



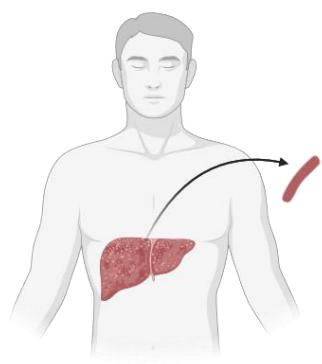
2nd Delta Cure Meeting

Pathogenesis: Insights from liver biopsies

Lena Allweiss - October 11, 2024

I have nothing to
disclose.

How does the intrahepatic landscape affect pathology?



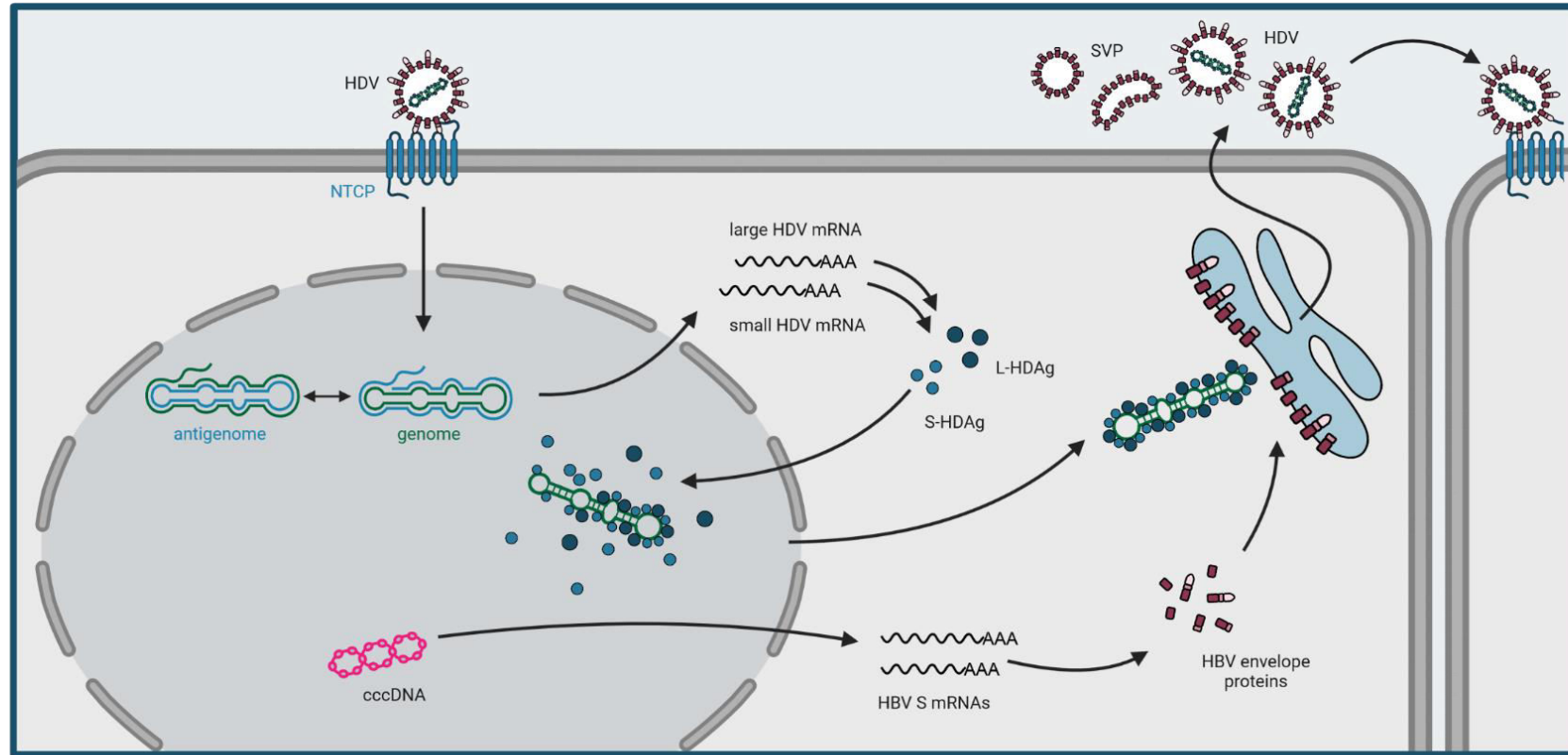
What is the distribution of mono or coinfecting hepatocytes in the liver?

What role do HDV monoinfected cells play?

What is the role of HBV DNA integrations for HDV spreading?

How does the intrahepatic landscape affect pathogenesis and treatment outcomes?

The intrahepatic landscape in CHD



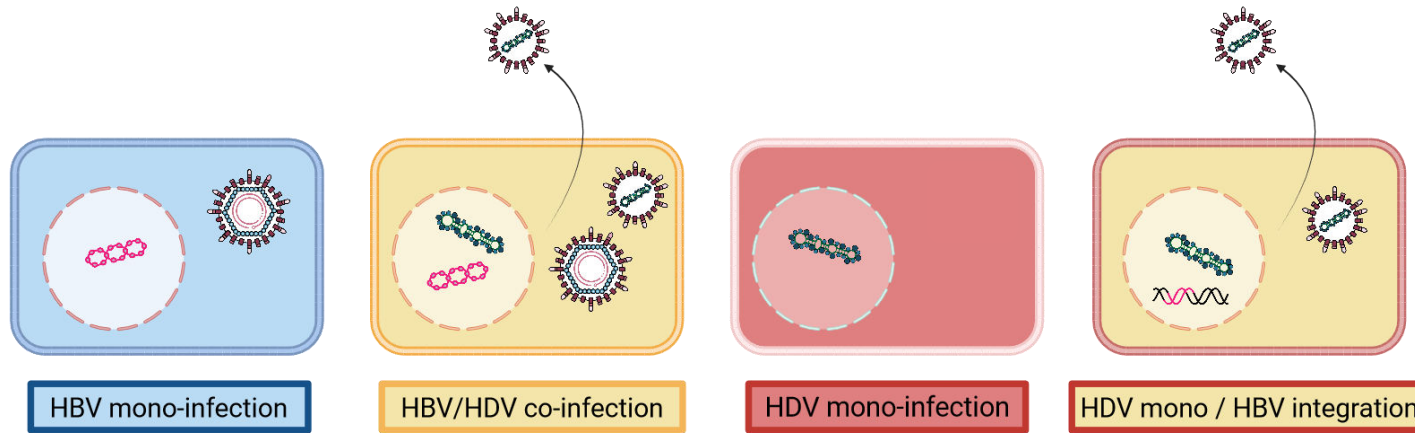
Adapted from Dandri et al J. Hepatol. 2022 Created with BioRender.com

1) HDV and HBV have the same envelope and infect hepatocytes via the same receptor: NTCP

