Medical Faculty Heidelberg

The scientific journey of bulevirtide / Hepcludex

Discovery development and mode of action

Stephan Urban, PhD

Department of Infectious Diseases, Molecular Virology, University Hospital Heidelberg, Germany

German Center for Infectious Diseases (DZIF), TTU Hepatitis, Heidelberg, Germany

Disclosures

consulting or speaking and/or research grants:

Gilead, Humabs, VirBio, Pepperprint, BMS, Galapagos; MSD, Hepatera, MYR GmbH;

I am a patent holder and inventor on patents protecting bulevirtide/Hepcludex

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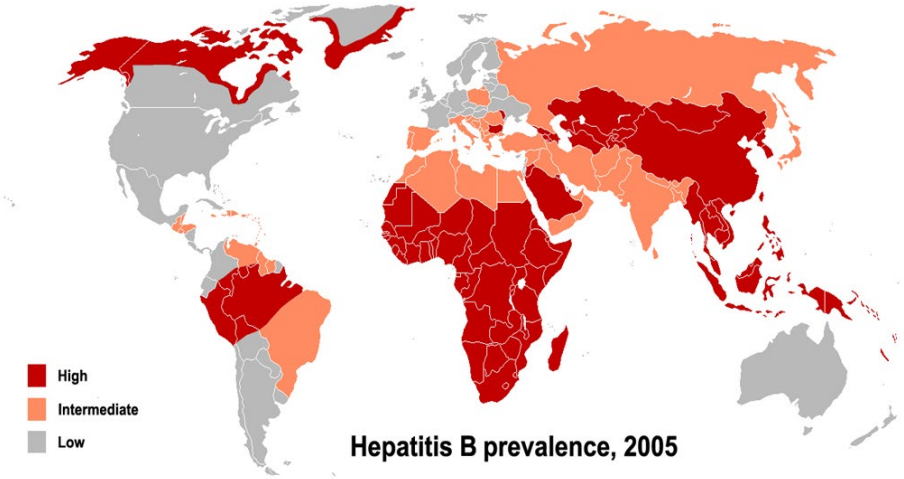
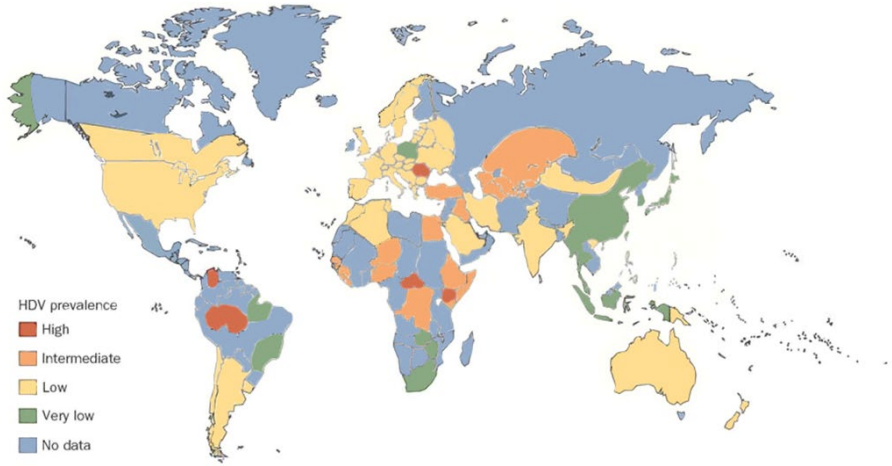
-
  - *Hepatera LLC (Mocsow, Russia)*
  - *MYR GmbH (Bad Homburg, Germany)*
  - *The German Center for Infectious Diseases (DZIF)*

Hepatitis B Virus (HBV) and Hepatitis D Virus (HDV) infection

- ~ 240 million people are chronically infected with HBV
- ~ 650.000 people die each year due to HBV-related liver diseases (cirrhosis and carcinoma)
- ~ 25 million people are HBV/HDV co-infected; chronic HDV-infection is the most severe form of hepatitis



WHO



Wedemeyer and Manns, Nature Reviews Gastroenterology 2010

Areas of high endemicity:
South East Asia, Africa , South America

Mother to child transmission in Asia and Africa:
Entry inhibition & early vaccination prevents chronification

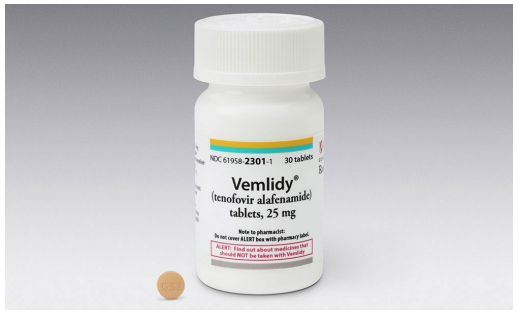
Areas of high endemicity:
Africa, South America, Pacific Islands, Mongolia, Turkey, Russia

HDV is probably "under-diagnosed"
Lack of accurate epidemiological data

Treatment regimens for chronic Hepatitis B and D

Entecavir/ETV/Baraclude
(BMS, approved 2006)

Tenofovir/TDF/Viread
(Gilead, approved 2002/2008)



TAF/Vemlidy
(Gilead, approved 2015)

Interferon a, PEG Interferon a
(Roche, MSD)

Nucleoside analogs: (Entecavir, Viread/Tenofovir, TAF)

⇒ suppression of viral load; ALT normalization; infinite therapy

⇒ very low rates of cure (HBsAg loss) ⇒ no effect on HDV infection

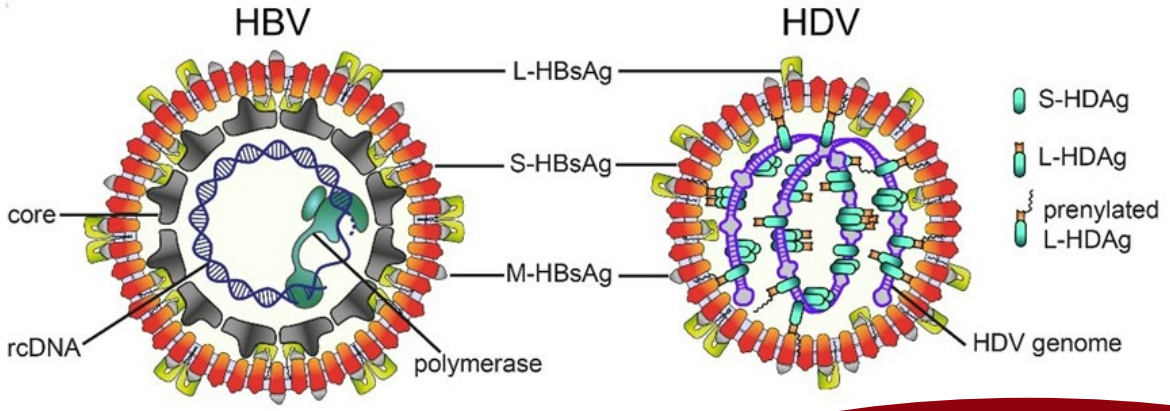
Interferons (IFNα /PEG-IFNα):

⇒ severe side effects ⇒ low rate of HBsAg loss

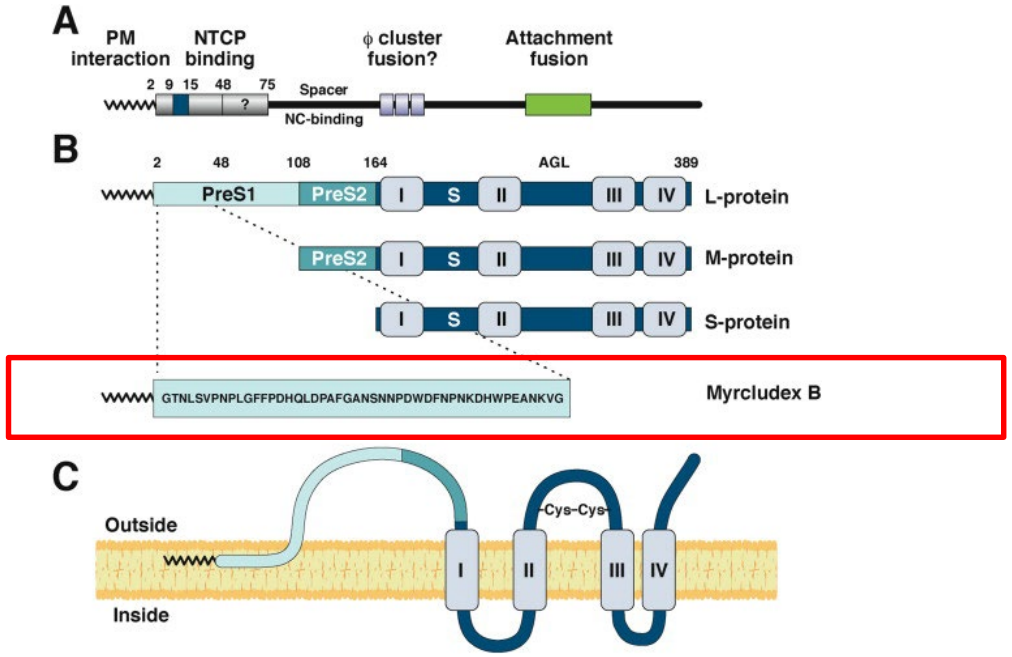
⇒ limited effect on HDV in eligible patients ⇒ long term relapses (not approved for HDV treatment)

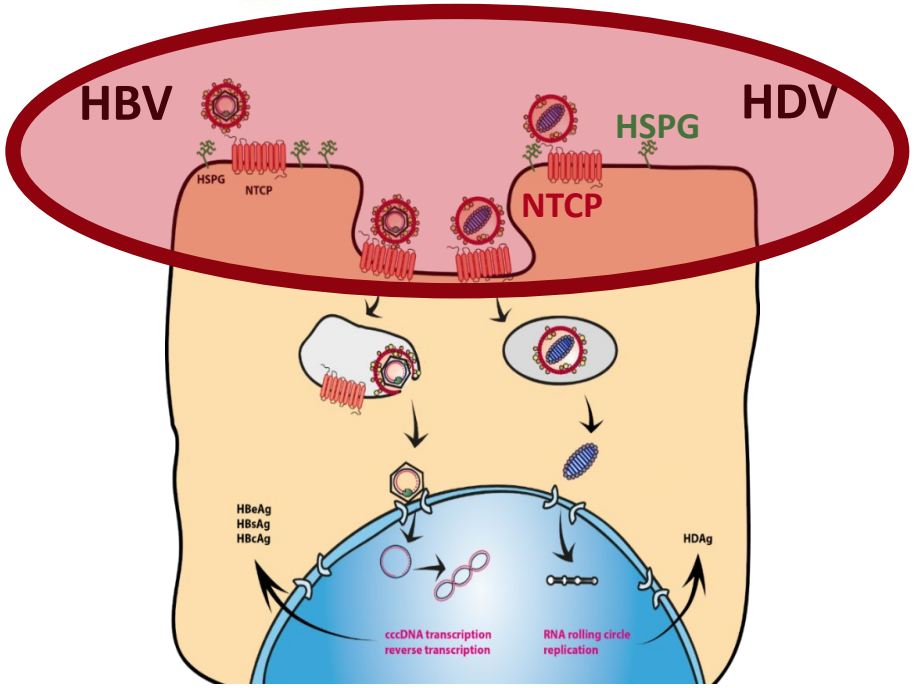
- *Currently approved therapeutic regimens for HBV are not curative*
- *No specific treatment for HDV available (until recently, when bulevirtide was approved in Europe)*
- *Medical need to improve HBV therapies; high medical need to develop effective HDV therapies*

