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The Hepatitis B Virus Life Circle: Achievements and Challenges

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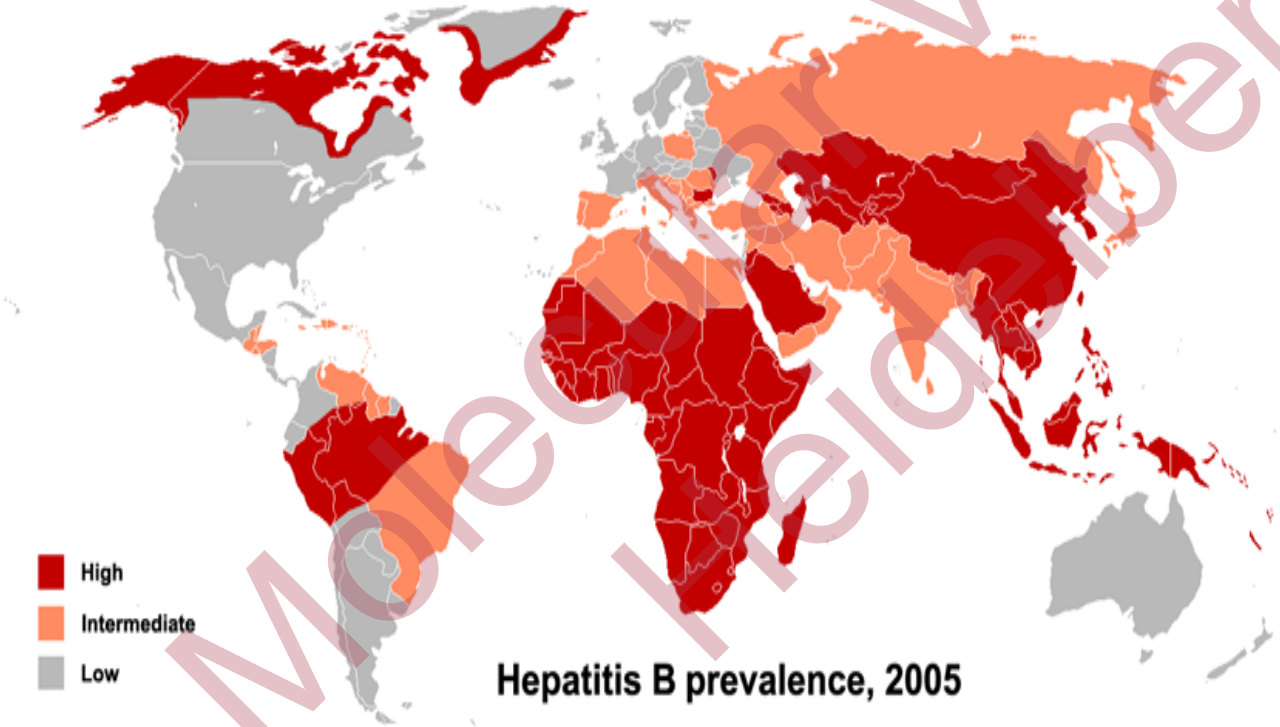
The global burden of Hepatitis B Virus (HBV) infection

- World wide ~2 billion people carry serological markers (HBsAg, anti-HBc) related to HBV infection.
- ~ 400 million are chronically infected with HBV (~ 170 million with HCV).
- ~ 1 million die (HBV-related liver cirrhosis, hepatocellular carcinoma (HCC)).
- currently there are no curative treatment options for chronic HBV infections; vaccination for prevention