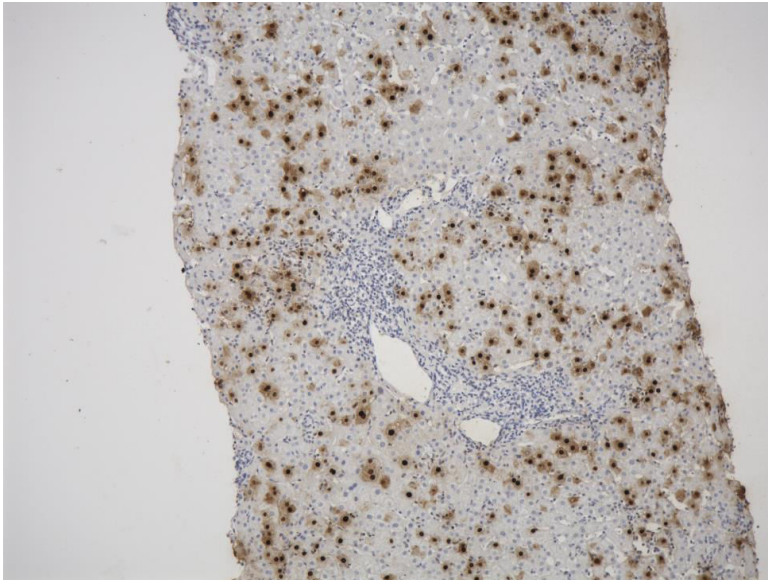
2) HDV replicates independently of HBV in the nucleus via redirection of host polymerase II

3) HDV is enveloped in HBs and released from the cells

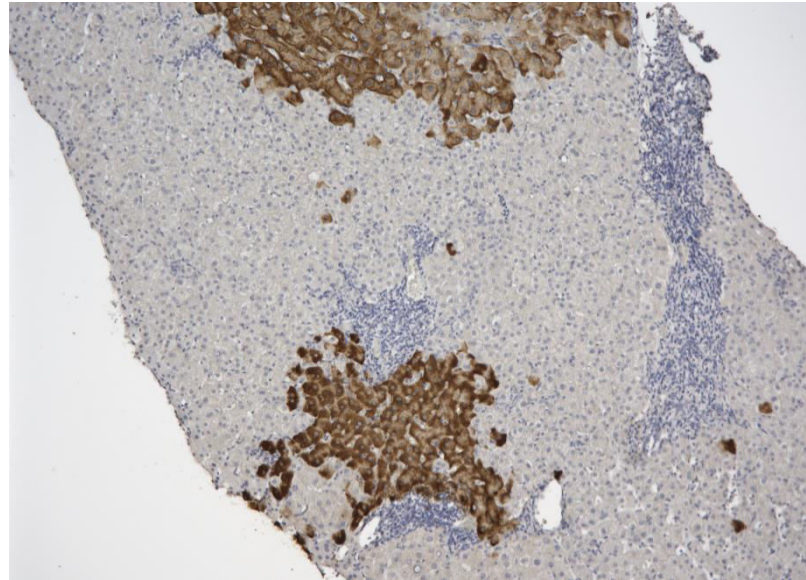
The intrahepatic landscape in CHD



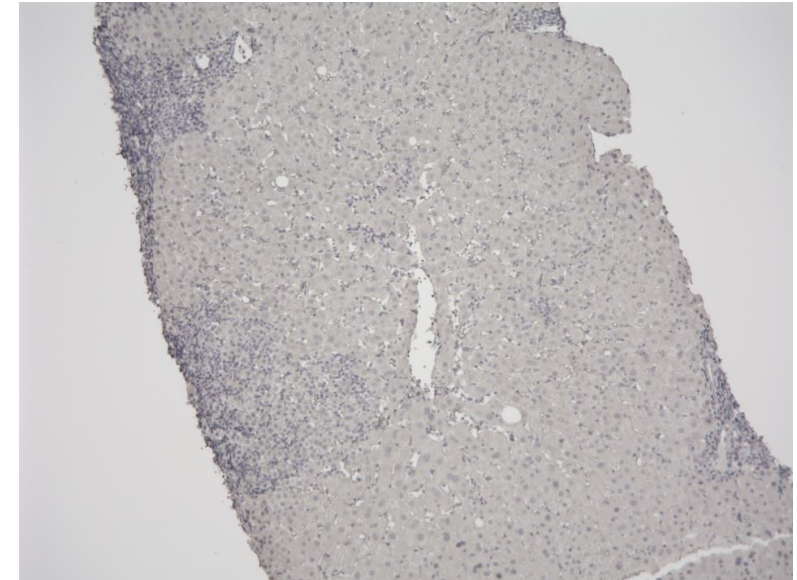
HDV Ag



HBsAg



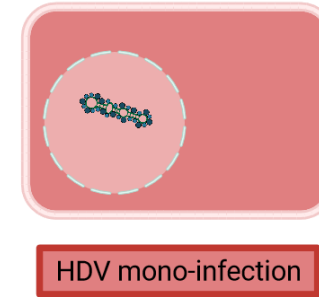
HBcAg



Existence of a diverse populations of non/mono/co-infected hepatocytes in the livers of CHD patients

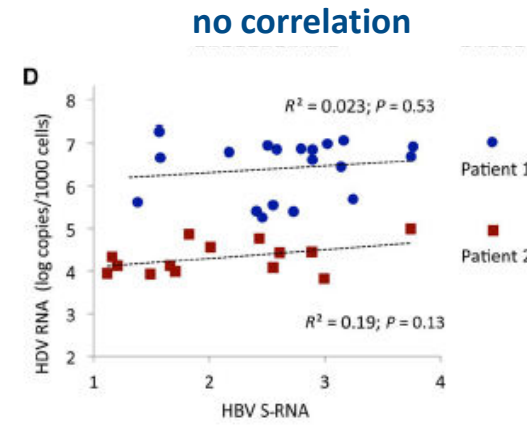
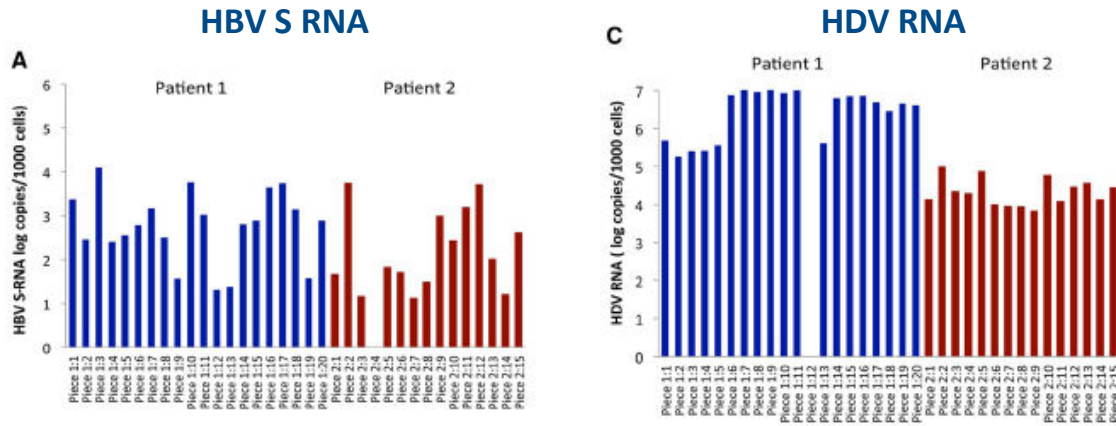
Abundance of HDV-monoinfected cells