HBV and HDV share the same envelope proteins and use identical receptors



Infectivity determinants within envelope proteins



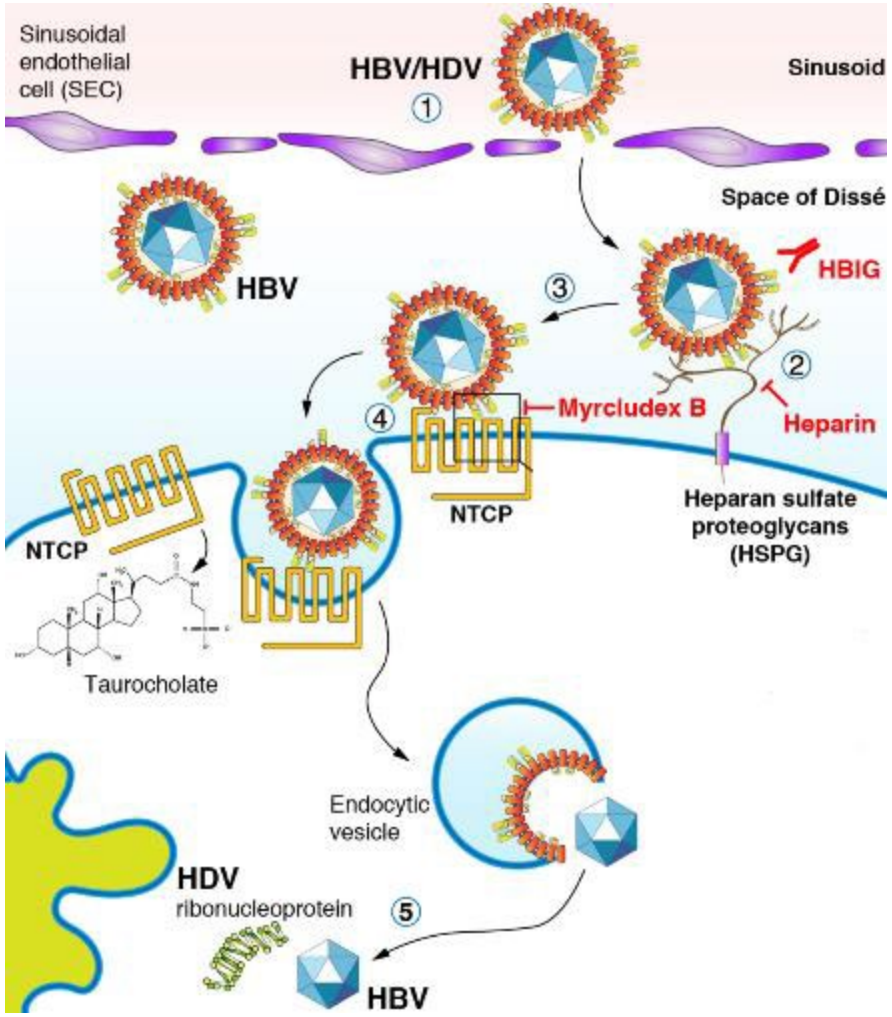


HBV and HDV persist as circular episomes in the nucleus of hepatocytes

Formation of episomes can be efficiently blocked by Hepcludex/bulevirtide

*Urban, Bartenschlager, Kubitz Zoulim, Gastroenterology, 2014

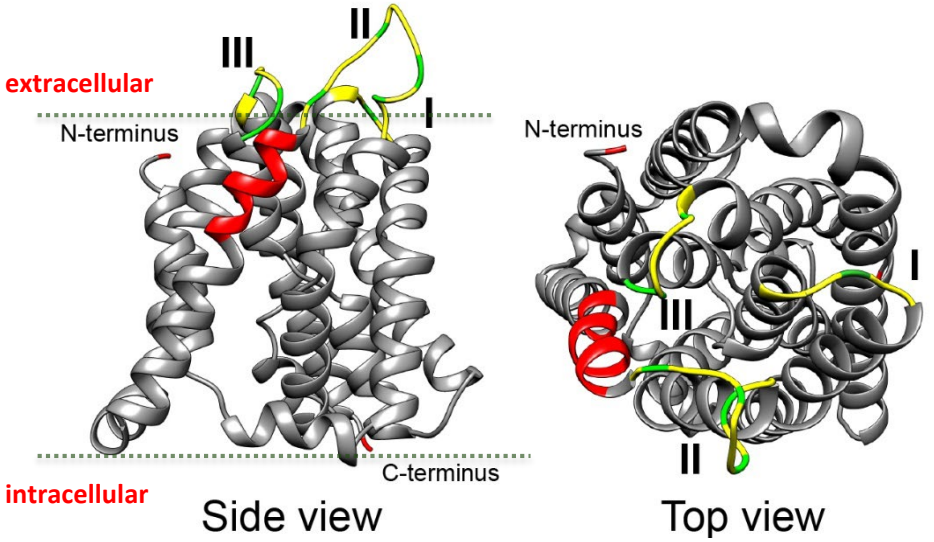
HBV and HDV entry and entry inhibition



Urban et al, Gastroenterology, 2014; Lempp & Urban, Intervirology, 2014, Urban, Hepatology 2015

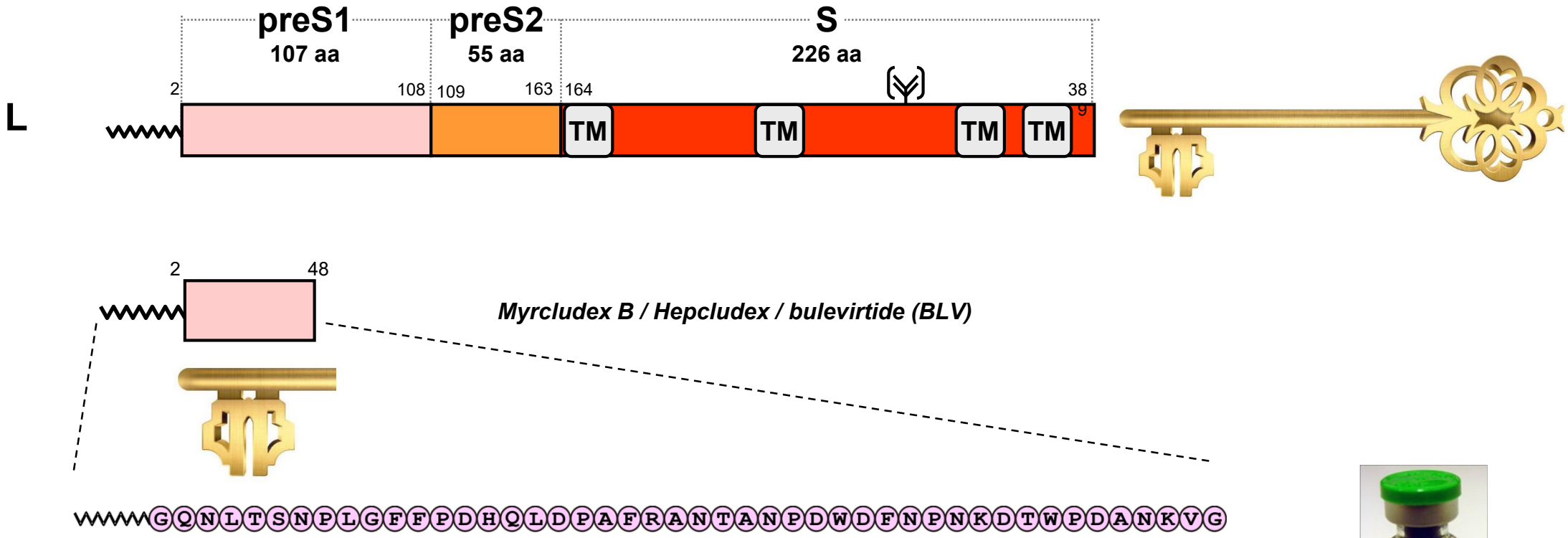
1. Viruses must enter the Space of Dissé ⇒ Size limitations by fenestrated endothelium
2. Attachment to Heparan Sulfate Proteoglycans (HSPG) ⇒ in part specific and mandatory
3. Envelope rearrangement, release of the receptor binding site ⇒ close or within the membrane
4. Formation of a highly specific Virus/NTCP complex ⇒ irreversible; probably requires trimerization

Sodium Taurocholate Co-transporting Polypeptide NTCP:
the major hepatic bile salt transporter



Yan et al., eLife 2012
Ni *et al.,* Gastroenterology 2013

The concept of receptor-targeted entry inhibition: The key bit irreversibly blocks the lock



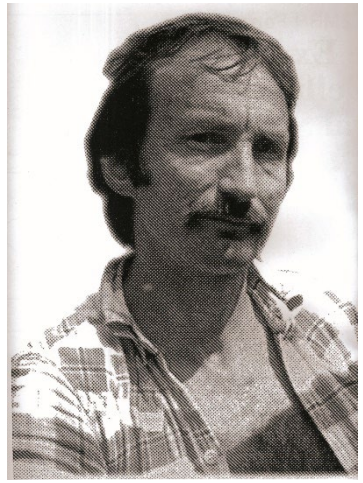
A chemically synthesized pepdidic receptor ligand (after screening hundreds of peptides):

Myrcludex B / Hepcludex / bulevirtide (BLV)

Gripon P, et al., *J.Virol*, 2005; Schulze A, et al., *J. Virol*, 2010

Myrcludex B

Once upon a time as a post-doc in the laboratory of Heinz Schaller at the ZMBH, 1996

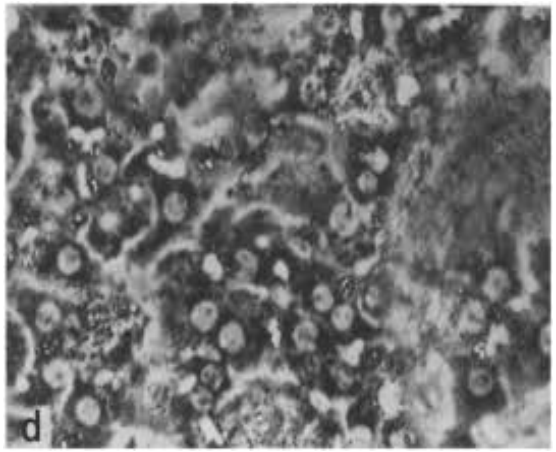


Heinz Schaller
ZMBH Heidelberg

Peter Hans
Hofschneider
MPI-Martinsried

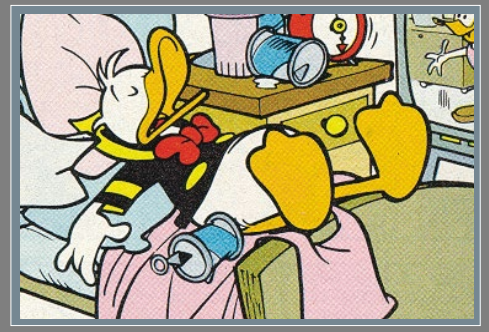
2-3 weeks old Pekin Duck

Primary duck hepatocytes

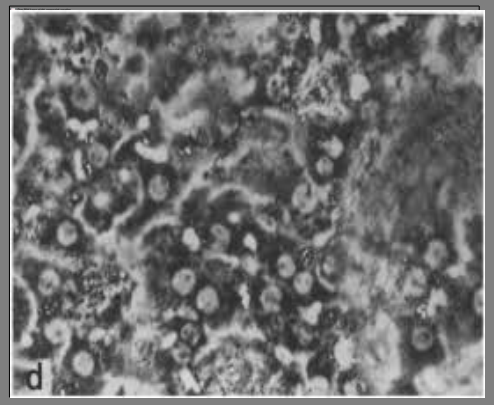


A duck hepatitis B virus (DHBV) based infection competition assay

2-3 weeks old Peking duck



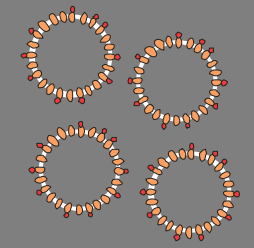
liver perfusion



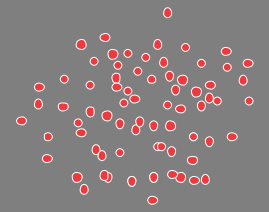
DHBV

infection
12 h, 37°C.

