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

...blocking both, intrahepatic spread of HDV through \textit{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

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 Myr202 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 *(Giersch et al., Gut, 2017, Ni et al., unpublished)*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.


What’s the contribution of extracellular and intracellular spread and how can this be counteracted by drugs?
The Myr202-trial

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

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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

Plasma HDV RNA decline correlated with a decrease of intrahepatic HDV RNA (Allweiss et al., unpublished)

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

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AASLD, San Francisco, 2018
MyrB/IFNa combination show synergistic antiviral effects on HDV

The Myr203-trial

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

Final results with follow up data in plenary III: GS-013
HDV infection induces an IFN response in HepaRG cells

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

• HDV infection of HepaRG cells induces ISGs responses following HDV infection

• Myrcludex B inhibits de novo induced HDV IFN responses

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

Zhang, et al. J. Hepatology, 2018
The PRR MDA5 selectively senses HDV replication and mediates induction of IFN-β 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

Exogenous IFN cannot abrogate HDV replication in hepatocytes

IFN-α/λ treatment

HDV

IFN-α2a/λ.1

d1-7

IFN-α2a: 100 IU/ml;
IFN-λ.1: 10 ng/ml

d5-11

HepaRGNTCP IFNs treatment

HDV RNA [x10^6 copies/30ng]

mock HDV HDV+IFN-α HDV+IFN-λ.

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

Zhang, et al. J. Hepatology, 2018

HDV d5 p.i.

Mx1 HDAg merge

HDAg Mx1 Nuclei
HDV spreads through cell division: spread is controlled by endogenous innate immune responses

HDV infection of HuH7NTCP (deficient for IFN activation)

HDV infection of HepaRGNTCP (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\textsuperscript{NTCP} cells

Control (shNT)

MDA5 knock down (shMDA5)

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, IFN-β, and IFN-λ profoundly suppress HDV cell to cell spread
Endogenous and exogenous IFN responses suppress HDV persistence during proliferation of hepatocytes in vitro

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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 responses [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].
- Independent of HBV envelope proteins.

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 synergy of investigational drugs (MyrB, Lonafarnib and IFN-α/λ1) 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 IFNAR1-mediated depletion of MDSCs or inhibitor targeting JAK1/2.
- Exogenous IFN-α/β/λ-/- were applied HDV infected cells.
- Immunofluorescence and imageJ were used for HDV antigen (HDAg) expression and HDAg positive cells were quantified using IF. HDAg positive cells and percentage were shown using IF. Repeated measurements were done to determine the mean and SD (n=8).

4. RESULTS

Cell division mediated HDV spread is restricted in innate immune competent HepaRG cells

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

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 [5] and [6].
- The system provides a cell culture model for the identification of novel synergistically acting immune modulators for future clinical combination therapies.

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7. REFERENCES


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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