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
HDAG and HBsAg					
Biopsy length (mm)*	27	8	27	18	25
No. of cells HDAG +ve	169	113	212	54	85
No. of cells HBsAg +ve	2000	8	913	28	20
HDAG + HBsAg +	23%	1%	39%	0%	4%
HDAG + HBsAg -	77%	99%	61%	100%	96%
HDAG and HBcAg					
Biopsy length (mm)*	6	—	29	—	—
No. of cells HDAG +ve	26	—	274	—	—
No. of cells HBcAg +ve	800	—	1176	—	—
HDAG + HBcAg +	8%	—	0%	—	—
HDAG + HBcAg -	92%	—	100%	—	—



There seems to be a high proportion of HDV-monoinfected cells in CHD.

Riley et al. Histopathology 1992



Prakash Hepatol. Commun. 2021

Correlation analysis in large CHD biopsy cohort

Baseline biopsies (MYR203/301)

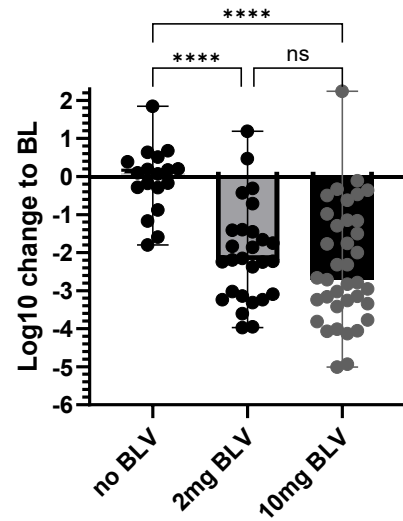
	HDV RNA liver	HDAg+ cells	HDV RNA serum	ALT serum	CXCL10 liver	pgRNA liver	cccDNA liver	S HBV RNA liver	total HBV DNA liver	HBsAg liver	HBsAg serum	HBV DNA serum
HDV RNA liver		0.79	0.52	0.41	0.10	-0.24	0.04	-0.18	-0.28	-0.11	-0.08	0.00
HDAg+ cells	****		0.44	0.52	0.38	-0.20	0.14	-0.17	-0.35	-0.22	-0.17	-0.15
HDV RNA serum	****	****		0.28	-0.10	0.20	0.34	0.41	0.29	0.13	0.38	0.01
ALT serum	****	****	**		0.55	-0.11	0.04	-0.14	-0.26	-0.20	-0.19	0.05
CXCL10 liver	ns	**	ns	****		-0.12	-0.23	-0.23	-0.32	-0.26	-0.32	-0.23
pgRNA liver	*	ns	ns	ns	ns		0.39	0.57	0.52	0.28	0.45	-0.15
cccDNA liver	ns	ns	*	ns	ns	**		0.45	0.48	0.15	0.37	-0.05
S HBV RNA liver	ns	ns	****	ns	*	****	***		0.72	0.42	0.59	-0.08
total HBV DNA liver	**	**	**	*	**	****	***	****		0.42	0.41	-0.11
HBsAg liver	ns	*	ns	ns	*	*	ns	****	***		0.41	0.08
HBsAg serum	ns	ns	****	ns	**	****	*	****	***	****		0.07
HBV DNA serum	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	

→ liver HDV RNA does not correlate with liver HBV parameters:
monoinfected hepatocytes
suppression of HBV replication?

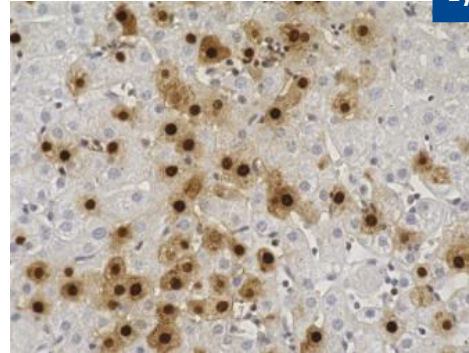
→ What is the relevance for liver pathology,
diagnostics and treatment outcomes?

Effect of BLV treatment on HDV-infected cells

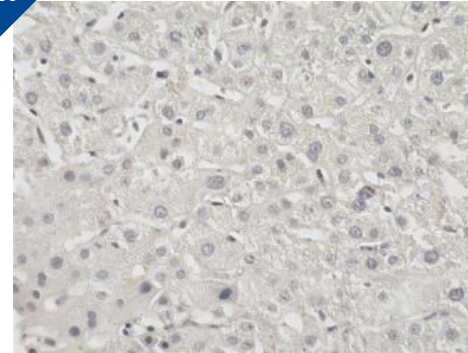
intrahepatic HDV RNA (MYR203/301)



HDAg IHC staining

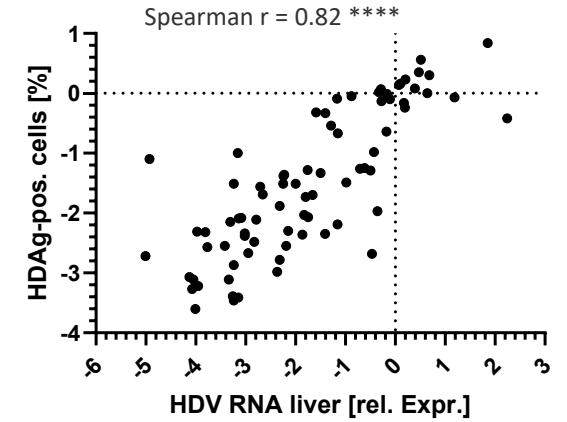


10 mg BLV (MYR203/301)