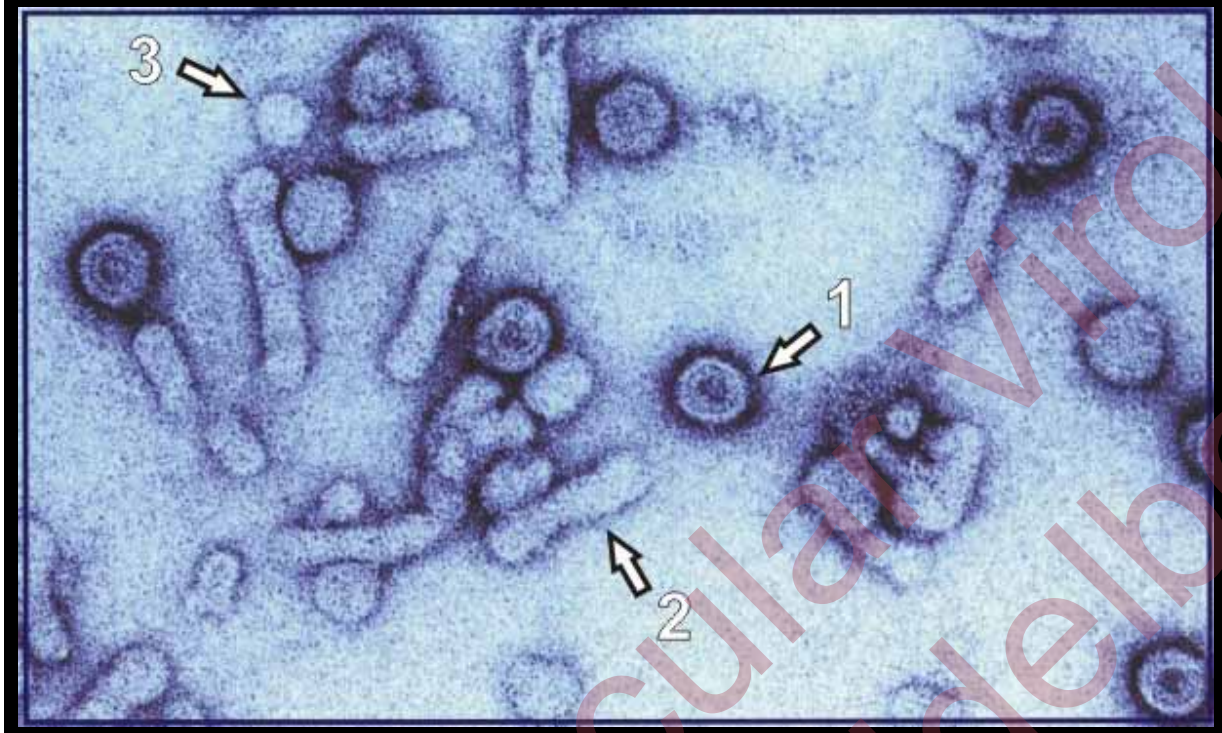


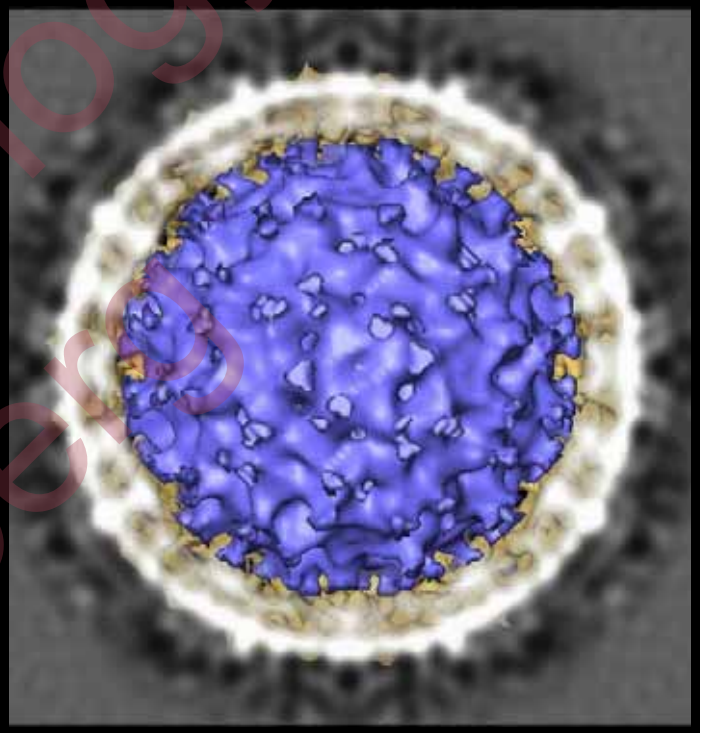
Highly endemic regions :
South East Asia, China, Central Africa.
Risk of infection > 60%
vertical transmission to children