subviral particles

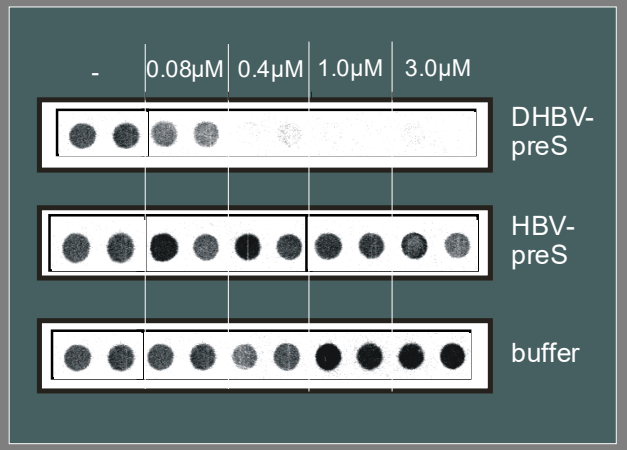


+

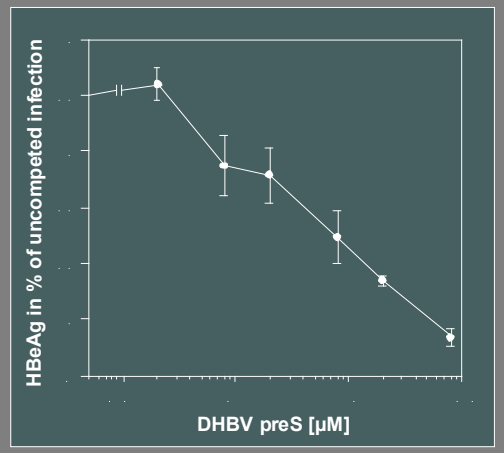


recombinant preS-polypeptides

Immuno/DNA dot blot



quantification



Aim: Identification of the DHBV receptor in order to get the human hepatitis B virus (HBV) receptor.

Carboxypeptidase D as an essential receptor for avian hepadnaviruses…

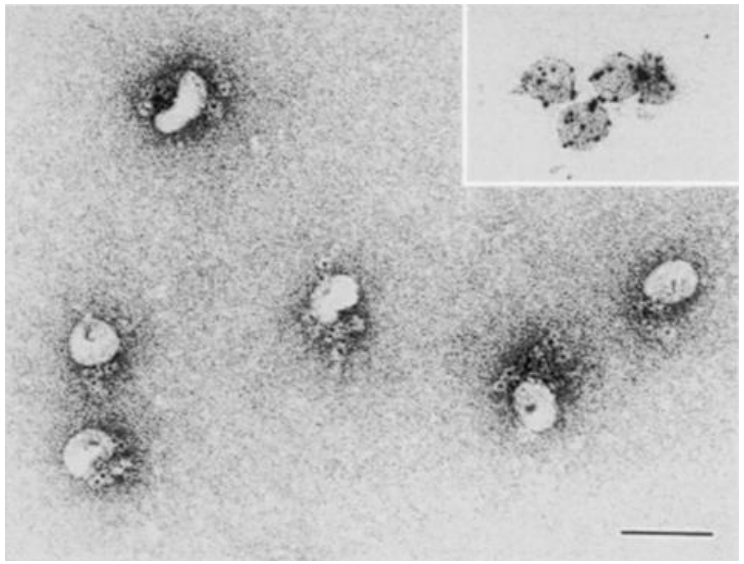
Avian hepatitis B virus infection is initiated by the interaction of a distinct pre-S subdomain with the cellular receptor gp180.
Urban S, Breiner KM, Fehler F, Klingmüller U, Schaller H.
J Virol. 1998 Oct;72(10):8089-97. doi: 10.1128/JVI.72.10.8089-8097.1998.

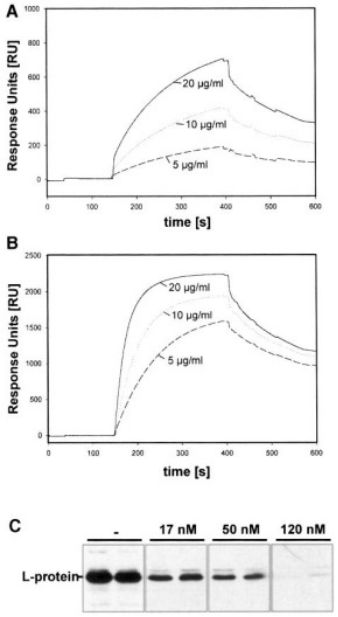
Carboxypeptidase D (gp180), a Golgi-resident protein, functions in the attachment and entry of avian hepatitis B viruses.
Breiner KM, Urban S, Schaller H.
J Virol. 1998 Oct;72(10):8098-104. doi: 10.1128/JVI.72.10.8098-8104.1998.

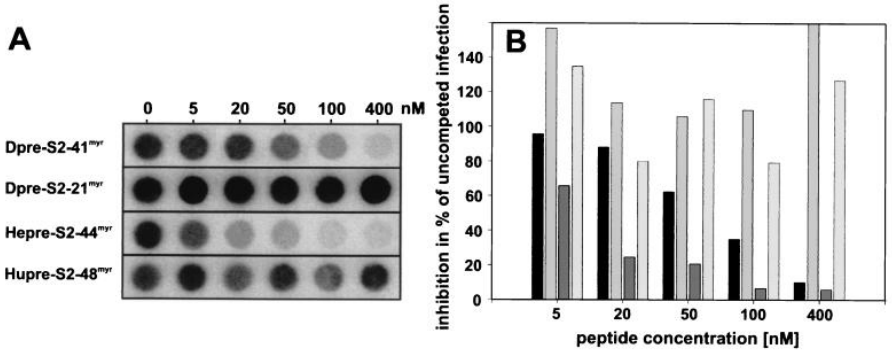
A soluble form of the avian hepatitis B virus receptor. Biochemical characterization and functional analysis of the receptor ligand complex.
Urban S, Kruse C, Multhaup G.
J Biol Chem. 1999 Feb 26;274(9):5707-15. doi: 10.1074/jbc.274.9.5707.

Receptor recognition by a hepatitis B virus reveals a novel mode of high affinity virus-receptor interaction.
Urban S, Schwarz C, Marx UC, Zentgraf H, Schaller H, Multhaup G.
EMBO J. 2000 Mar 15;19(6):1217-27. doi: 10.1093/emboj/19.6.1217.

Inhibition of duck hepatitis B virus infection by a myristoylated pre-S peptide of the large viral surface protein.
Urban S, Gripon P.
J Virol. 2002 Feb;76(4):1986-90. doi: 10.1128/jvi.76.4.1986-1990.2002.