Allweiss et al. J Hepatol 2024

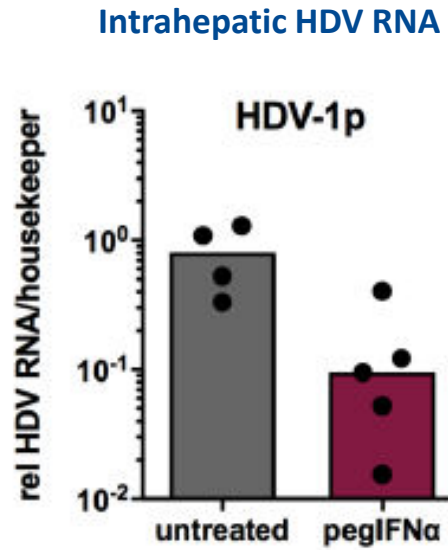
correlation Log10 change to BL



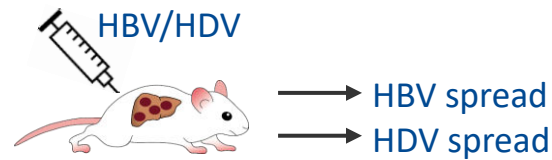
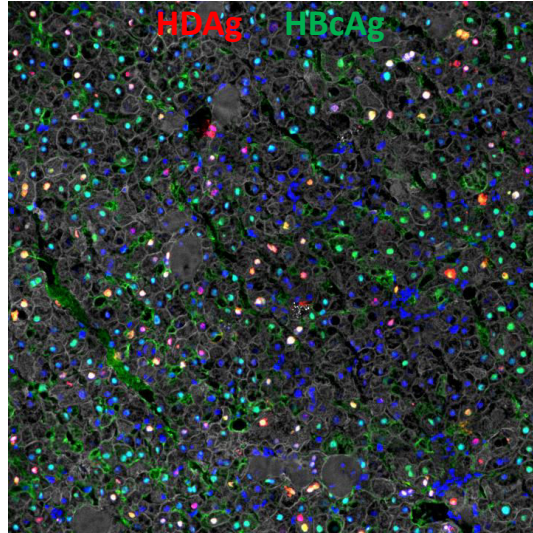
BLV treatment leads to a strong reduction of intrahepatic HDV RNA and HDAg+ cells, indicating that BLV reduced the number of infected cells.

→ Does the intrahepatic landscape influence treatment outcome?

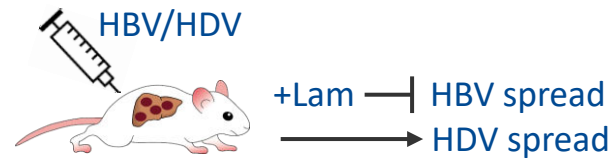
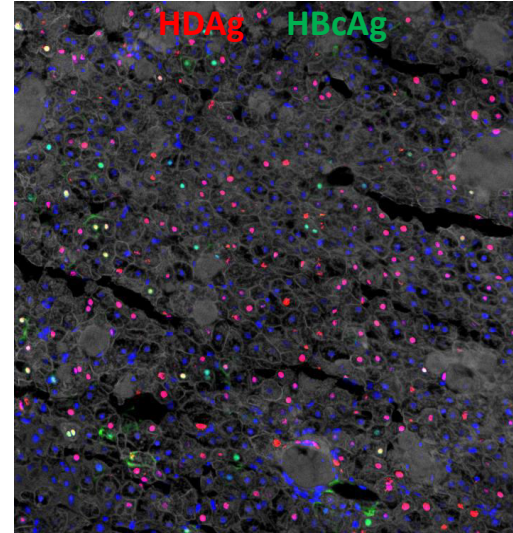
Effect of IFN α treatment on HDV-monoinfected cells



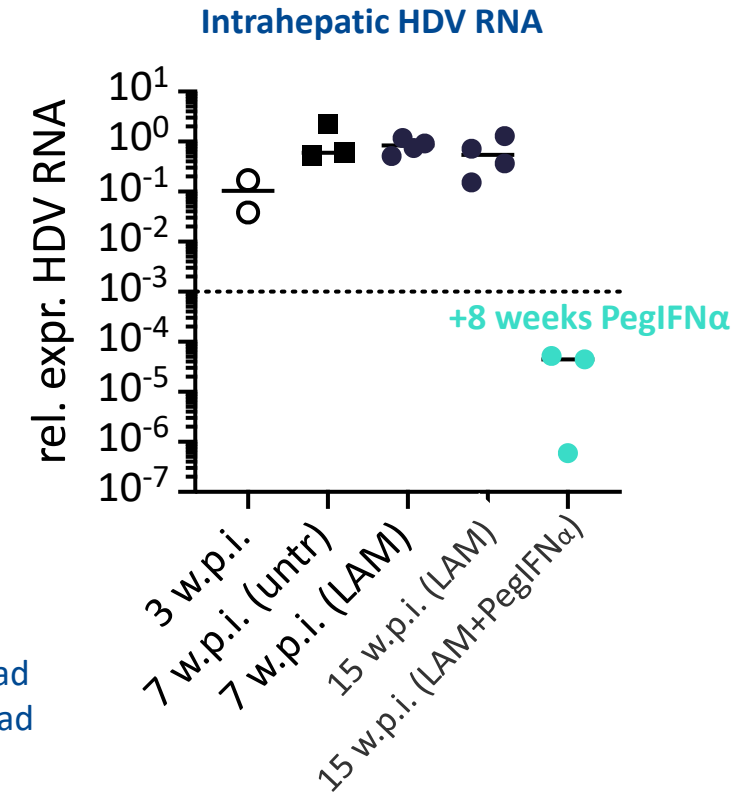
Giersch et al. JHEP Rep. 2023



co-infected cells



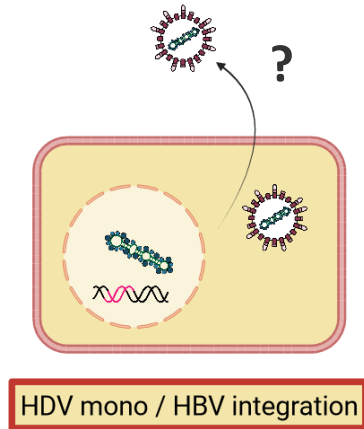
mono-infected cells



A. Volmari/T. Volz et al. unpublished

PegIFN α treatment appears to be more effective in mice with high numbers of HDV-monoinfected cells.

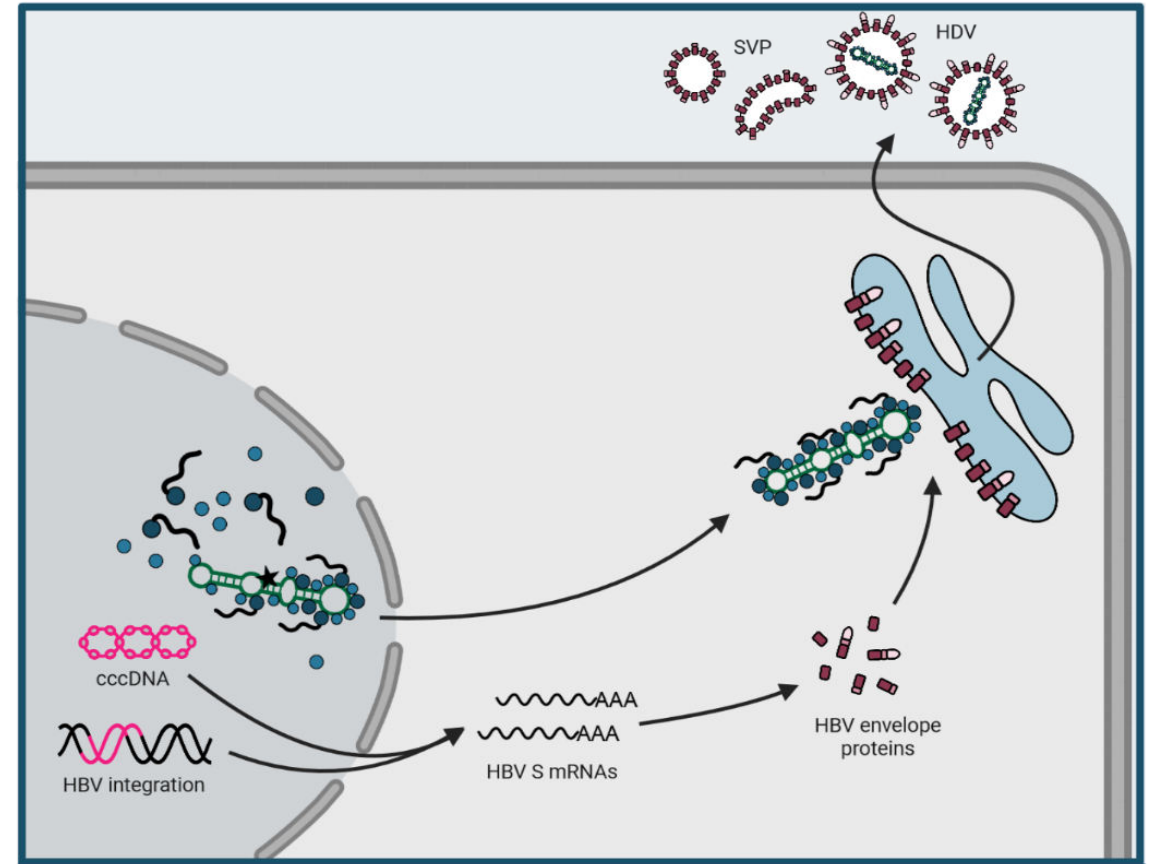
→ What is the mechanism of IFN α treatment? How does this relate to other proposed mechanisms such as inhibiting cell-division-mediated spread or cell entry?



