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MOLECULAR VIROLOGY HEIDELBERG

HDV and the Interferon Response

Synergistic suppression of HDV persistence *in vitro* by co-treatment with investigational drugs targeting both extracellular and cell-division-mediated spreading pathways

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Disclosures

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Patent holder and inventor on patents protecting Myrcludex B

HDV and the IFN-response

Gastroenterology 2018;154:1791–1804

HBV Bypasses the Innate Immune Response and Does Not Protect HCV From Antiviral Activity of Interferon

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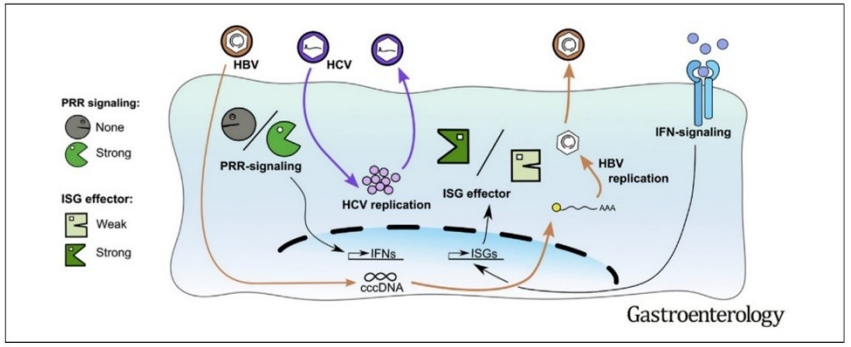
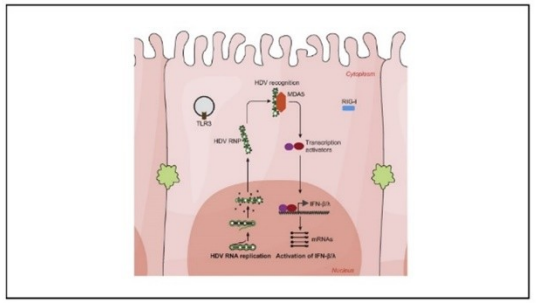


Research Article
Viral Hepatitis

JOURNAL OF HEPATOLOGY

Hepatitis D virus replication is sensed by MDA5 and induces IFN-β/λ responses in hepatocytes

Graphical abstract



Highlights

- In contrast to HBV, HDV activates the IFN response in hepatocytes.
- MDA5 is the key pattern recognition receptor sensing HDV replication.
- HDV replication is insensitive to the MDA5-mediated self-induced IFN response.
- IFN treatment doesn't abolish intracellular HDV replication *in vitro*.

Authors

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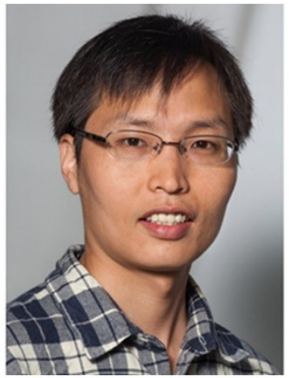
Lay summary

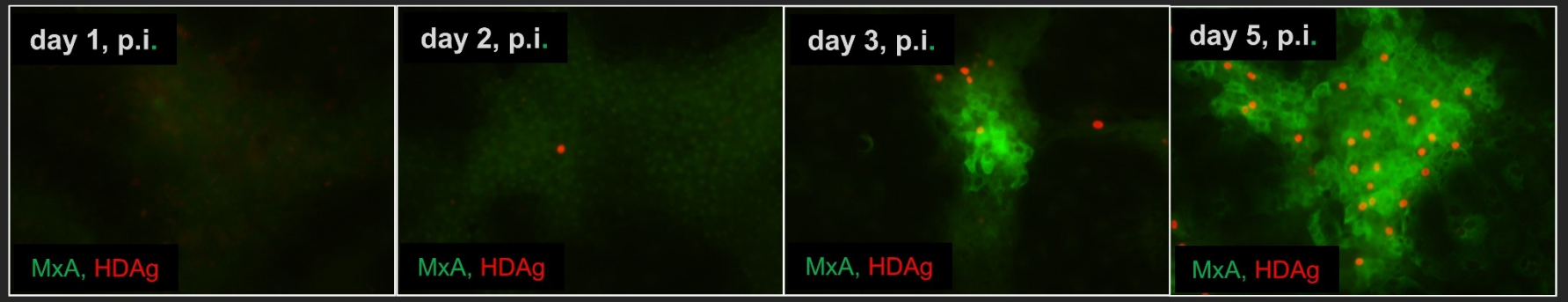
In contrast to hepatitis B virus, infection with hepatitis D virus induces a strong IFN-β/λ response in innate immune competent cell lines. MDA5 is the key sensor for the recognition of hepatitis D virus replicative intermediates. An IFN-activated state did not prevent hepatitis D virus replication *in vitro*, indicating that hepatitis D virus is resistant to self-induced innate immune responses and therapeutic IFN treatment.



HBV is a stealth virus while HDV induced profound IFN-β/λ responses

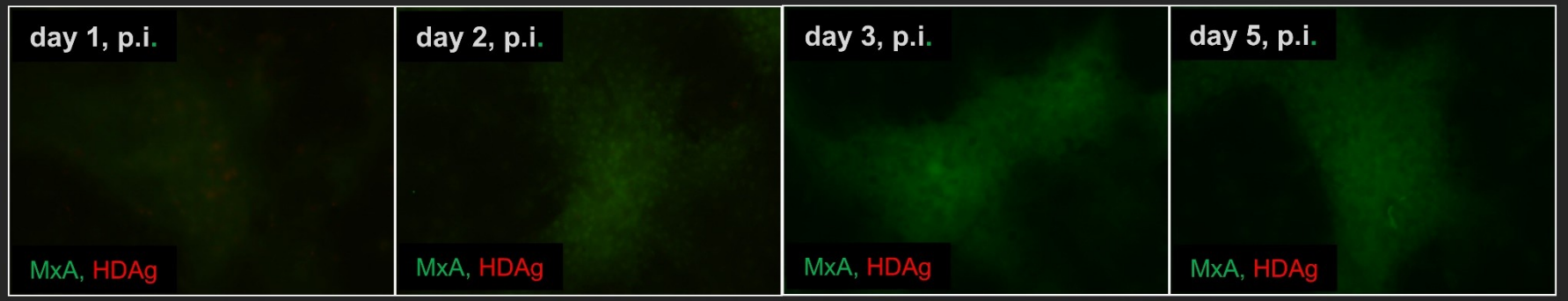
HDV infection induces an IFN response in HepaRG cells

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



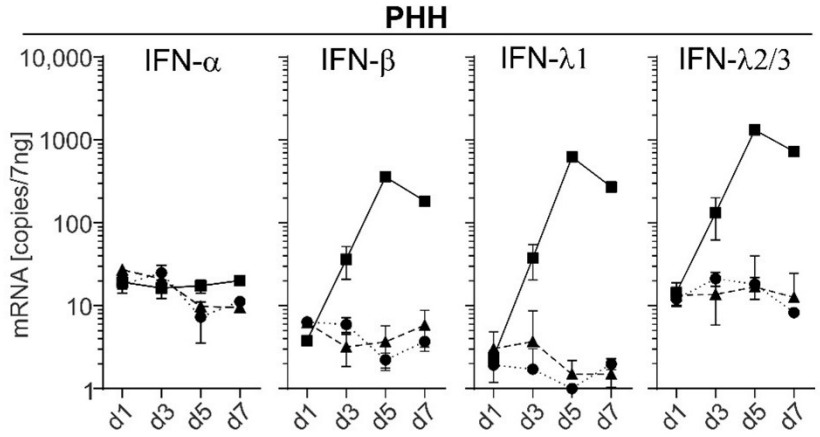
Zhang, et al. J. Hepatology, 2018

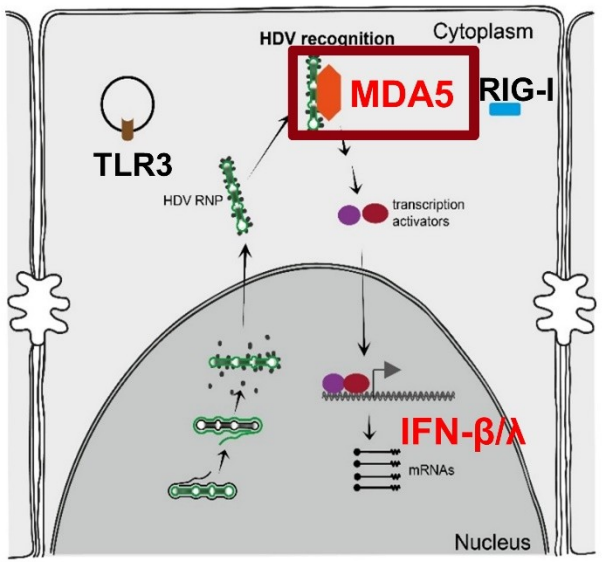
……and in the presence of the entry inhibitor Myrcludex B