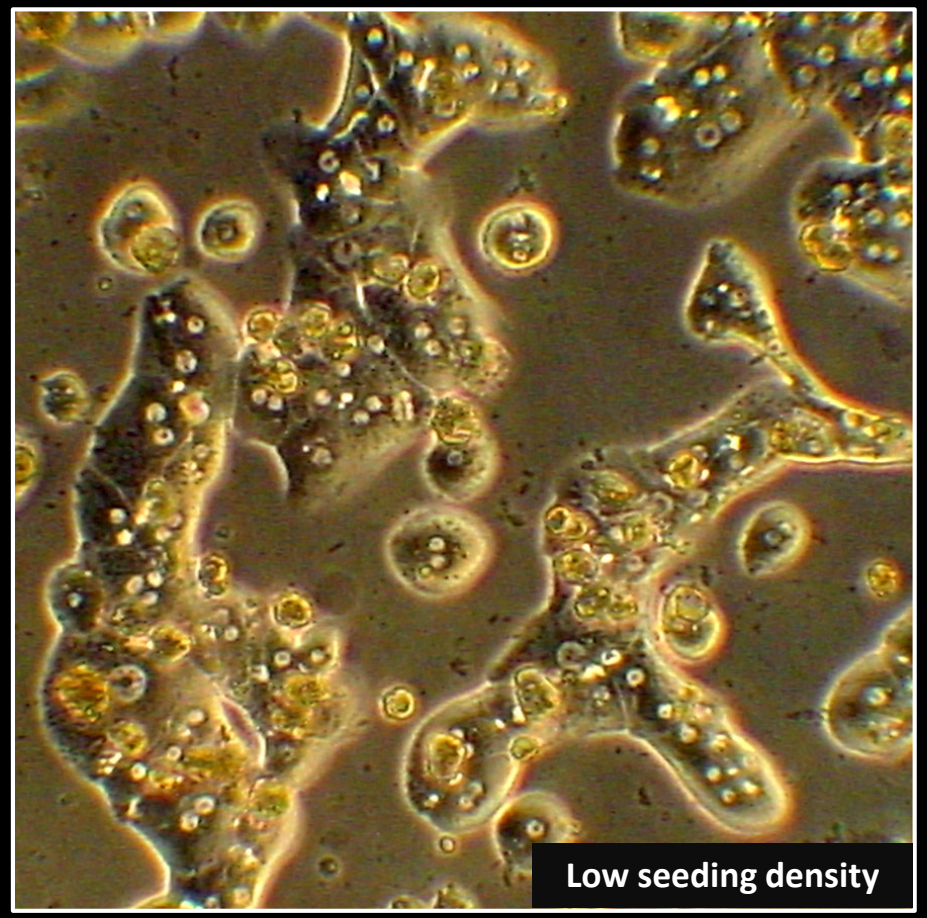


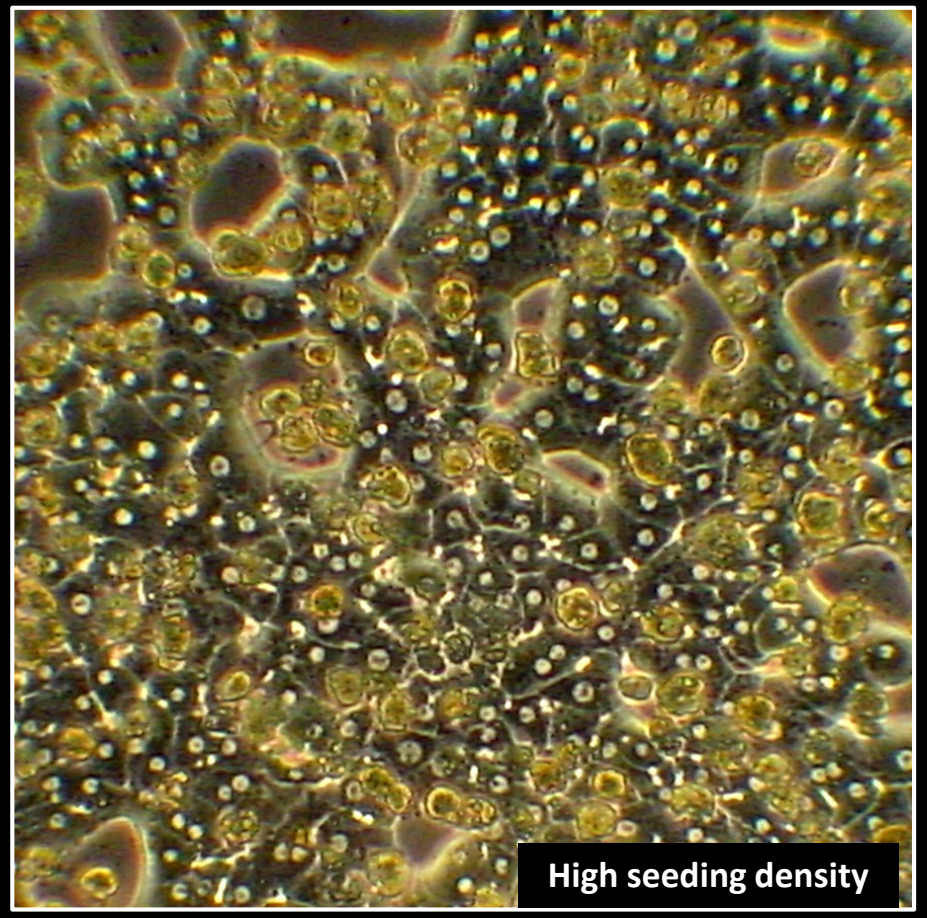


=> The HBV receptor was not identified but the concept of entry inhibition by envelope derived peptides was born.

A big problem at that time: The lack of cell culture systems for Hepatitis B Virus

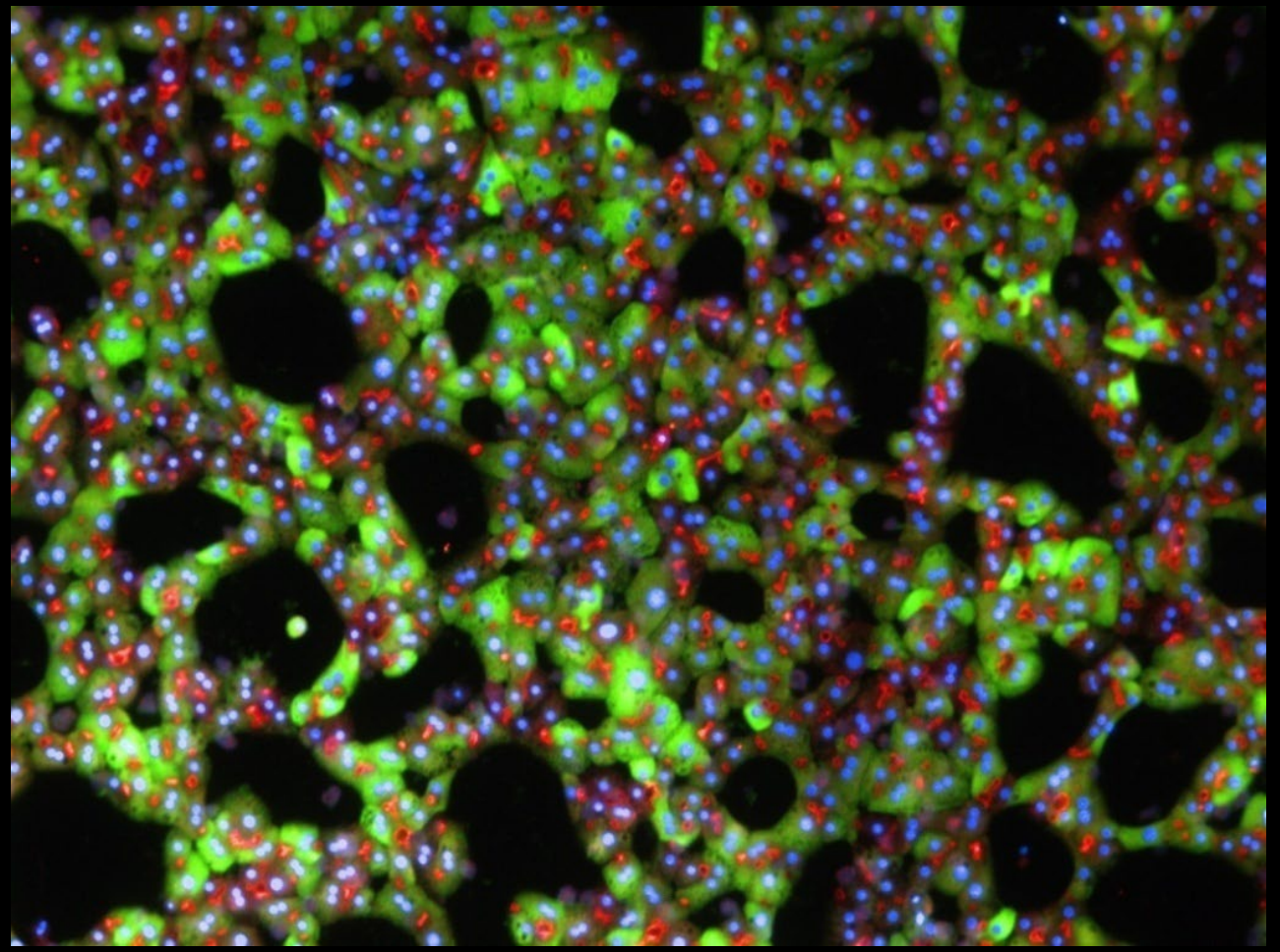
Primary human hepatocytes….





Primary human hepatocytes were required to study HBV/HDV infection

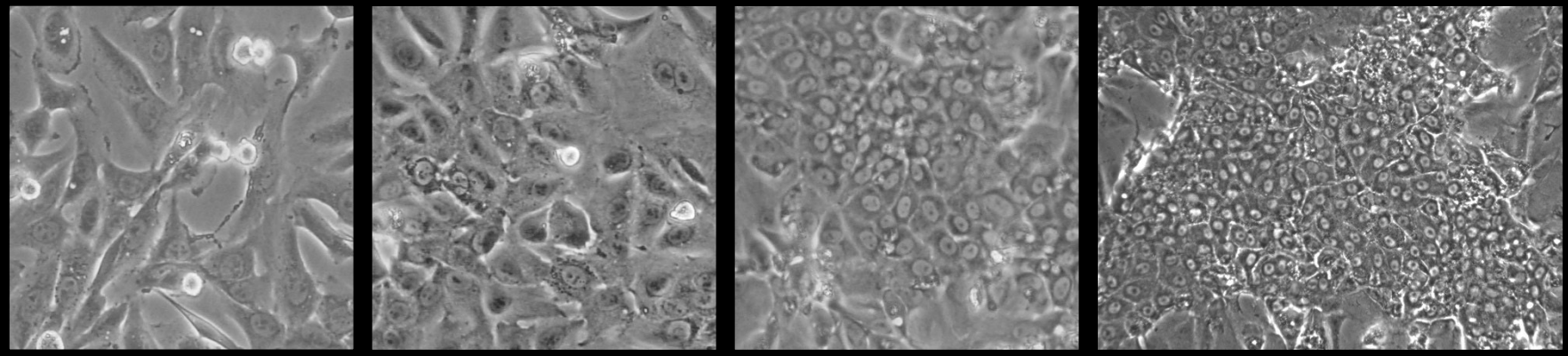
….are highly susceptible for HBV infection but poorly available



HBsAg = infizierte Zellen
DAPI = Zellkerne
MRP2 = Gallenkanäle

In 2002, HepaRG cells were described as the first cell culture system to study HBV infection

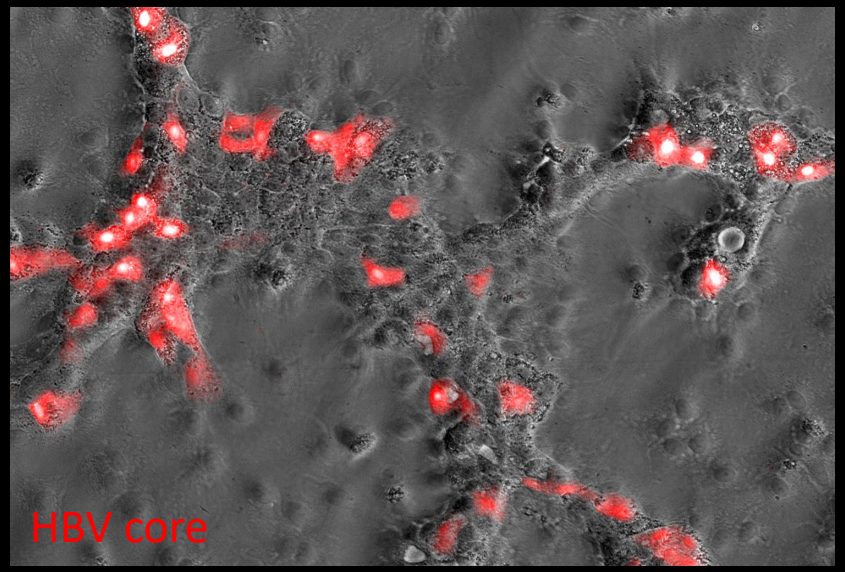
experimental differentiation during 3-4 weeks….



…. induces susceptibility to HBV infection.

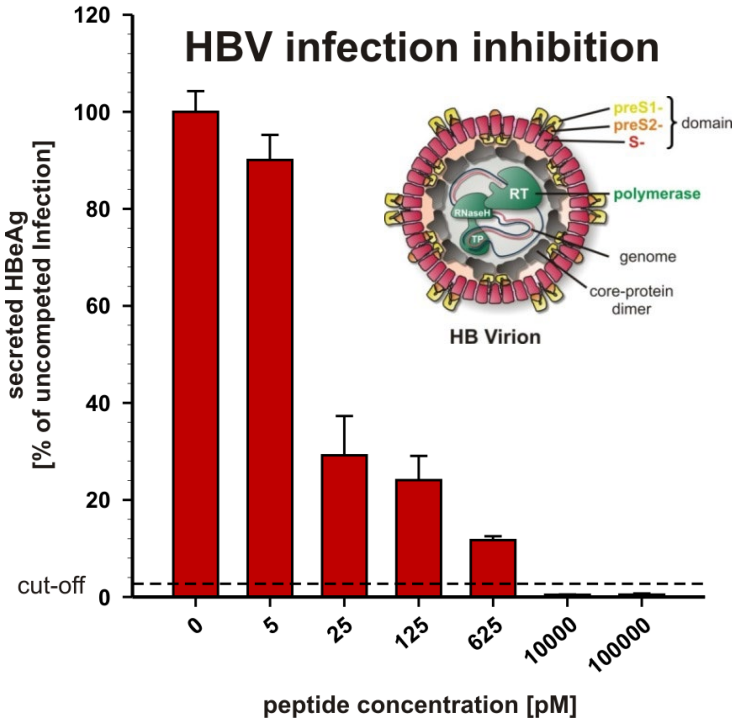
Gripon, Rumin, Urban *et al.,* PNAS, 2002