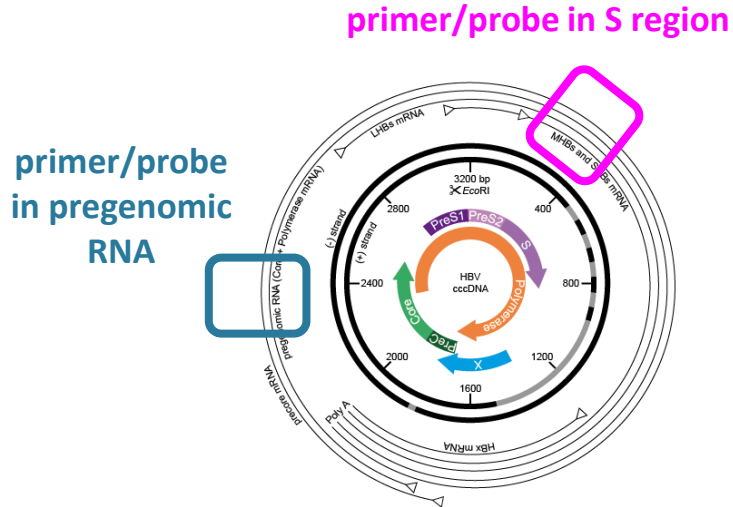
Can HDV use HBVs from integrated HBV DNA?

→ Hepatoma cell lines containing natural HBV DNA integrations support HDV assembly and release

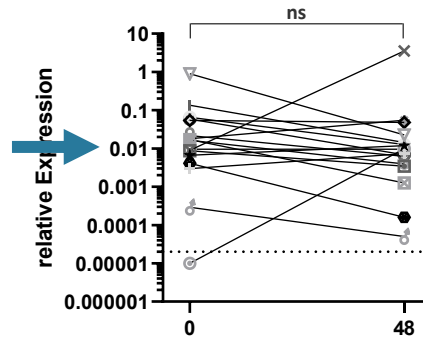
Freitas et al. J Virol. 2014



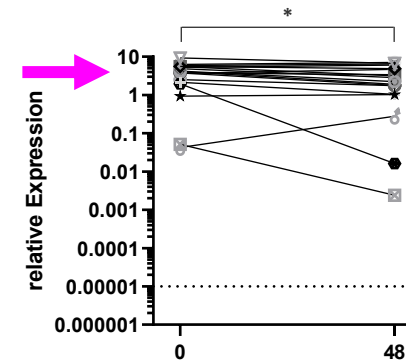
HBV DNA integrations in CHD



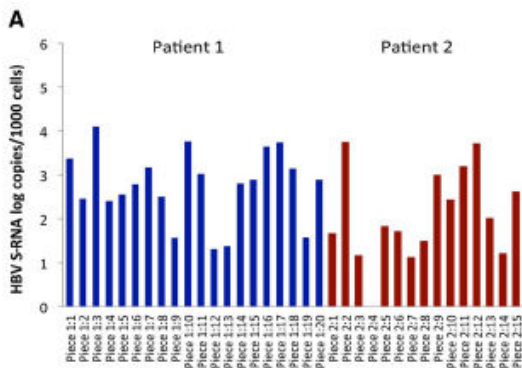
Liver biopsies from MYR301 - no treatment arm:



Allweiss et al. J Hepatol 2024

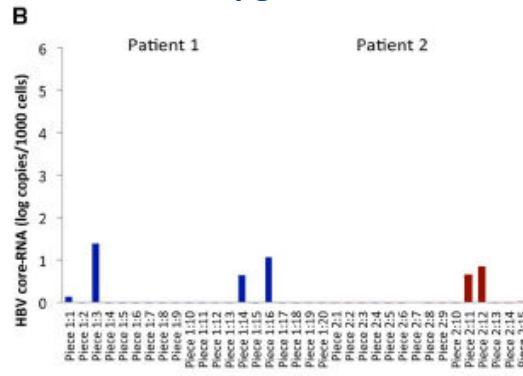


Cirrhotic explant liver tissue:
HBV S RNA



Prakash Hepatol. Commun. 2021

HBV pgRNA



High levels of HBV S transcripts but low pregenomic RNA levels suggest the presence of integrations

Deep sequencing reveals extensive integrations in cirrhotic CHD patient livers

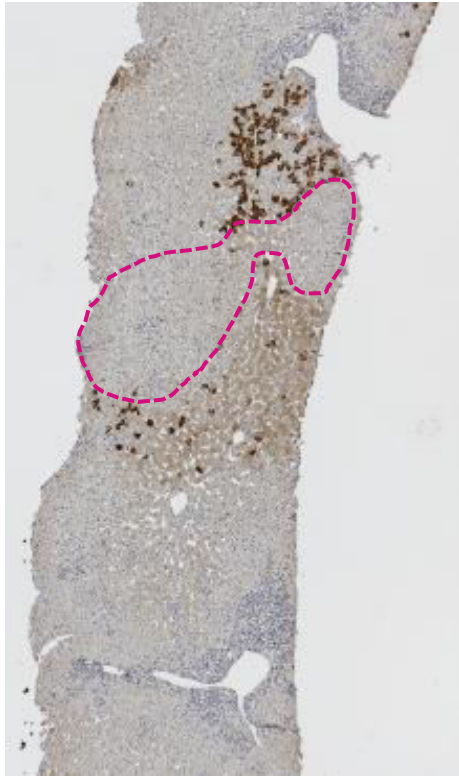
Ringlander et al. J viral Hepat. 2020; J Infect Dis 2024