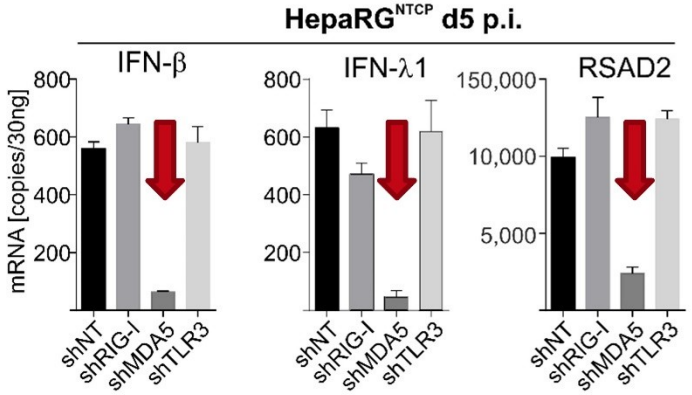
- HDV infection of HepaRG cells induces ISGs responses following HDV infection
- Myrcludex B/Bulevirtide inhibits de novo induced HDV IFN responses

MDA5 selectively senses HDV replication and let to induction of IFN-ß/λ





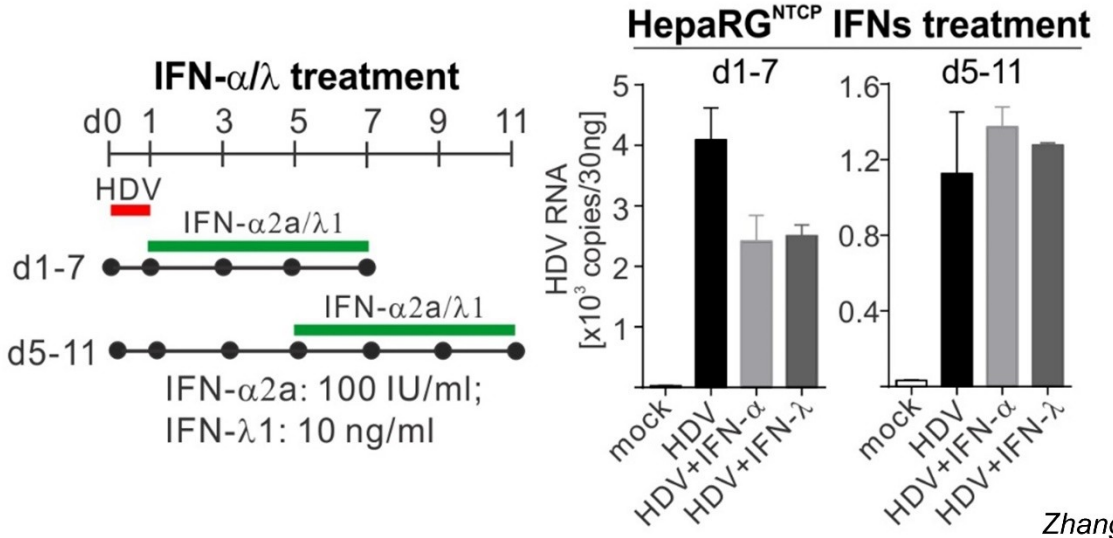
- **HDV infection activates IFN-β/λ responses in primary human hepatocytes**
- **Knock down of MDA5 abolishes IFN activation**



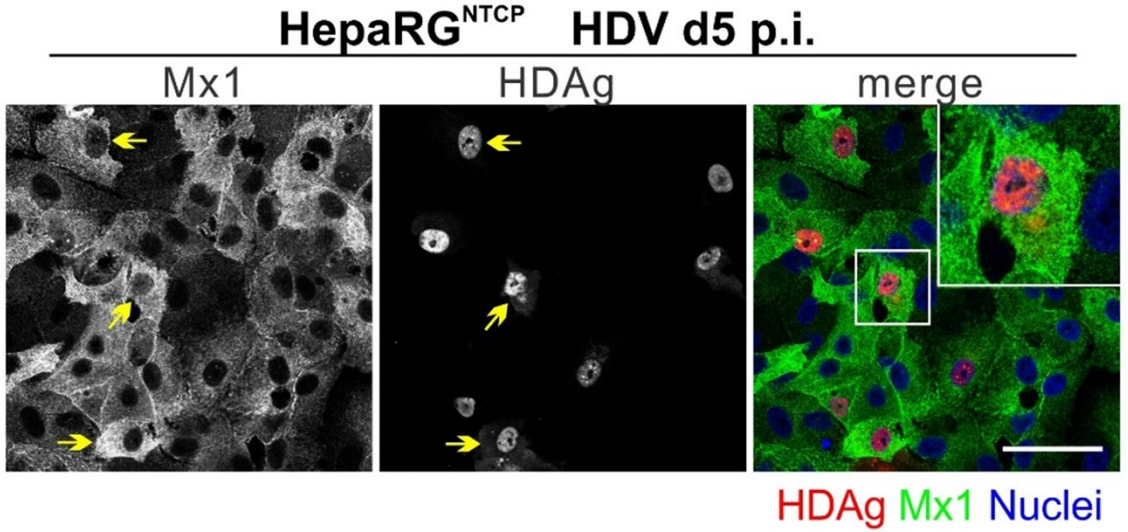
⇒ **MDA5 is the key sensor (PRR) for HDV replication**