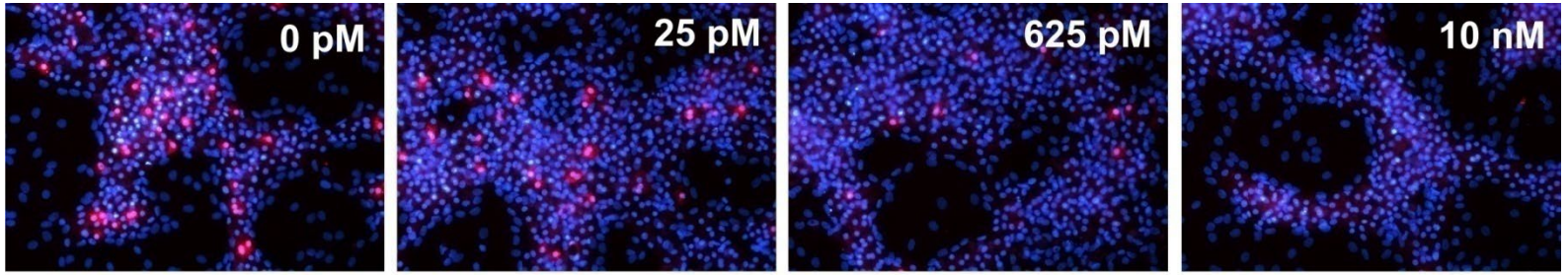
Philippe Gripon 2005



⇒ **Limited susceptibility of a subpopulation of hepatic cells**

Bulevirtide/Hepcludex inhibits both HBV and HDV infection with high potency at subnanomolar concentrations



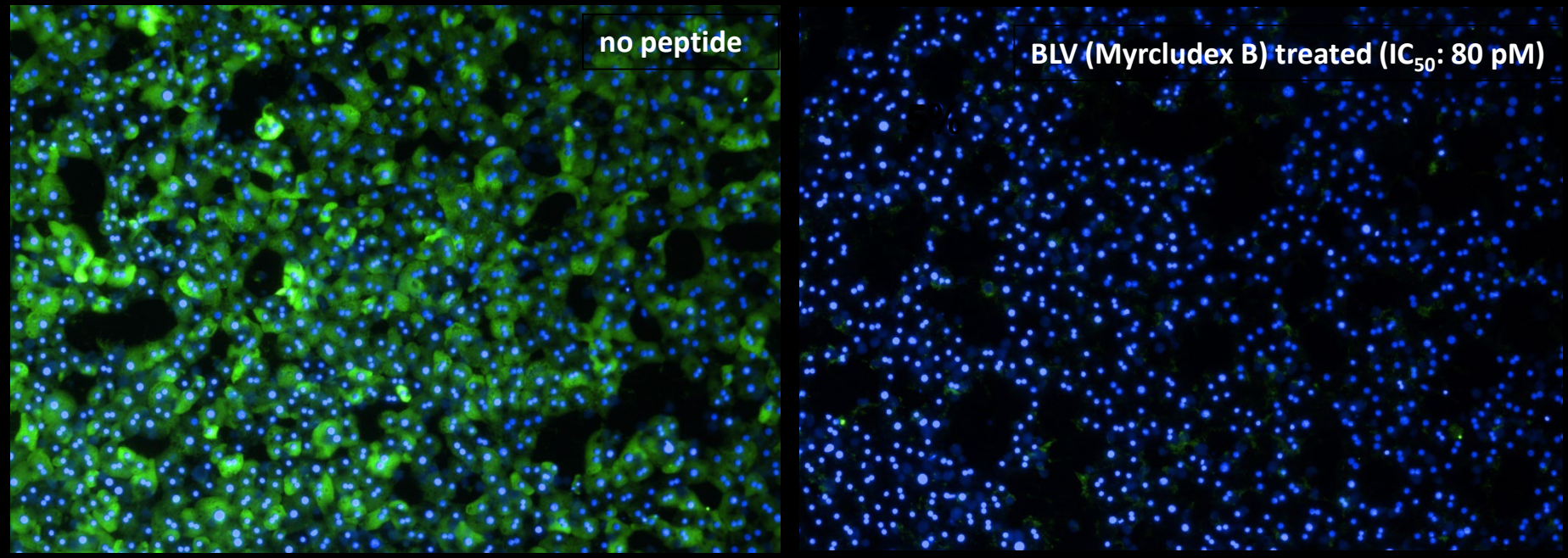


HBcAg, DAPI

Gripon et al., PNAS, 99 (24) 2002
Urban et al., J. Virol, 79 (3), 2005
Glebe et al., Gastroenterology, 129, 2005
Engelke et al., Hepatology, 43, 2006
Schulze et al., Hepatology, 46, 2007

Bulevirtide (BLV) completely blocks HBV infection of primary human hepatocytes (PHH)

Infection of PHH with HBV in the absence and the presence of BLV

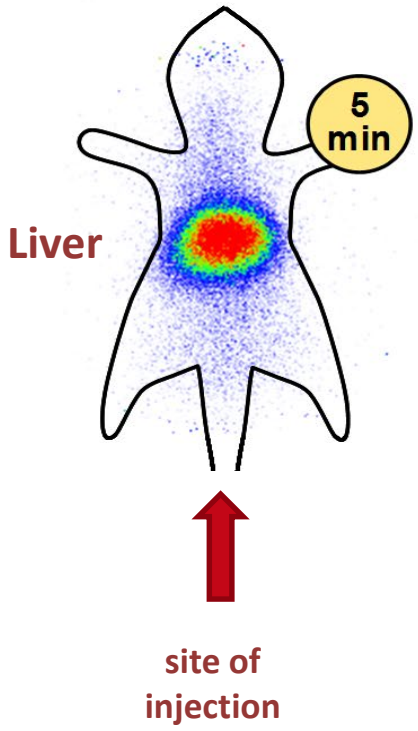


HBsAg = day 15 p.i.
DAPI = nuclei

Bulevirtide accumulates in the liver of mice

conserved domain

Stearoyl-GQNLSTSNPLGFFPDHQLDPAFRANTANPDWDFNPNKDTWPDANKVGy-I^{125}



Which molecule is addressed ?

Sodium taurocholate co-transporting polypeptide (NTCP) is the receptor for HBV/HDV and the target of Bulevirtide

Wenhui Li, Baruch Blumberg Prize 2021

Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus

Huan Yan[1,2†], Guocai Zhong[2†], Guangwei Xu[2], Wenhui He[2,3], Zhiyi Jing[2], Zhenchao Gao[1,2], Yi Huang[2,3], Yonghe Qi[2], Bo Peng[2], Haimin Wang[2], Liran Fu[2,3], Mei Song[2,3], Pan Chen[2,3], Wenqing Gao[2], Bijie Ren[2], Yinyan Sun[2], Tao Cai[2], Xiaofeng Feng[2], Jianhua Sui[2], Wenhui Li[2]*

[1]Graduate program in School of Life Sciences, Peking University, Beijing, China; [2]National Institute of Biological Sciences, Beijing, China; [3]Graduate program in Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Yan et al., Elife, 2012

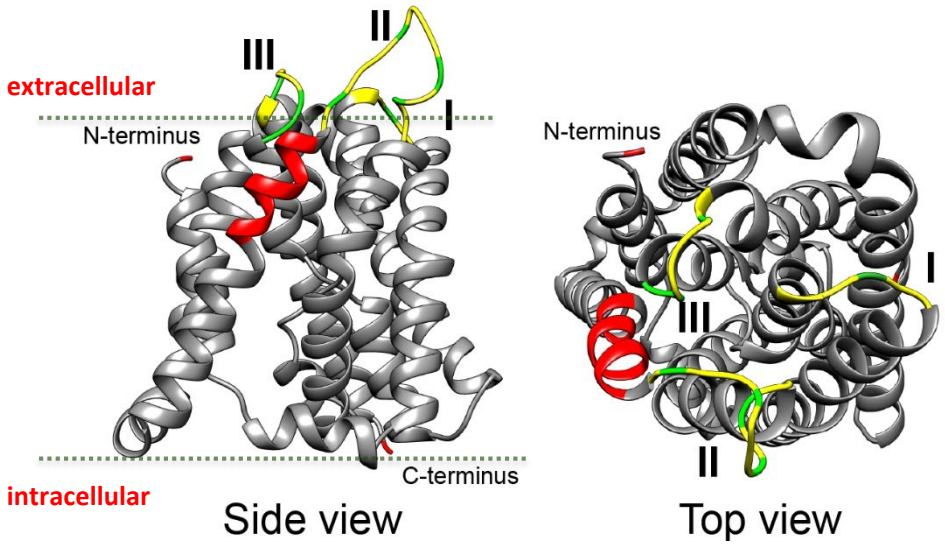
Hepatitis B and D Viruses Exploit Sodium Taurocholate Co-transporting Polypeptide for Species-Specific Entry nto Hepatocytes

Yi Ni,[1] Florian A. Lempp,[1] Stefan Mehrle,[1] Shirin Nkongolo,[1] Christina Kaufman,[1] Maria Fälth,[2] Jan Stindt,[3] Christian Königer,[4] Michael Nassal,[4] Ralf Kubitz,[3] Holger Sültmann,[2] and Stephan Urban[1]

[1]Department of Infectious Diseases, Molecular Virology, University Hospital Heidelberg, Heidelberg, Germany; [2]German Cancer Research Center and National Center for Tumor Diseases, Unit Cancer Genome Research, Heidelberg, Germany; [3]Clinic for Gastroenterology, Hepatology and Infectiology, University Hospital Düsseldorf, Düsseldorf, Germany; and [4]Department of Internal Medicine II, University Hospital Freiburg, Freiburg, Germany

Ni *et al.*, Gastroenterology 2013

NTCP, the major hepatic bile salt transporter



- ***NTCP is an integral transmembrane protein.***
- ***transports bile salts from blood into hepatocytes.***
- ***bulevirtide/Hepcludex inhibits bile salt transport (IC_{50} 50 - 100 nM).***

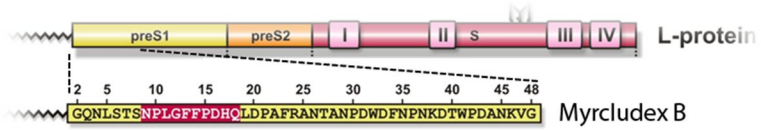
⇒ ***BLV treatment induces elevated bile salt levels at high doses***

⇒ ***Possible side effects might be related to impairment of bile acid and NTCP substrate transport.***

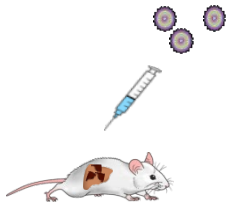
Urban et al., Gastroenterology, (review) 2014