Baseline biopsies (MYR203/301)

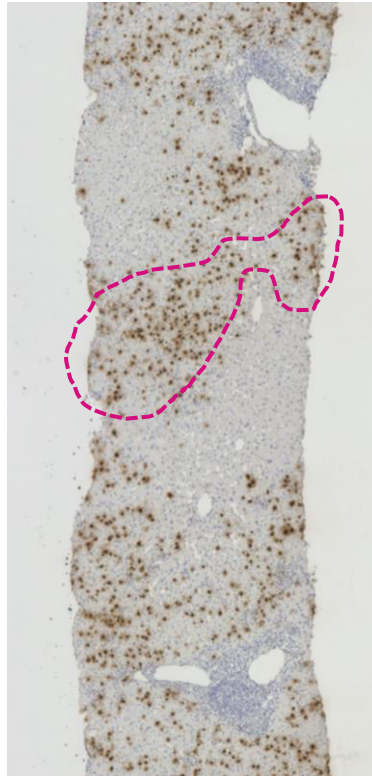
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total HBV DNA liver	**	**	**	*	**	****	***	****		0.42	0.41	-0.11
HBsAg liver	ns	*	ns	ns	*	*	ns	****	***		0.41	0.08
HBsAg serum	ns	ns	****	ns	**	****	*	****	***	****		0.07
HBV DNA serum	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	

→ serum HDV RNA correlates with serum HBsAg and liver HBV: release of HDV through integration and cccDNA-containing cells

IHC on adjacent biopsy sections (MYR203 BL)