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

IFN treatment of HDV infected hepatocytes marginally affects HDV replication

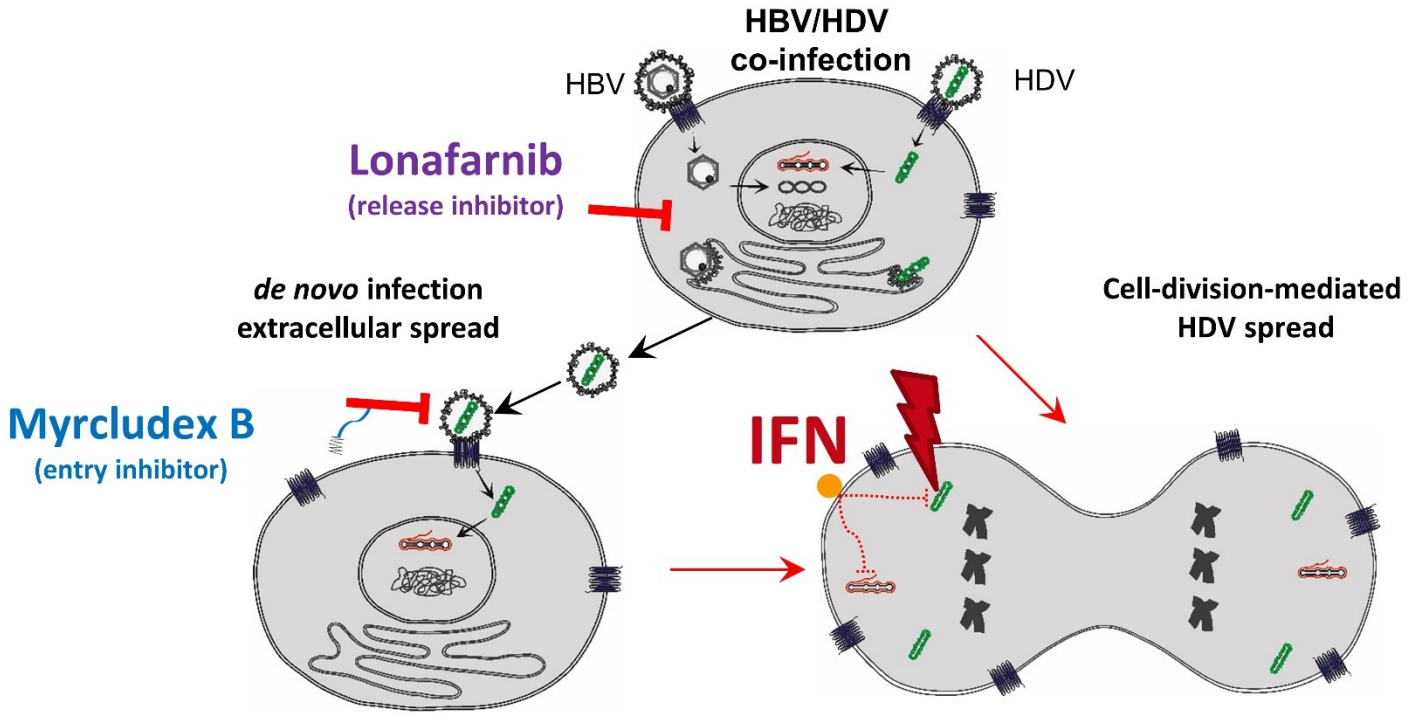


Zhang, et al. J. Hepatology, 2018



HDV replication is insensitive to IFNα and IFNλ treatment in resting hepatocytes

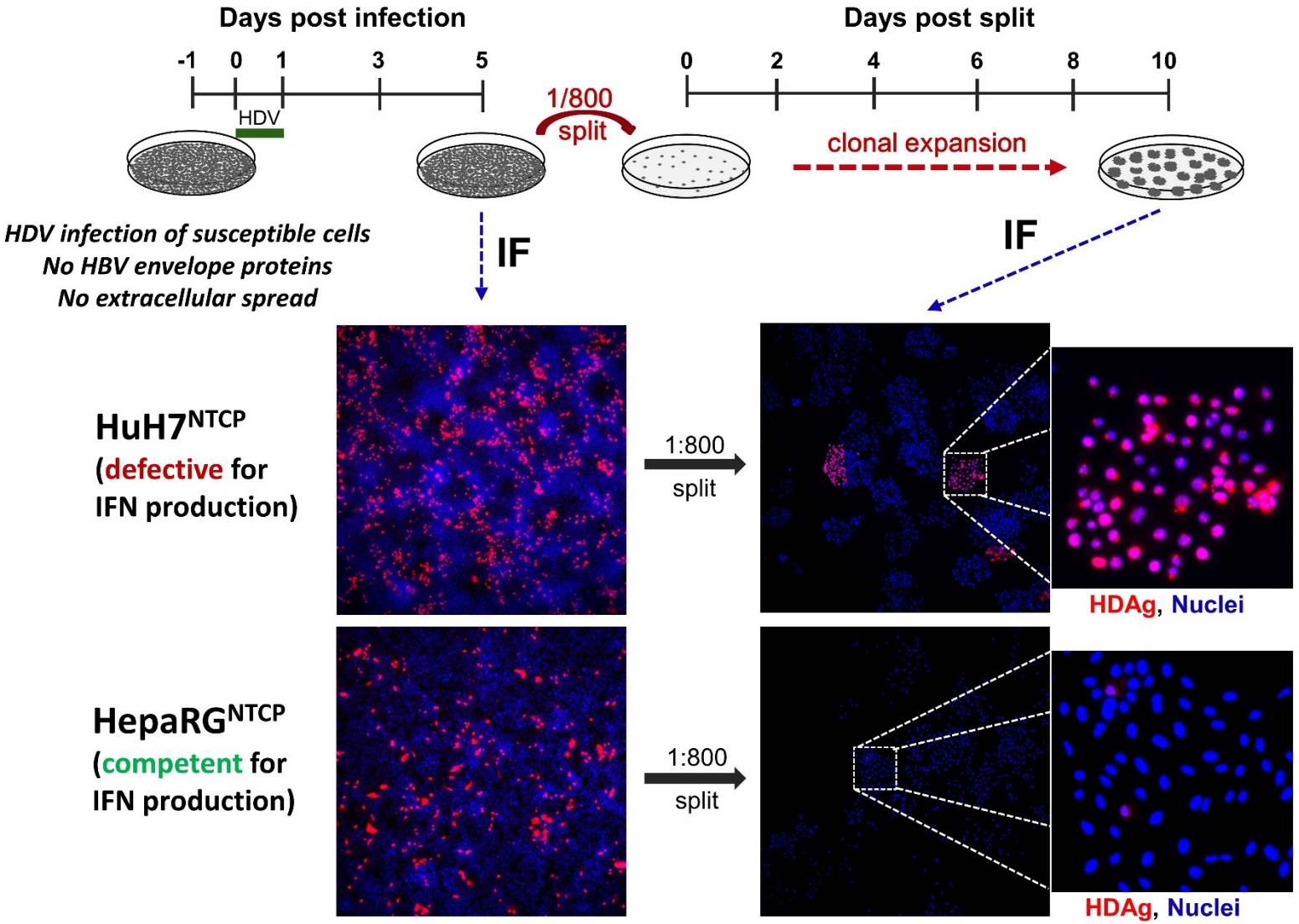
The effect of IFNs on dividing hepatocytes



Giersch *et al*., Gut, 2019.

Zhang *et al*. Int. HBV meeting. 2018. Taormina.

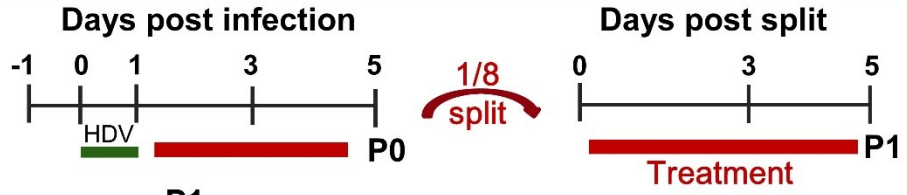
Cell-division-mediated HDV spread in innate immune defective and competent cells



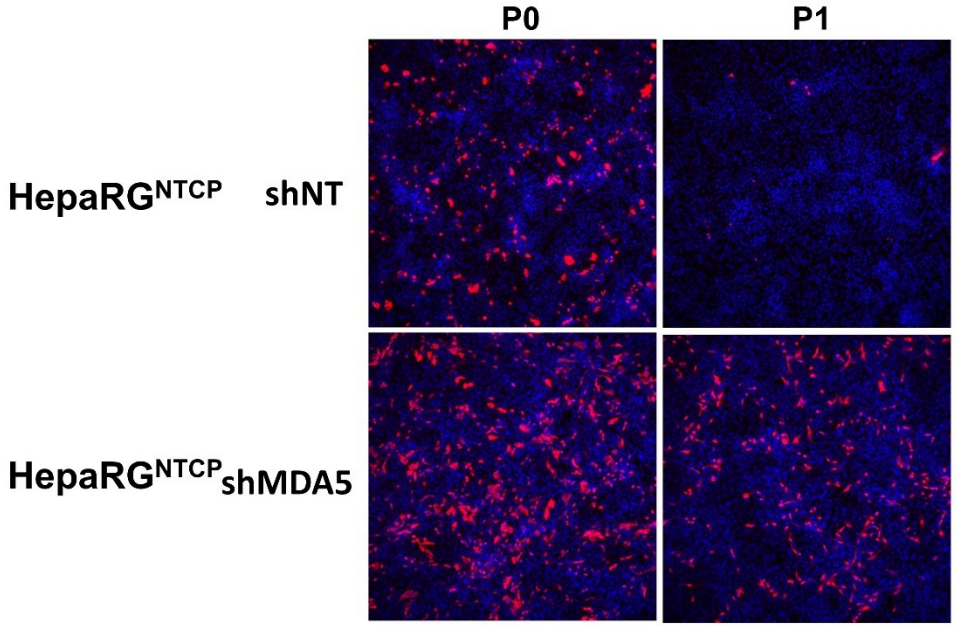
Zhang et al. Int. HBV meeting. 2018. Taormina.