The long way to the first in man trial: Safety in phase Ia clinical trial

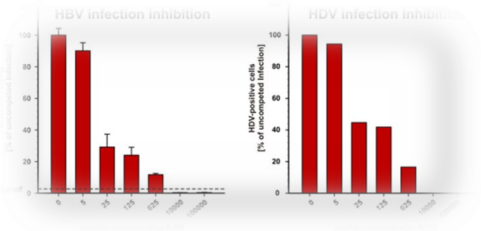
2001 **2009** **2011**

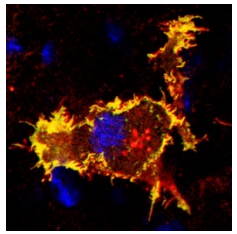


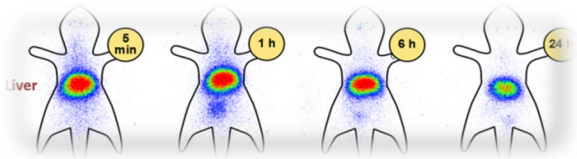
HBV



HBV Dane particle



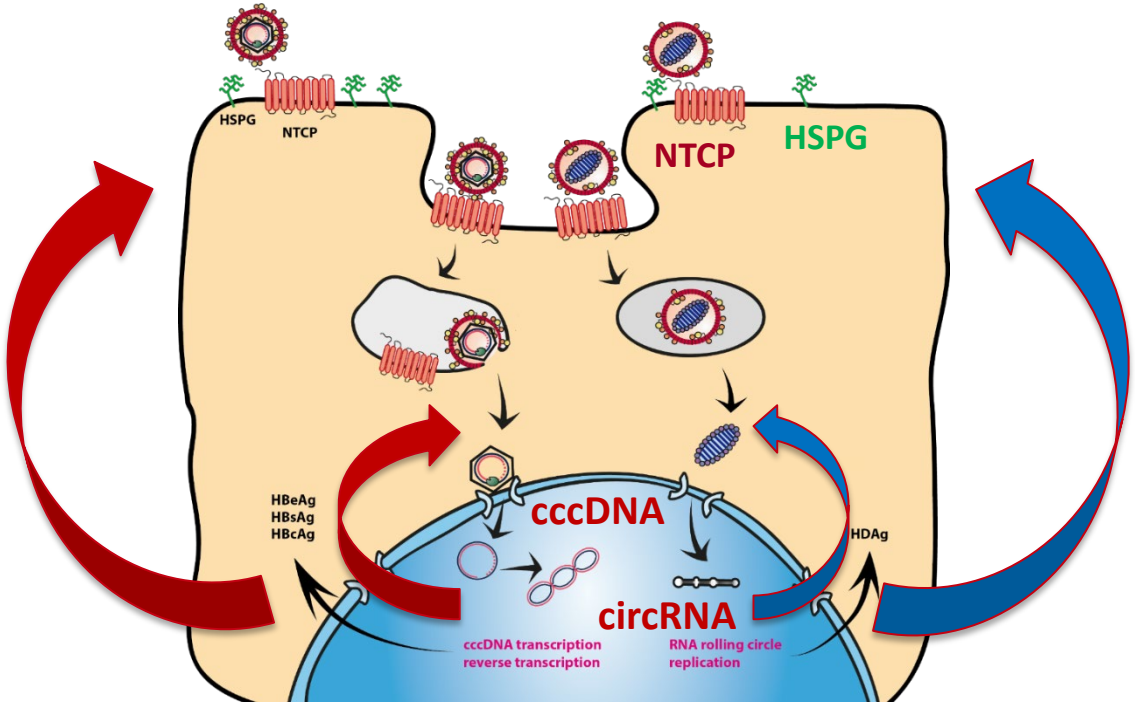




Clinical efficacy of Hepcludex / bulevirtide (formerly Myrcludex B)

in HBV-infected and HBV/HDV co-infected patients

The mode of action of an entry inhibitor in a persistant HBV/HDV infection



Schulze et al., Hepatology 2007; Yan et al., eLIFE 2012; Ni et al., Gastroenterology 2013

- Both HBV and HDV establish circular episomes (cccDNA, circRNA) in the nucleus of infected hepatocytes
- Dynamic replenishment of episomes (by intra and extracellular routes) is crucial for persistence

Does entry inhibition (= blocking the extracellular route) contribute to clearance of HBV/HDV episomes in chronically infected patients?

The Myr202-trial: Blocking „only“ de novo infection in HDV infected patients

Articles

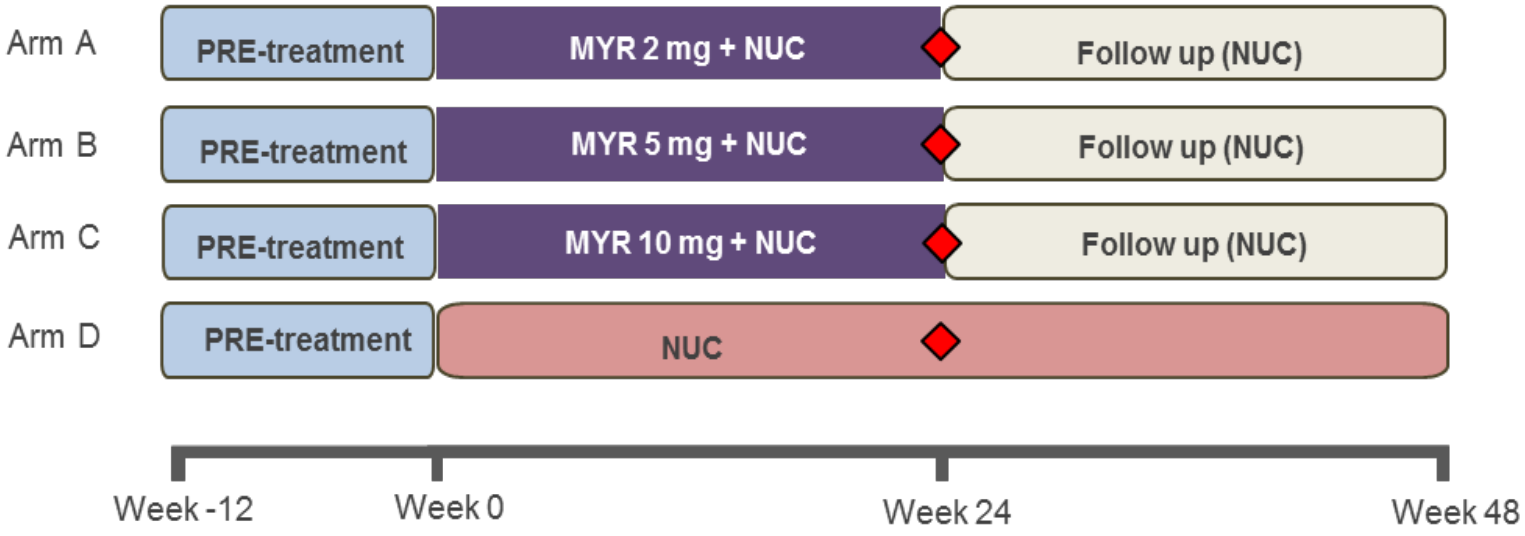
Safety and efficacy of bulevirtide in combination with tenofovir disoproxil fumarate in patients with hepatitis B virus and hepatitis D virus coinfection (MYR202): a multicentre, randomised, parallel-group, open-label, phase 2 trial

Heiner Wedemeyer, Katrin Schöneweis, Pavel Bogomolov, Antje Blank, Natalia Voronkova, Tatiana Stepanova, Olga Sagalova, Vladimir Chulanov, Marina Osipenko, Viacheslav Morozov, Natalia Geyvandova, Snezhana Sleptsova, Igor G Bakulin, Ilsiyar Khaertynova, Marina Rusanova, Anita Pathil, Uta Merle, Birgit Bremer, Lena Allweiss, Florian A Lempp, Kerstin Port, Mathias Haag, Matthias Schwab, Julian Schulze zur Wiesch, Markus Cornberg, Walter E Haefeli, Maura Dandri, Alexander Alexandrov, Stephan Urban

Lancet Inf. Diseases, 2022

The Myr-202 study design

- 120 HBeAg-neg. patients; randomized into 4 arms - 30 patients per arm
- Pretreatment with tenofovir for at least 12 weeks
- Bulevirtide (Myrcludex B, 2, 5, 10 mg) was self administered (s.c.) once daily
- Patients received tenofovir (TDF, oral qd) during the entire study period*

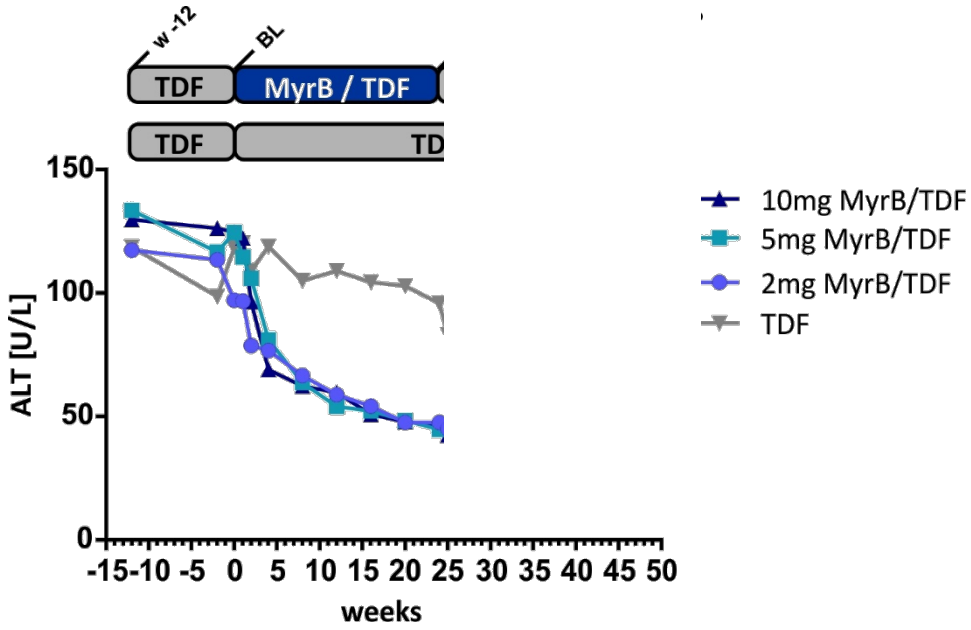


Wedemeyer et al., ILC, 2018, Paris, GS-005

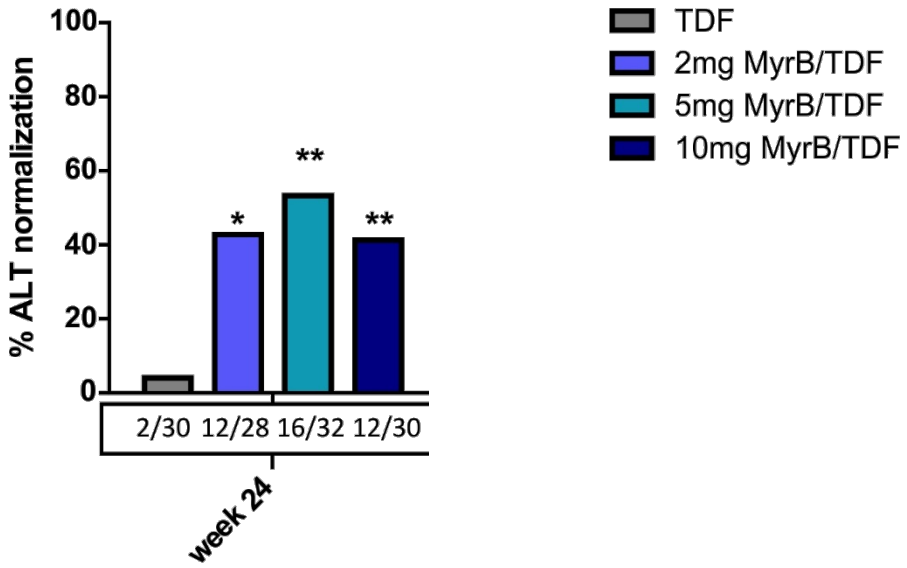
* no drug drug interaction between TDF and bulevirtide has been clinically demonstrated – Blank et al., Clin Pharmacol Ther. 2018

Normalization of ALT during therapy (biochemical responses)