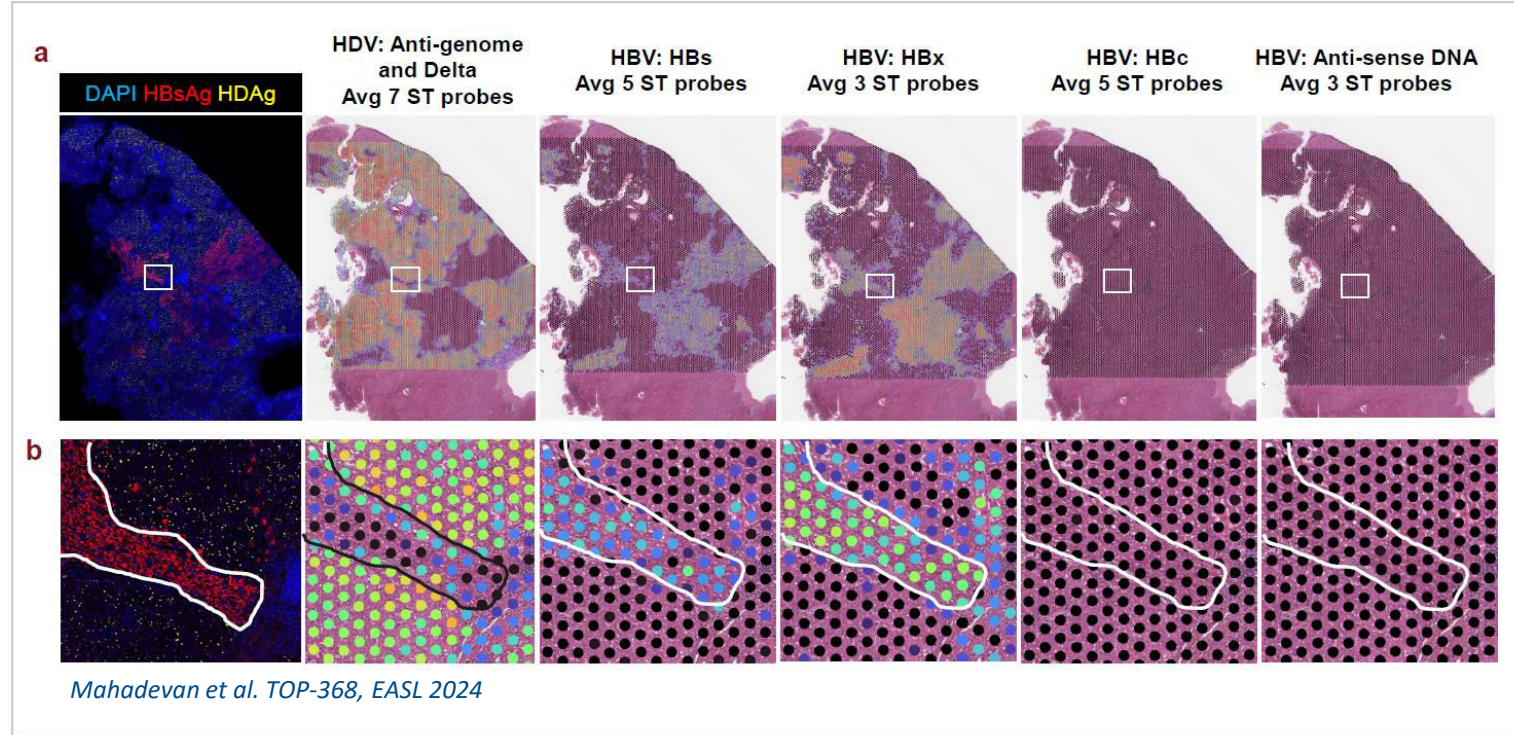


HBsAg



HDAg

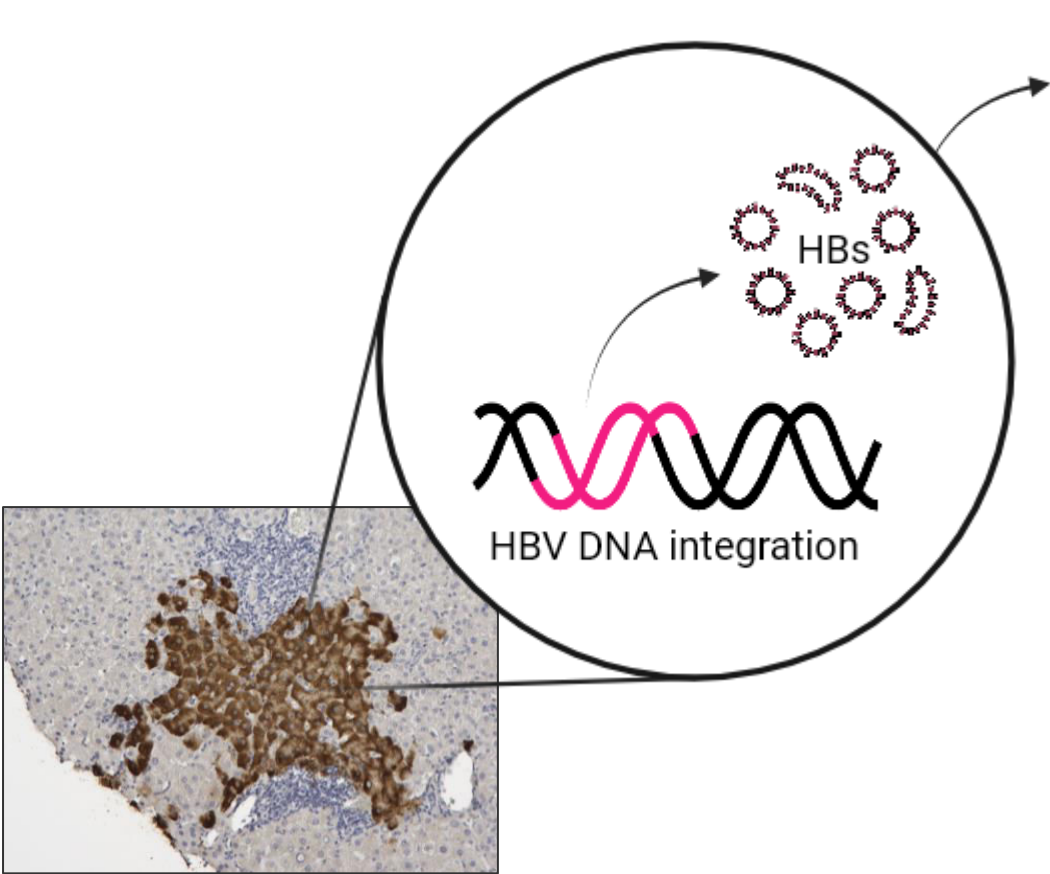
Allweiss et al. unpublished



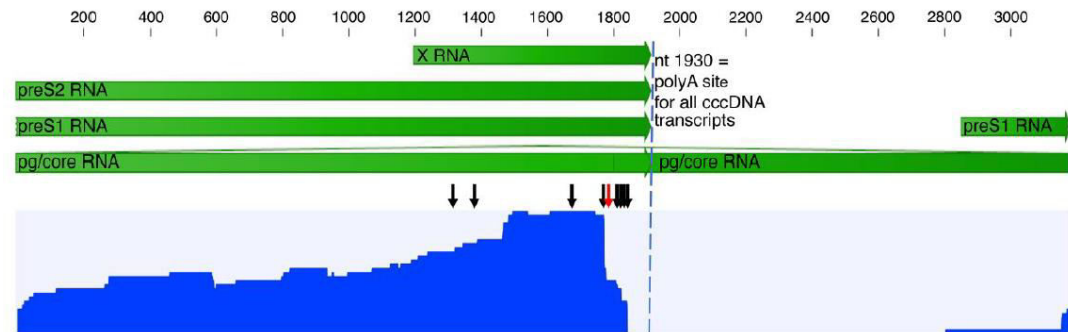
Spatial transcriptomics in 1 CHD liver biopsy identifies HBsAg+ HBV integration clusters

HDAg+ and HBsAg+ cells appear mutually exclusive by IF and spatial transcriptomics

Role of integrated HBs for HDV spreading



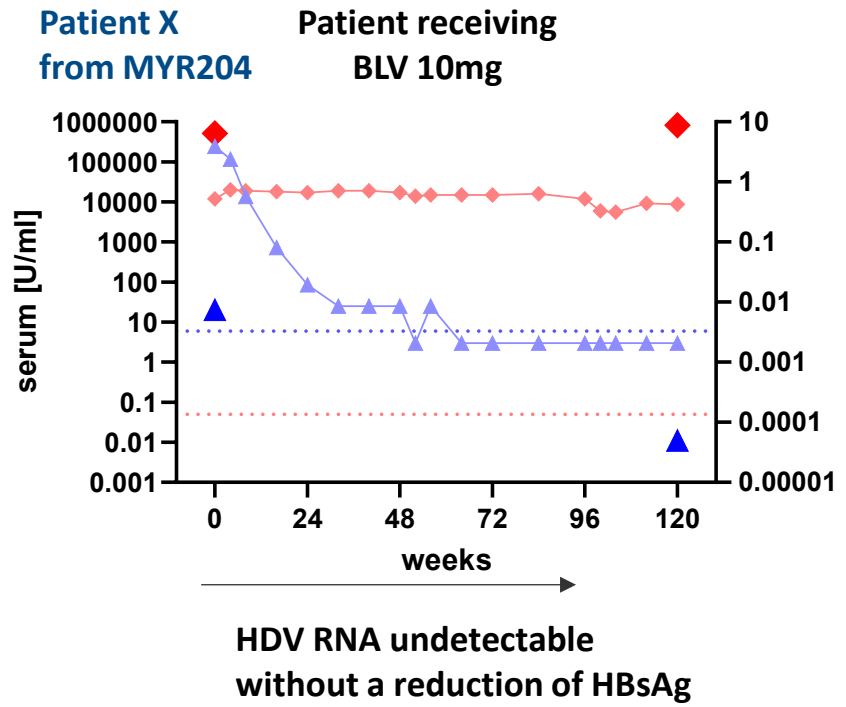
- Can HDV infect cells with HBV integrations efficiently?
- Do these HDV virions contain preS1 and are they infectious?



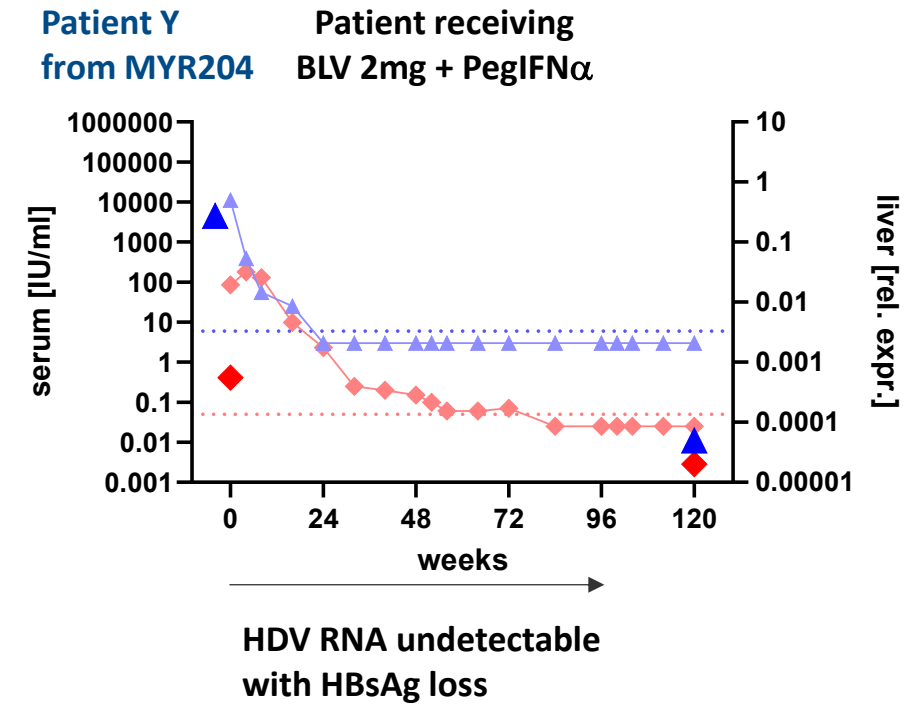
Ringlander et al. J Infect Dis 2024

→ What is the relevance for liver pathology, persistence and treatment outcomes?