⇒ Cell-division-mediated HDV spread is suppressed in innate immune competent cell lines

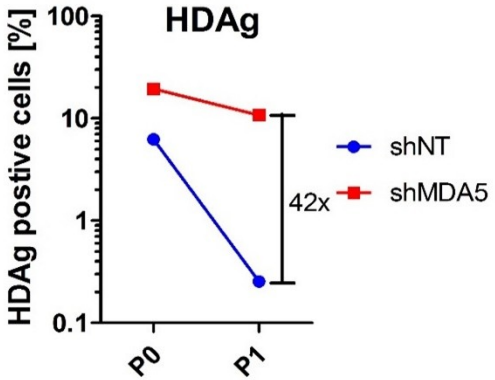
Blocking the endogenous IFN response promotes HDV spread in HepaRG^{NTCP} cells

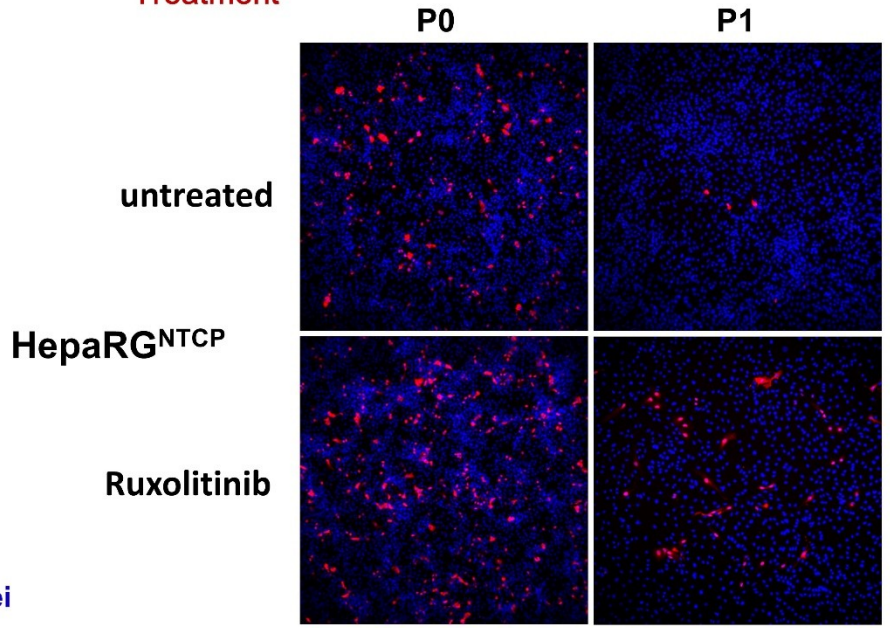


Ruxolitinib :
JAK1/2 inhibitor
blocks ISG induction

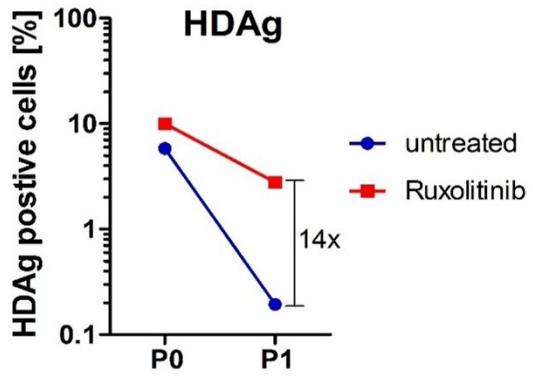


HDAg, Nuclei



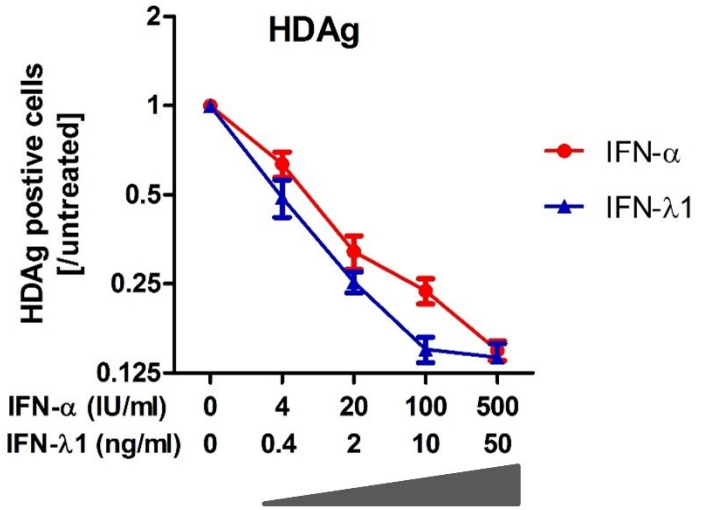


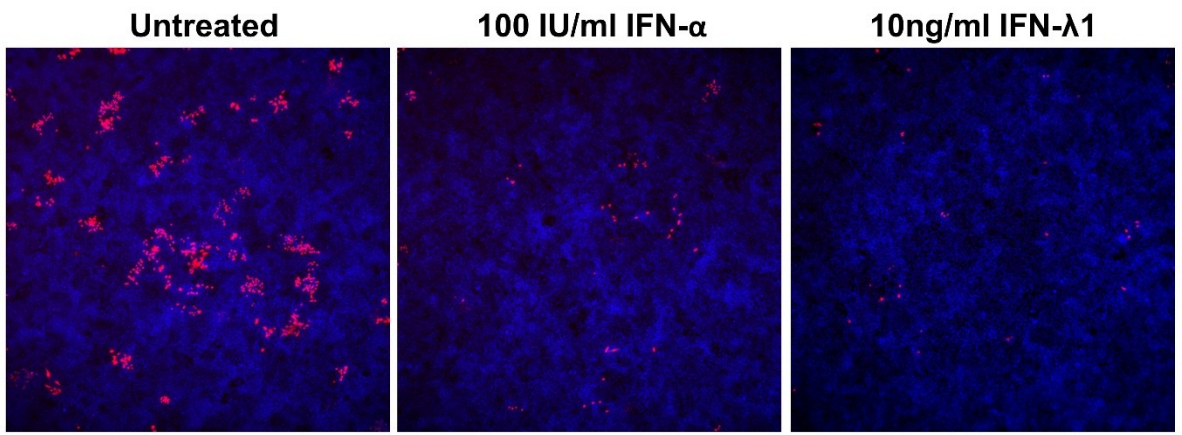
HDAg, Nuclei



IFN treatment suppresses cell-division-mediated HDV spread in HuH7^{NTCP} cells

HuH7^{NTCP}
deficient in IFN induction
sensitive to IFN

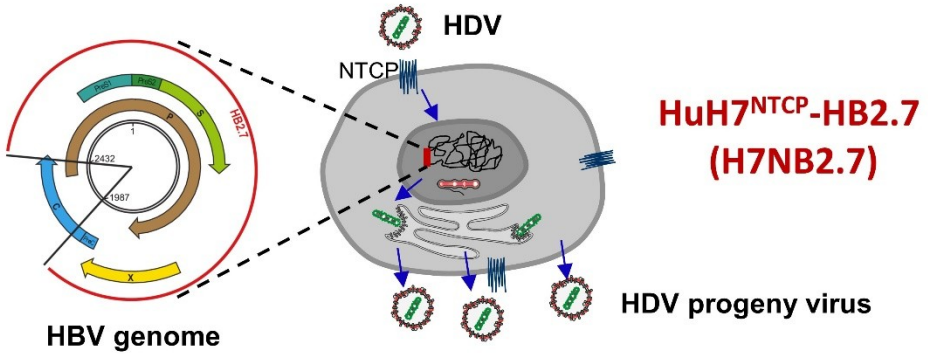




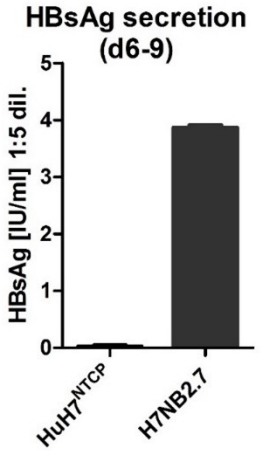
⇒ Both, HDV-induced endogenous IFN responses **and** exogenous IFNα/λ treatment suppresses cell-division-mediated HDV spread

