MYR203) to Assess Safety and Efficacy of Myrcludex B in Combination with Peg-Interferon Alpha 2a in Patients with Chronic HBV/HDV Co-Infection. *https://aasldpubs.onlinelibrary.wiley.com/doi/10.1002/hep.30256.*

8 CONTACT

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Conclusions

- Persistence of HDV in the liver requires extracellular (Myrcludex B sensitive) and cell to cell-mediated (IFN-sensitive) replenishment pathways.
- HDV infected hepatocytes have a very short half life time.
- Addressing both routes (either directly or indirectly) will result in strong synergisms.
- Since non of the developmental drugs target HDV RNA directly it may be difficult to eradicate HDV as long as HBsAg is expressed (from cccDNA or integrates).
- This may require long term (indefinite) treatment with drugs that repress HDV replication in the liver