Role of integrated HBs for HDV spreading



- ▲— serum HDV RNA
- ▲— liver HDV RNA
- ◆— serum HBsAg
- ◆— liver HBsAg RNA

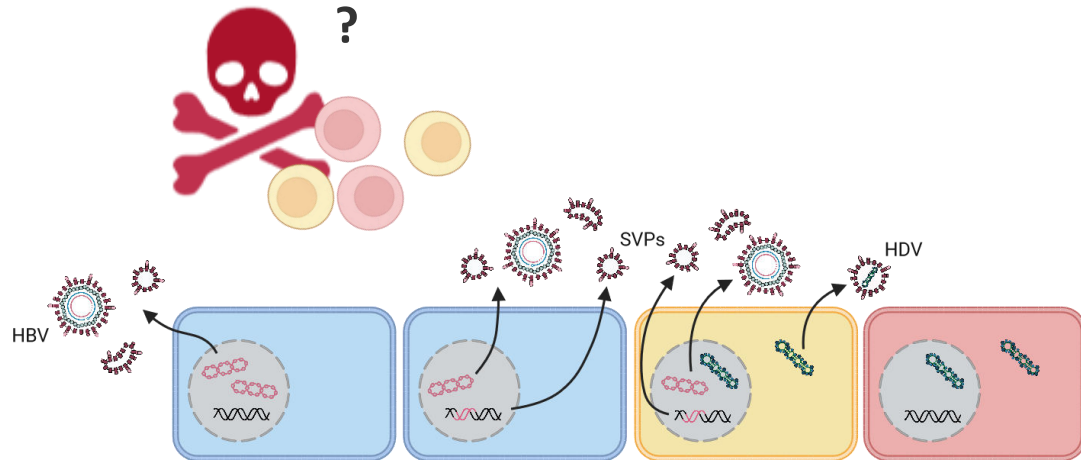


Allweiss et al. EASL 2024

BLV or BLV+PegIFN α treatment led to undetectable HDV RNA post-treatment in many patients without HBsAg loss.

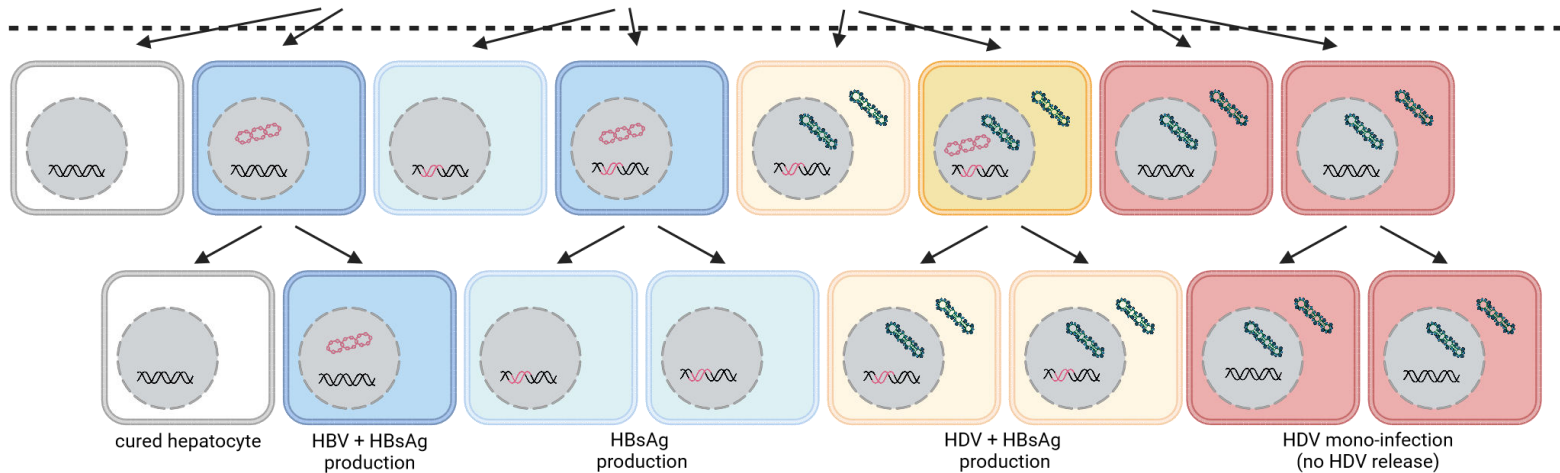
→ Is a reduction/elimination of integrated HBs necessary for longterm control of HDV?

Impact of infection heterogeneity



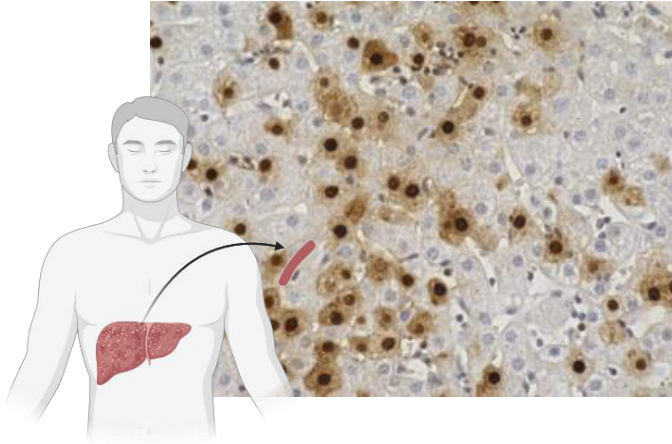
CHD induces liver inflammation and damage through several not completely defined pathways

→ hepatocyte populations might respond differently



Liver inflammation and cell death leads to proliferation

→ Proliferation of these populations will affect HDV, integrations, and cccDNA in different ways

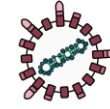


- Heterogenous populations of non/HDV/HBV/integrated hepatocytes
- High abundance of HDV-monoinfected cells
- High abundance of HBV integrations which are able to support HDV release
- These populations will affect persistence, pathology and treatment outcome

Many open questions...

- Preclinical and clinical research – also in liver biopsies – is essential to understand HDV pathology and optimize treatment options
- Single cell analyses/spatial transcriptomics/multiplex stainings in biopsies will be key to solve this complexity
- Biomarkers to detect HBs and HDV from different sources are needed for monitoring treatment outcomes

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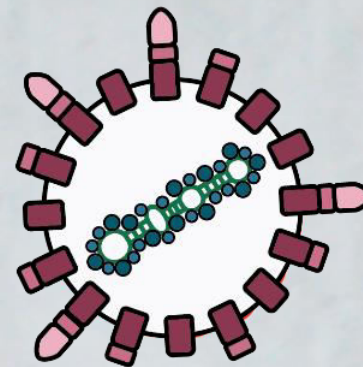


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BIOLOGY OF THE HEPATITIS B
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University Medical Center
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