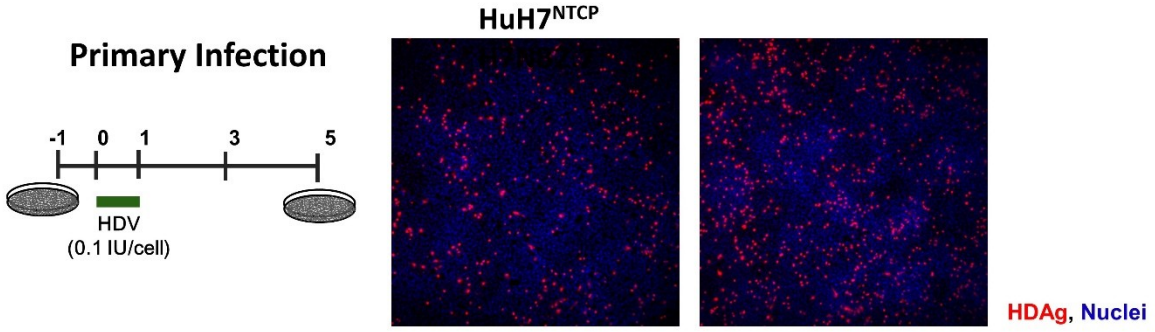
Establishment of in vitro model supporting extracellular spread of HDV

Stable integration of a 2.7 kb subgenomic HBV fragment and NTCP:
Provision of HBV envelope proteins and the receptor



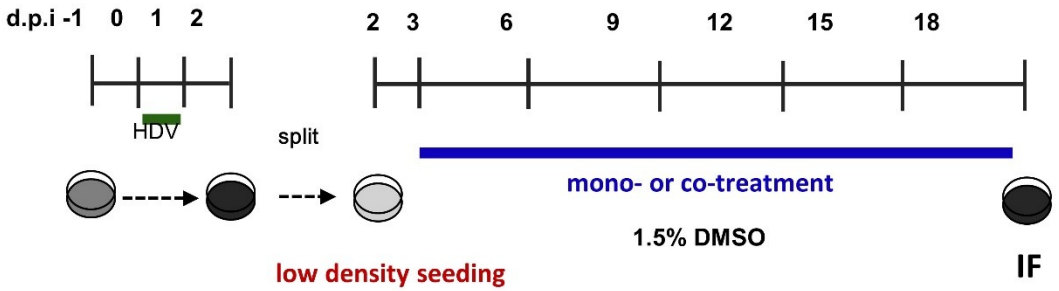
HuH7^{NTCP}-HB2.7
(H7NB2.7)





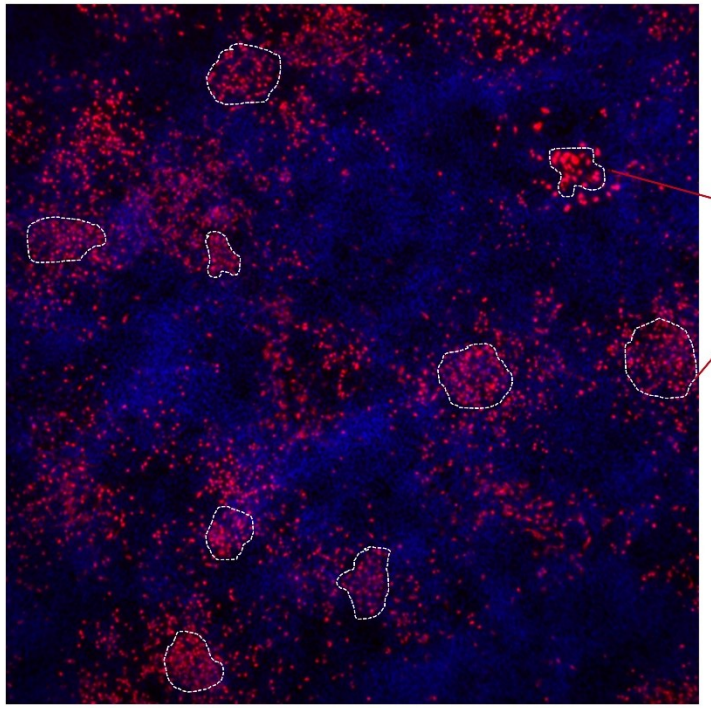
Lempp, *et al*. Nature Communications. 2019.
Zhang, *et al*. Unpublished.