Change of mean ALT levels during the study



ALT normalization

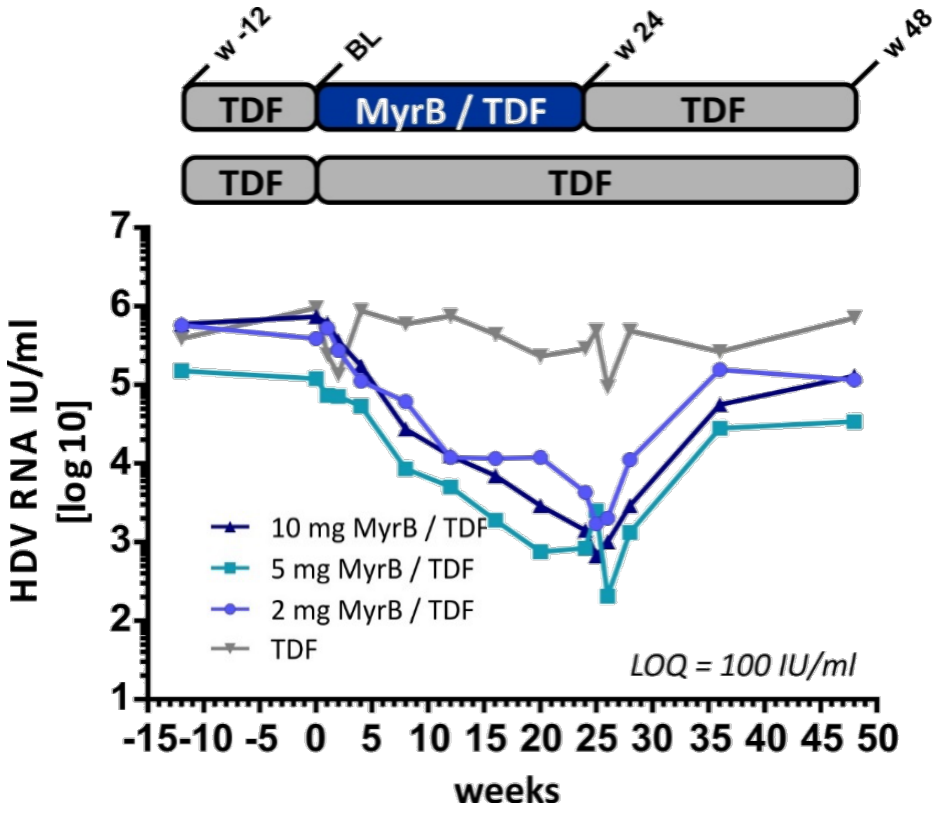


MyrB 2mg vs TDF * p=0.0013; MyrB 5mg vs TDF ** p=0.0002
MyrB 10mg vs TDF ** p=0.0023

- Fast and dose independent normalization of ALT in all BLV treatment arms (no ALT response in TDF arm)
- Increase of ALT levels during follow up (under TDF)

BLV monotherapy induces profound reductions of HDV serum RNA levels

The Myr202-trial



Median RNA log10 change to BL at week 24

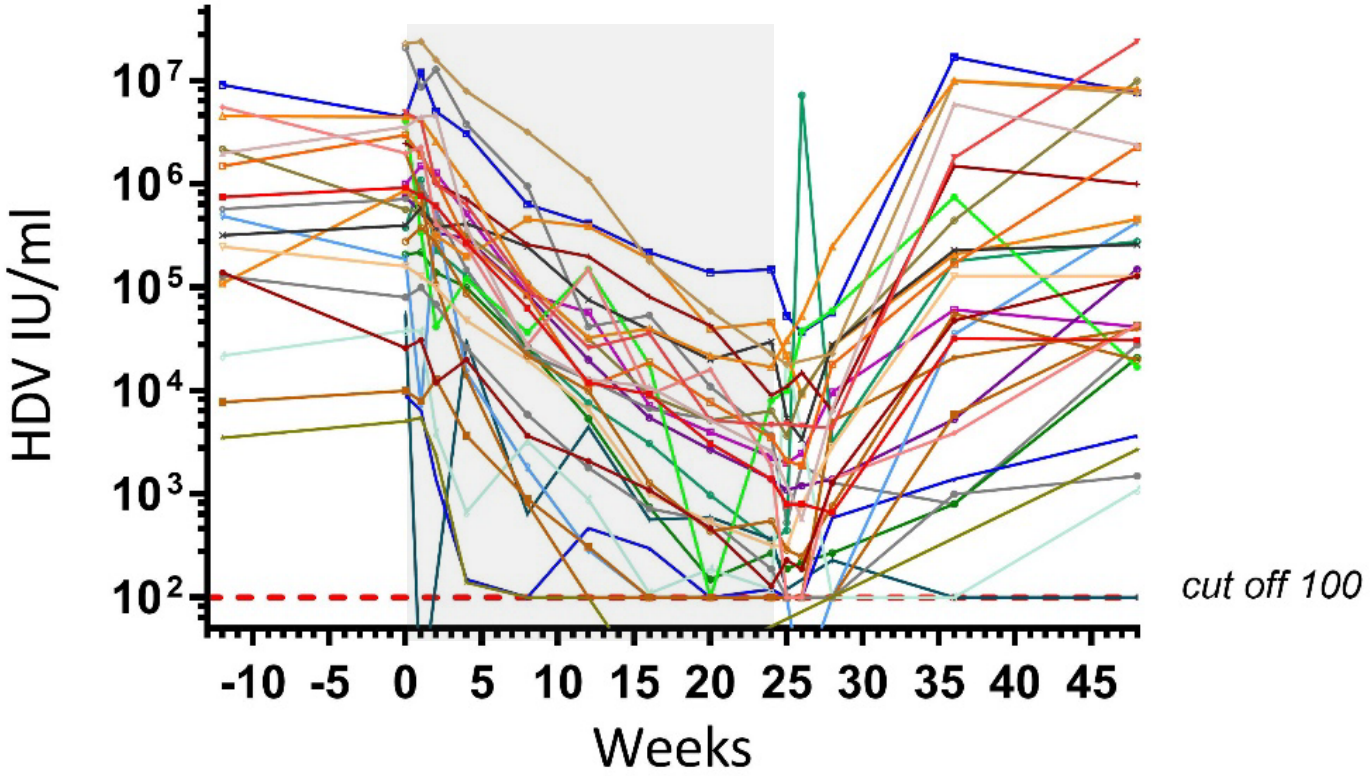
MyrB 2mg: -1.75 **MyrB 10mg: -2.70**

MyrB 5mg: -1.60 TDF: -0.18

Blocking de novo HDV infection results in 500-fold reduction of HDV serum RNA at week 24

⇒ Hypothesis: Rapid turnover (weeks, not months) of HDV-infected hepatocytes

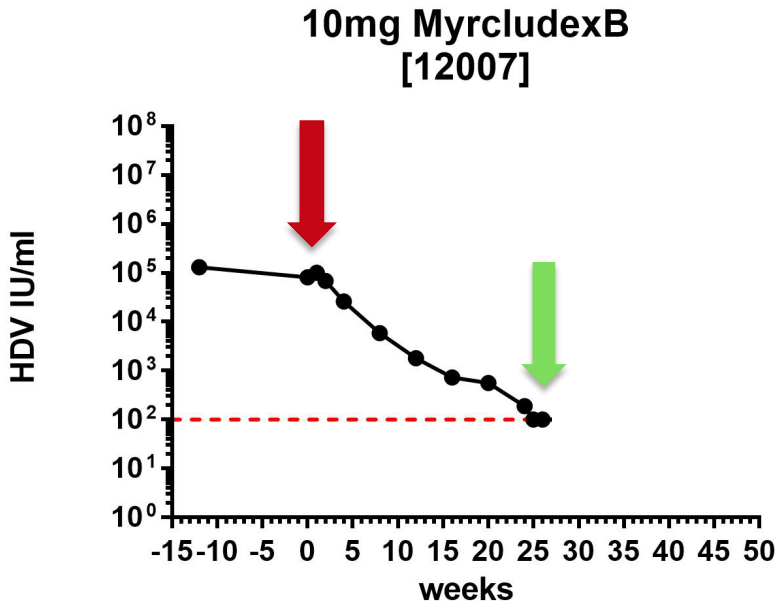
Virological responses in individual patients (10 mg BLV)



- **Patients responded differently to bulevirtide treatment**
- **No break-through under therapy in the 10 mg arm; No resistance**
- **Tendency of HDV rebound to initial levels. Memory for replication space ?**

Schöneweis et al., ILC, 2018, Paris, SAT-369

BLV leads to elimination of HDV infected hepatocytes in patients



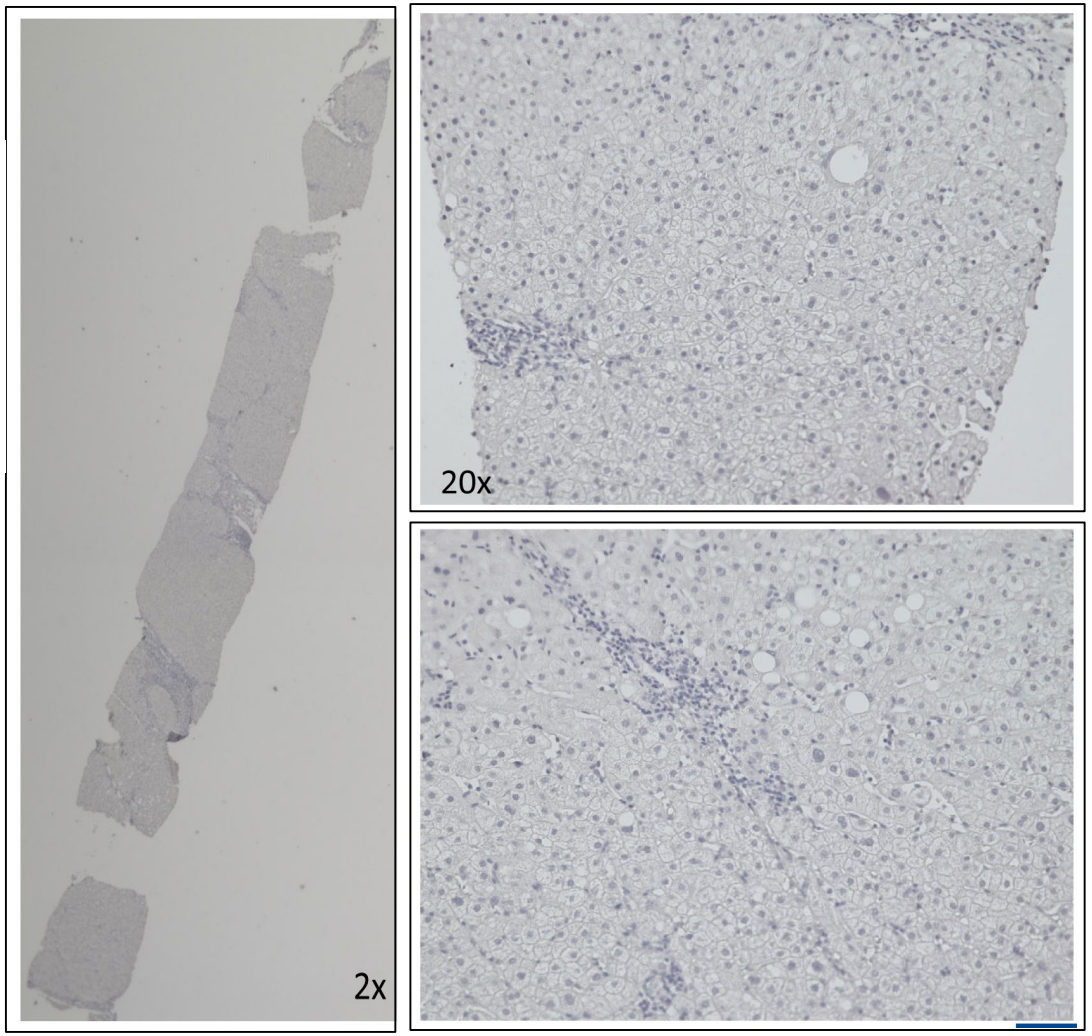
Intrahepatic HDV RNA decline:

0.78log IU/ml in A (n=7) 2mg

1.07log IU/ml in B (n=5) 5 mg

1.34log IU/ml in C (n=7) 10 mg

0.30log IU/ml in D (n=3) TDF

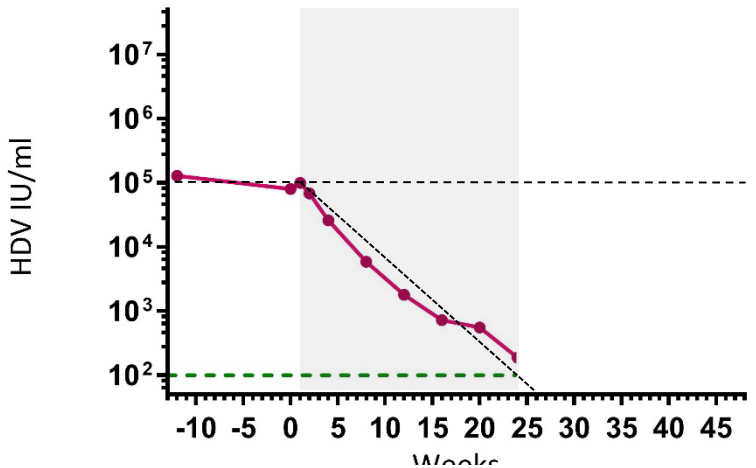


Allweiss et al., ILC, 2018, Paris, PS-162

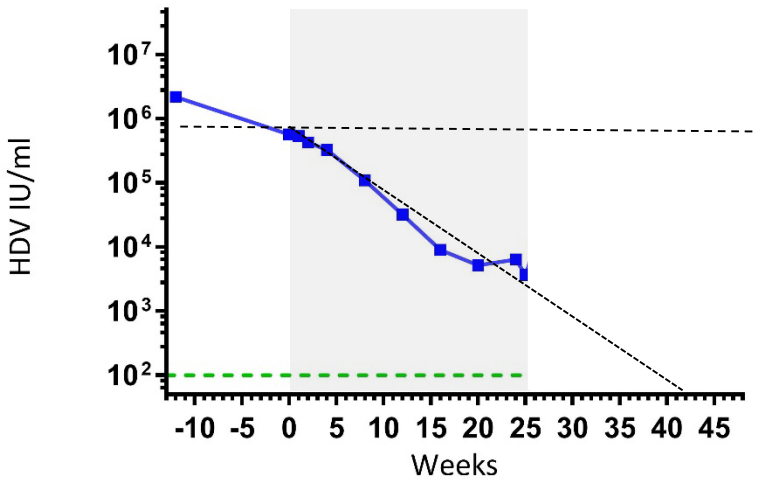
Curative potential of BLV long term monotherapy in HDV infected patients

HDV serum RNA declines in most patients follow a zero order kinetics

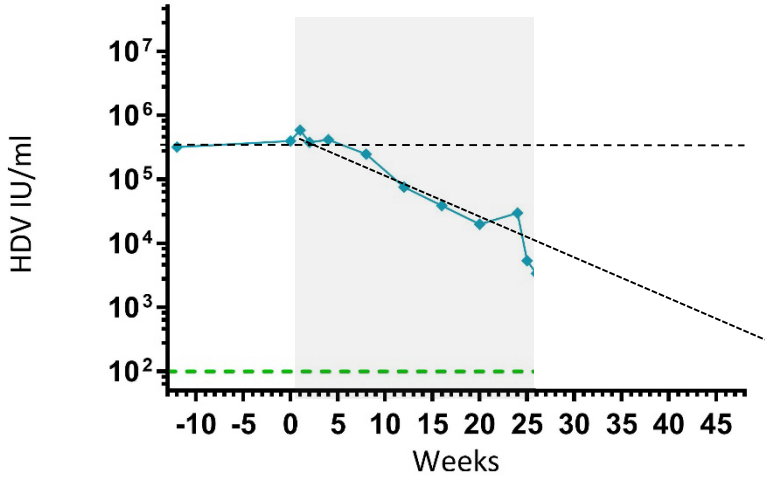
10mg Myrcludex B, patient 12007



10mg Myrcludex B patient 12008



10mg Myrcludex B patient 01016



HDV serum RNA-decline follows zero order elimination kinetics (as expected for an entry inhibitor)

Individual differences in elimination rates observed

Treatment extension predicts virus elimination

Time under Myrcludex Treatment*	Percentage of Patients with HDV Load = 0
2 Years	> 60%
3 Years	> 87%

*calculation based on Snoeck E, *et al.*,Clin Pharmacol Ther. 2010…

State of Hepcludex/bulevirtide for chronic hepatitis D infections (CHD); Design of the phase III trial Myr-301 (NCT03852719)

Bulevirtide (Myrcludex B) was approved under the trade name Hepcludex® in the EU August 4th 2020.

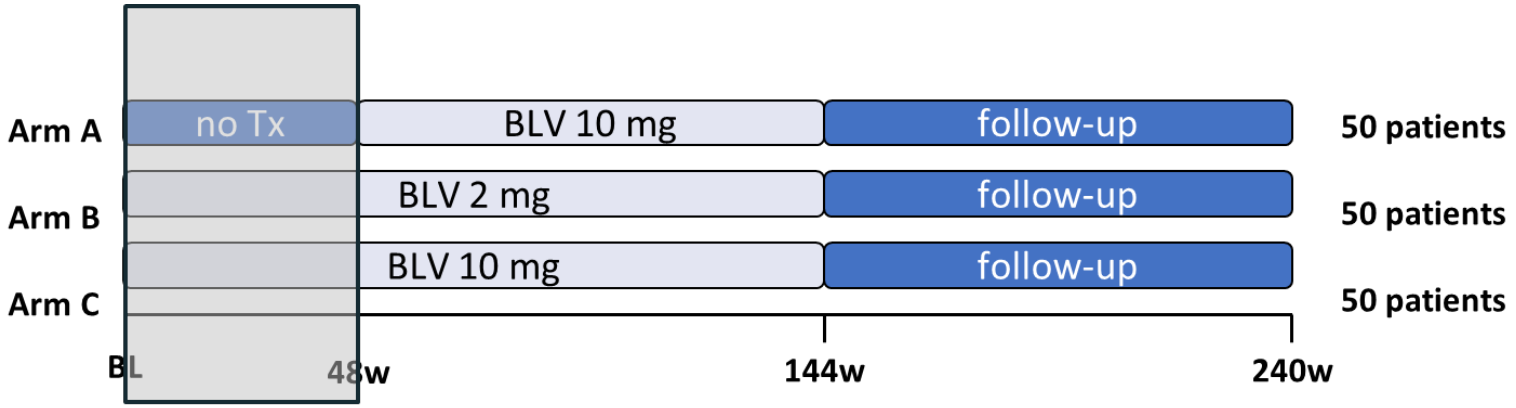
Myr-Pharmaceuticals has been acquired by Gilead, December 10th 2020 (1.45 billion Euro).

Gilead submitted a Biological License Application for bulevirtide to the FDA on November 22nd 2021.

FDA approval is pending (CRL by FDA received), improvement of manufacturing required, no additional trials demanded.

Is there curative potential ?

Phase III study design



50% of patients achieve > 2 log decline within 24 weeks; elimination expected within 2-3 years of treatment

Real world data presented at EASL 2022, London and AASLD 2022 Washington

A comprehensive Review: Degasperi E, Anolli MP, Lampertico P.; J Viral Hepat. 2023

GS006
Efficacy and safety of bulevirtide monotherapy given at 2 mg or 10 mg dose level once daily for treatment of chronic hepatitis delta: week 48 primary end point results from a phase 3 randomized, multicenter, parallel design study

OS093
Real life study of bulevirtide in chronic hepatitis delta: preliminary results of the ANRS HD EP01 BuleDelta prospective cohort

OS149
Treatment with bulevirtide improves patient-reported outcomes in patients with chronic hepatitis delta: An exploratory analysis of a Phase 3 trial at 48 weeks

THU194
Fate of HDV-specific CD8+ T cells during bulevirtide monotherapy in patients with chronic hepatitis delta

THU302
Bulevirtide is broadly active against all HDV genotypes expressing envelopes from HBV genotypes A-H and a large panel of clinical isolates

THU309
Polymorphic analysis of bulevirtide sequence in PreS1 of large HBsAg across HBV genotypes A-H

SAT341
Bulevirtide treatment of hepatitis D in Germany: multicentre real-world experience

SAT345
Improvement of liver-stiffness after 6 months of therapy: real-life data for HBV/HDV co-infected patients treated with bulevirtide

SAT351
Integrated efficacy analysis of 24-week data from two phase 2 and one phase 3 clinical trials of bulevirtide monotherapy given at 2 mg or 10 mg dose level for treatment of chronic hepatitis delta

SAT352
Integrated safety analysis of 24-week data from three phase 2 and one phase 3 clinical trials of bulevirtide monotherapy given at 2 mg and 10 mg dose level for treatment of chronic hepatitis delta

SAT353
Virologic response to bulevirtide is delayed in cirrhotic HDV patients with clinically significant portal hypertension

SAT354
Response-guided long-term treatment of chronic hepatitis D patients with bulevirtide-Results of a "real world study"

SAT360
Predictive factors of virological response at one year in patients with chronic HBV/HDV co-infected treated with Bulevirtide

SAT373
Treatment with Bulevirtide in patients with chronic HBV/HDV co-infection. Safety and efficacy at month 18 in real-world settings

SAT379
Bulevirtide 2 mg/day monotherapy in patients with chronic hepatitis delta with or without cirrhosis: a multicenter european cohort real-life study

SAT381
Comparative performance analysis between manual and automatic RNA extraction to quantify HDV RNA by RoboGene 2.0 kit in untreated and bulevirtide-treated HDV patients

SAT385
No detectable resistance to bulevirtide in participants with chronic hepatitis D (CHD) through 24 weeks of treatment

SAT408
Baseline bile acid levels but not bile acid increases during bulevirtide treatment of hepatitis D are associated with HDV RNA decline

SAT414
Real-world data on treatment with bulevirtide in patients with chronic hepatitis B and D coinfection

SAT417
Impact of patient-related factors on the pharmacokinetics of Bulevirtide

SAT429
Bulevirtide monotherapy for 48 weeks in HDV patients with compensated cirrhosis and clinically significant portal hypertension

SAT430
Bulevirtide avoids future clinical events and related costs compared to pegylated-interferon alpha in chronic hepatitis D in Spain

SAT440
Most patients with advanced cirrhosis treated with bulevirtide monotherapy have a non-monophasic HDV RNA decline patterns: an interim kinetic analysis of real-life setting

SAT450
Off-therapy cure of hepatitis delta after 3 years of bulevirtide monotherapy in a patient with compensated advanced cirrhosis

12 further presentations at the AASLD meeting in Washington

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