Use of H7NB2.7 cell culture model to investigate both HDV spreading pathways



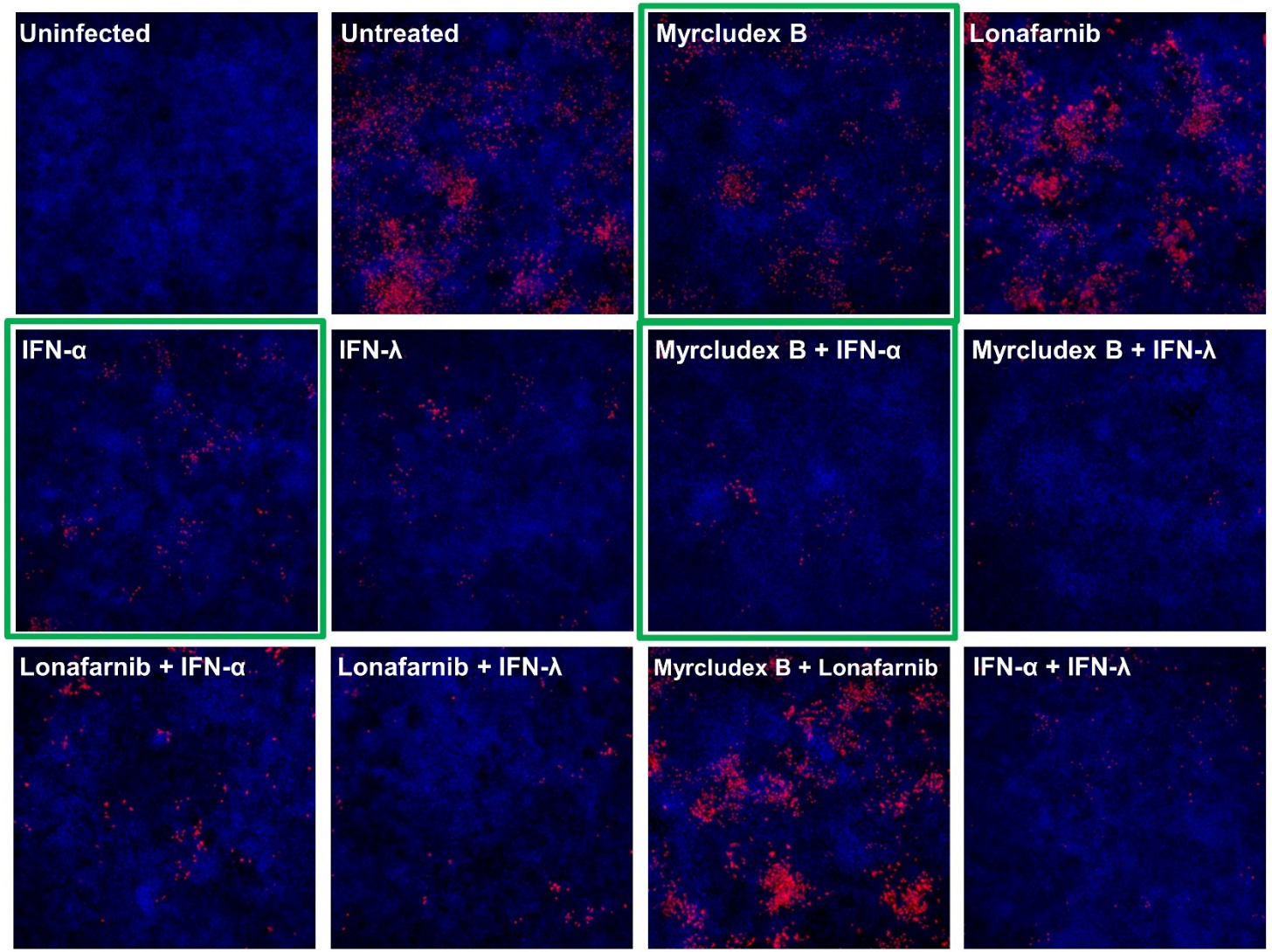
Investigated Combinations

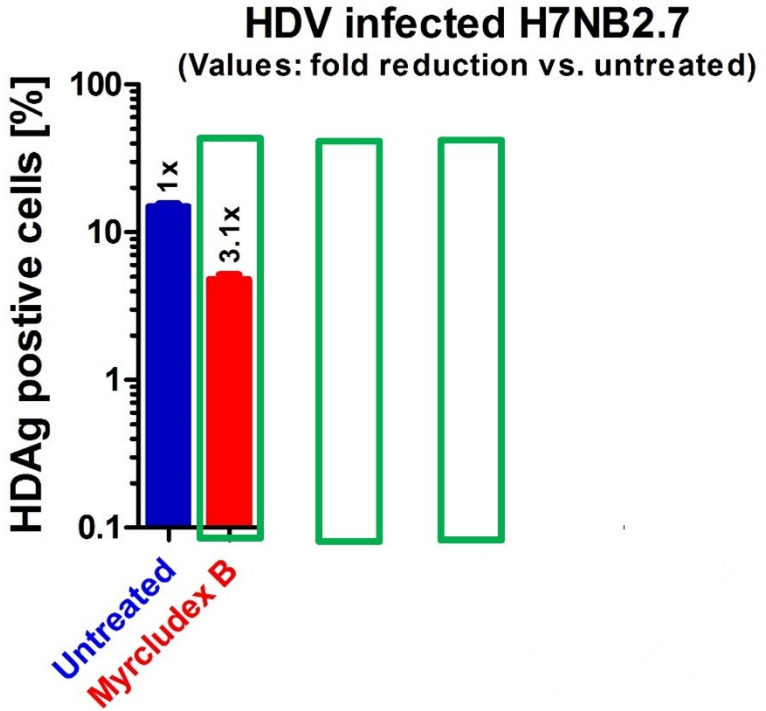
mono-treatment	co-treatment
MyrB	MyrB + IFN-α
Lonafarnib	MyrB + IFN-λ1
IFN-α	Lonafarnib + IFN-α
IFN-λ1	Lonafarnib + IFN-λ1
	MyrB + Lonafarnib
	IFN-α + IFN-λ1



Quantification of all HDAg positive cells

Evaluation of in vitro synergisms of drug using H7NB2.7 cells





- Lonafarnib promotes cell-division-mediated HDV spread probably by enhancing HDV replication.

Lempp, *et al*. Nature Communications. 2019

- IFN-α/-λ suppresses cell-division-mediated HDV spread

Zhang, et al. HBV Meeting. 2018. Taormina

⇒ **Drug combinations targeting both spreading pathways act synergistic**

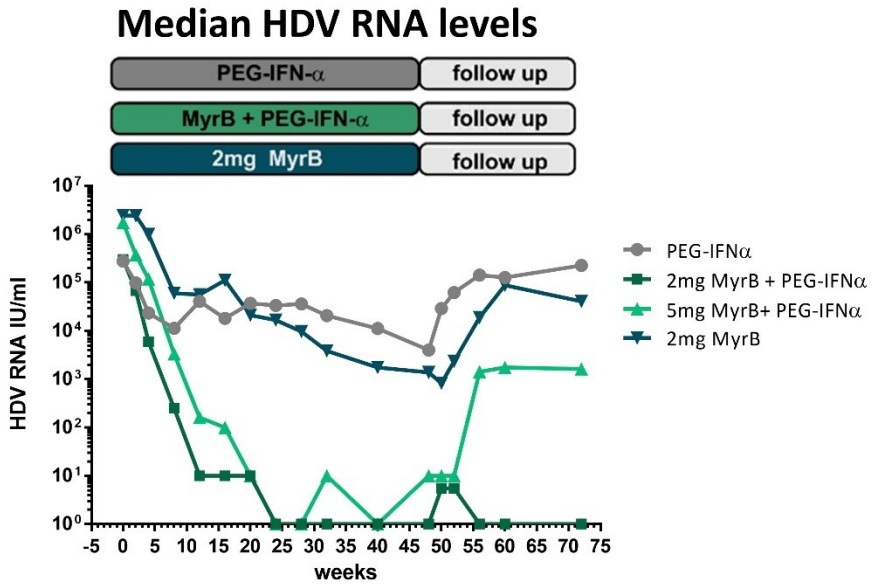
Conclusion and clinical implications

Conclusions:

- HDV can spread by an extracellular route and by cell-division-mediated spread
- An *in vitro* infection model supporting both pathways has been established
- Co-treatment with drugs targeting the two different spreading pathways blocks HDV synergistically
- The system can be used to predict the strength of synergisms of drug combinations

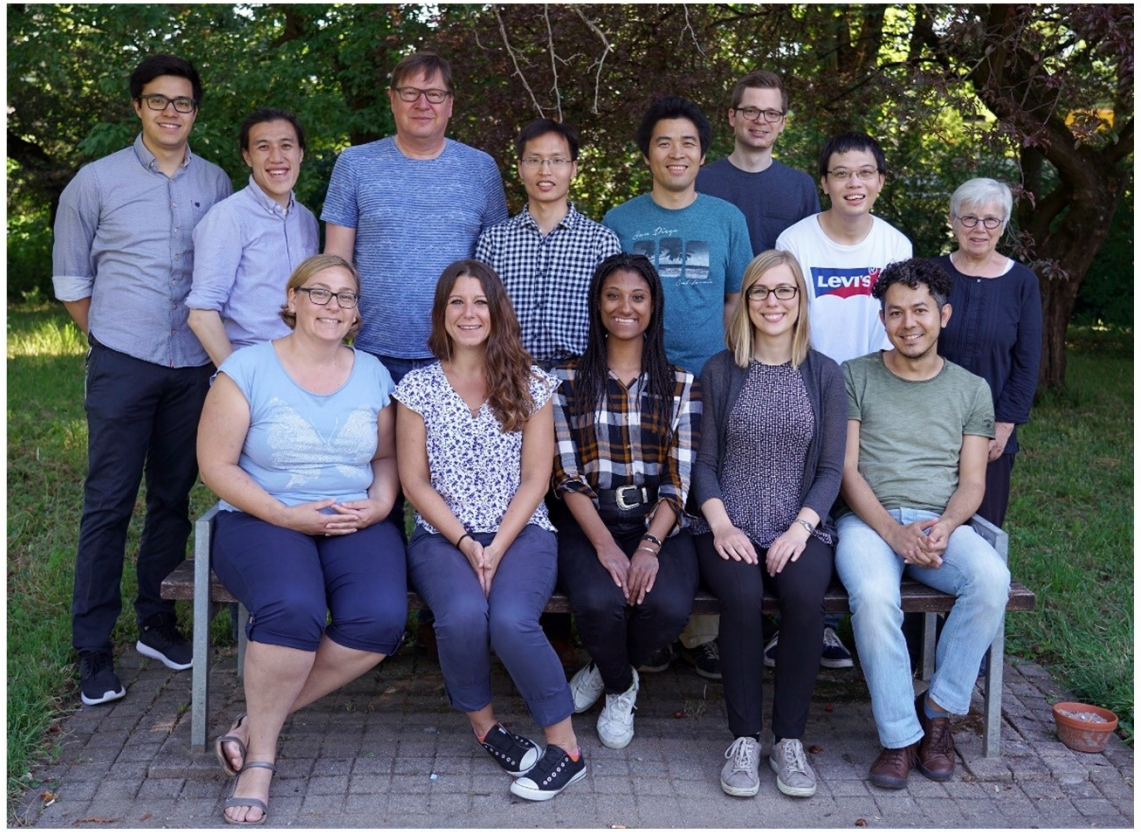
Clinical implication for Myrcludex B

- The finding confirms the clinical observation of the Myr-203 study demonstrating a strong synergism of Myrcludex B/peg-IFN-α combination therapy



O-85, Wedemeyer et al.,: SAFETY AND EFFICACY OF 10mg (HIGH-DOSE) BULEVIRTIDE (MYRCLUDEX B) IN COMBINATION WITH PEG-INTERFERON ALPHA 2a OR TENOFOVIR IN PATIENTS WITH CHRONIC HBV/HDV CO-INFECTION: WEEK 24 INTERIM RESULTS OF THE MYR203 EXTENSION STUDY.

Acknowledgements



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Group **Bartenschlager**: Pascal Mutz, Nadine Gillich

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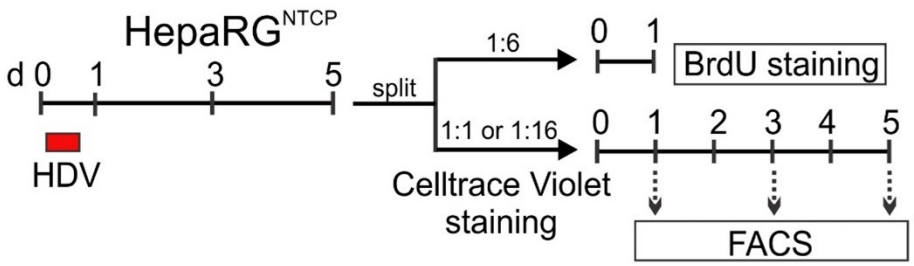
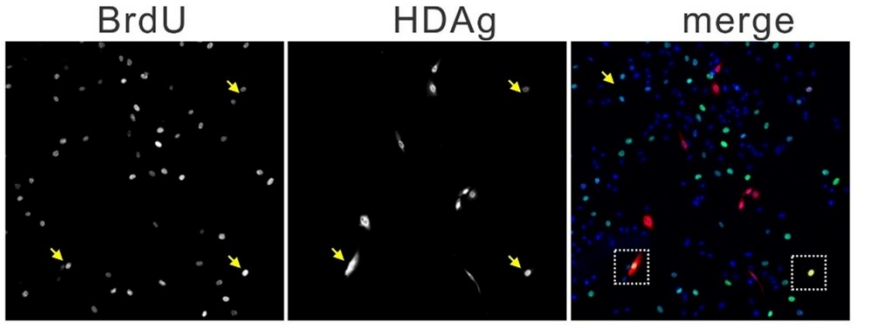
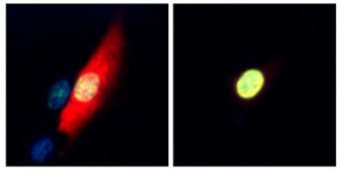
Backup

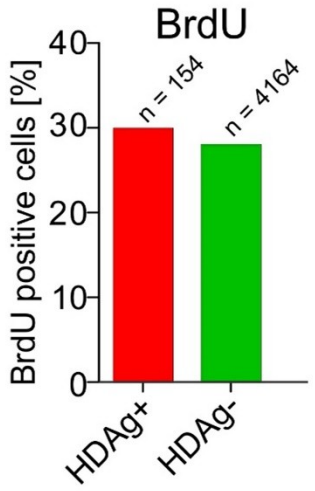
HDV replication does not impair the division of HepaRG^{NTCP} cells

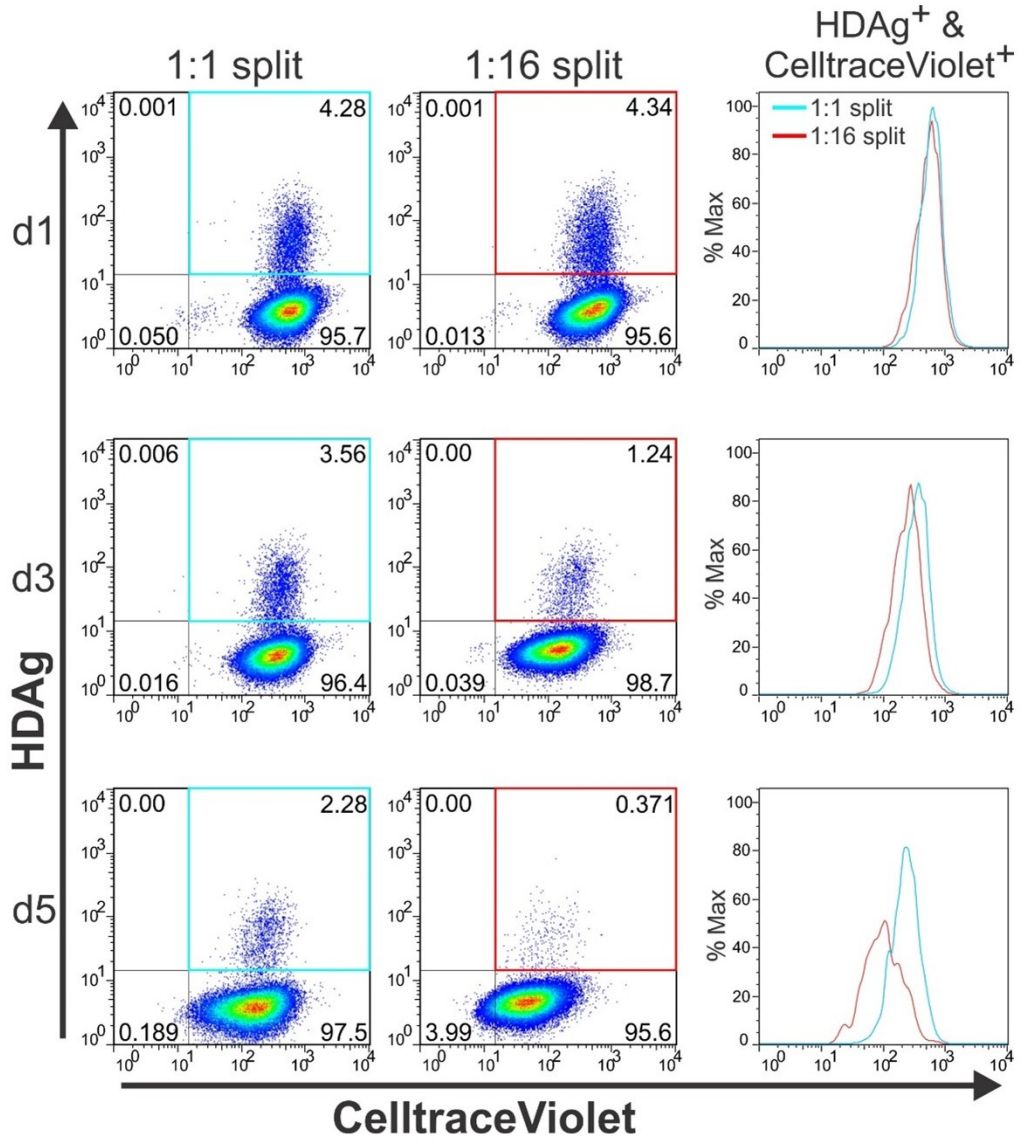




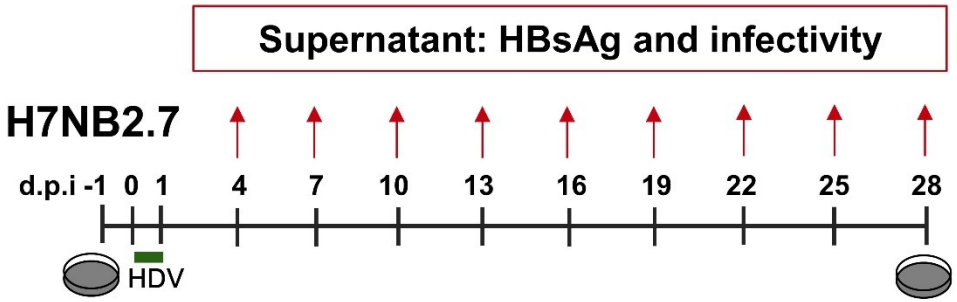


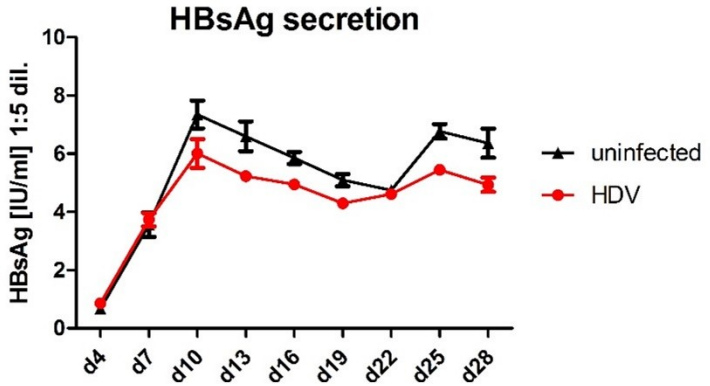
HDAg, BrdU, Nuclei

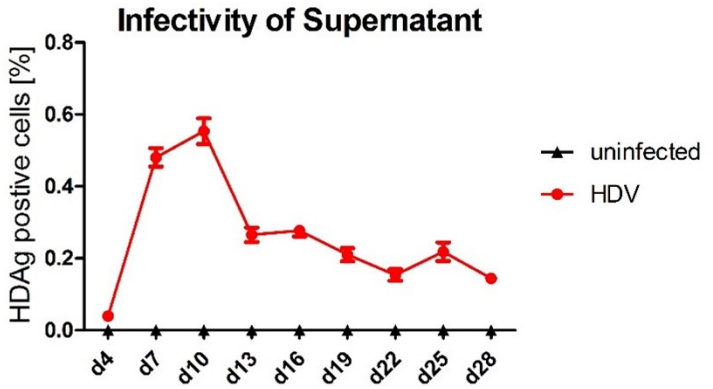




H7NB2.7 cells support HDV spread by the extracellular pathway



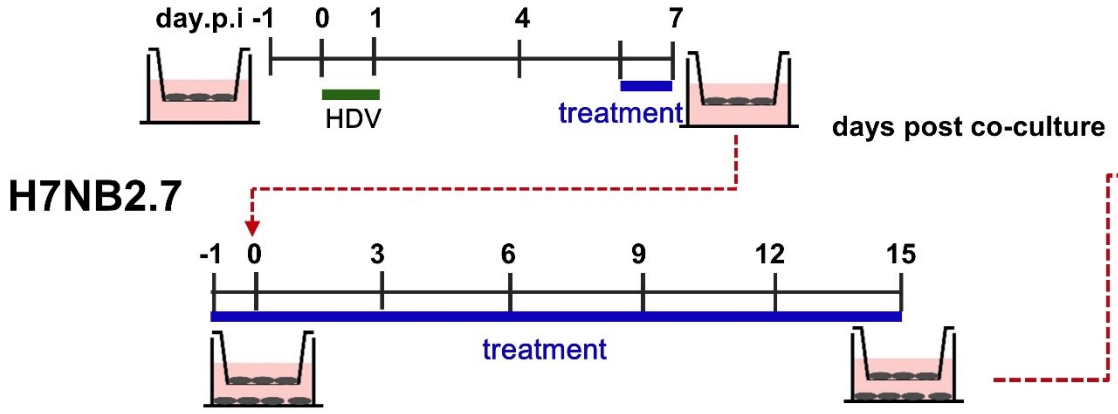


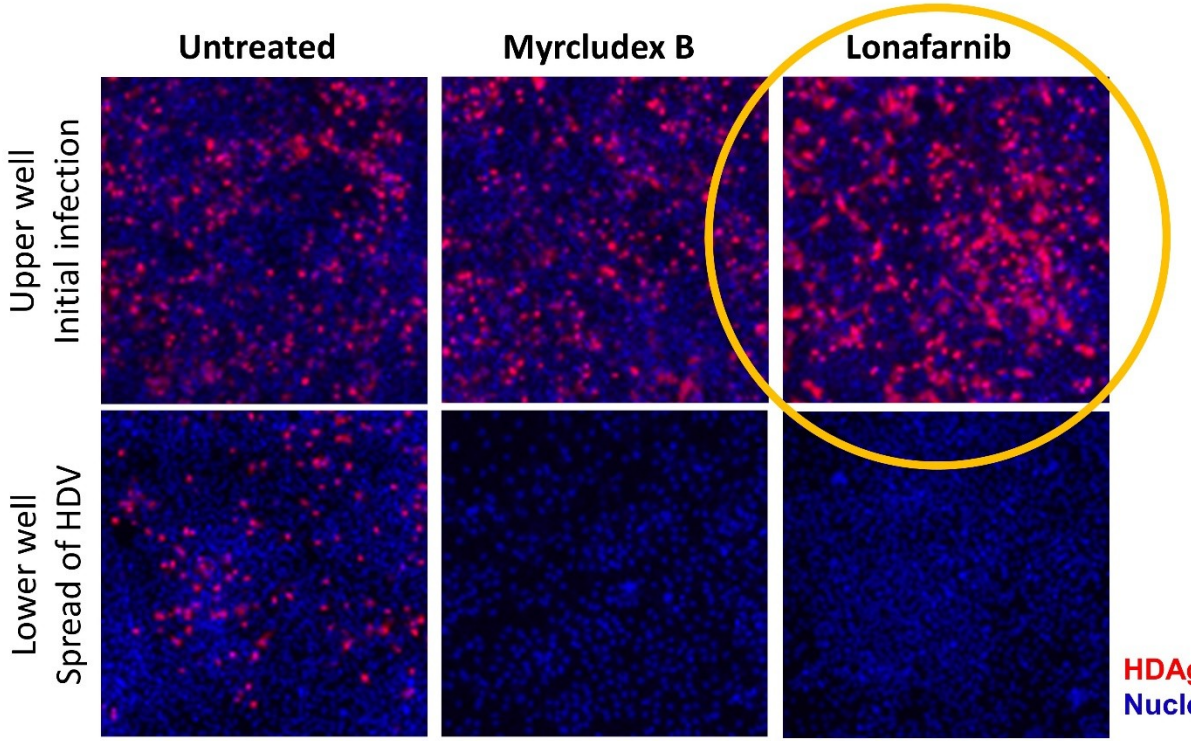


HBsAg secretion is not affected by HDV replication

HDV infected H7NB2.7 produce progeny HDV

A transwell assay to measure HDV spread





- Extracellular spread of HDV by H7NB2.7 cells can be blocked by Myrcludex B and Lonafarnib
- Lonafarnib **enhances (!)** intracellular HDV replication

Lempp, *et al*. Nature Communications. 2019.

Proviral and antiviral modulators affecting intracellular HDV replication