Areas with medium endemicity:
South- Eastern Europe, Middle East, Northern Africa, Japan, Mesoamerica, Russia.
Risk of infection 20-60%

Low prevalence areas:
Northern America, West Europe, Australia, Parts of South America.
Risk of infection < 20%
(horizontal transmission, sexual contacts)

Hepatitis B virions and subviral particles





Seitz *et al.*, EMBO J., 2007

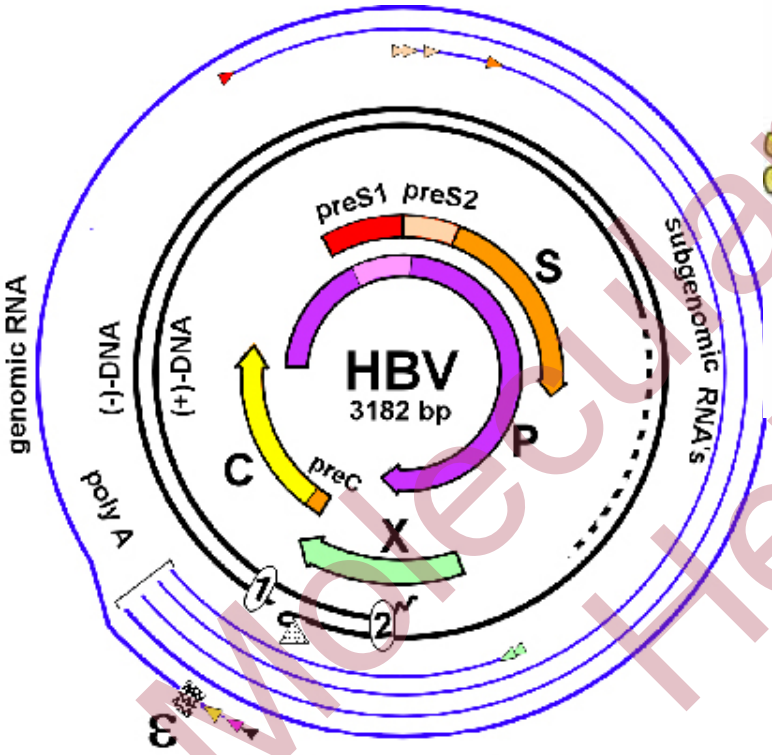
HBV forms different types of infectious and non-infectious particles

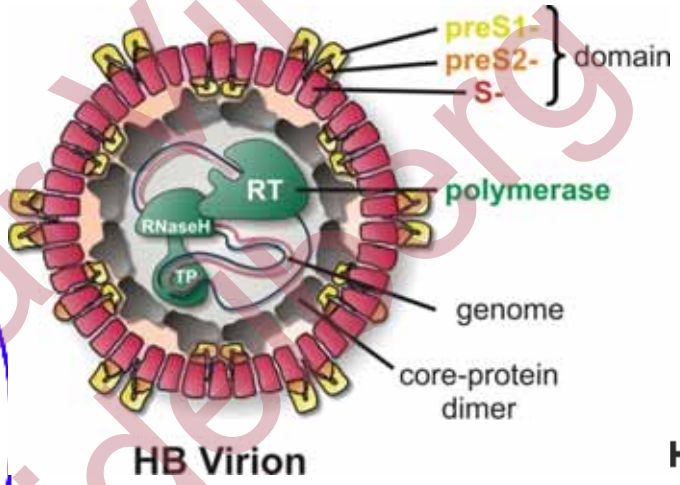
- 10^4-10^{10}/ml infectious virions of 42-47 nm diameter with nucleocapsid (1)
- Long and short nucleocapsid-free filaments; 22-25 nm in diameter (2)
- spherical, nucleocapsid-free particles with 22-25 nm diameter (3);

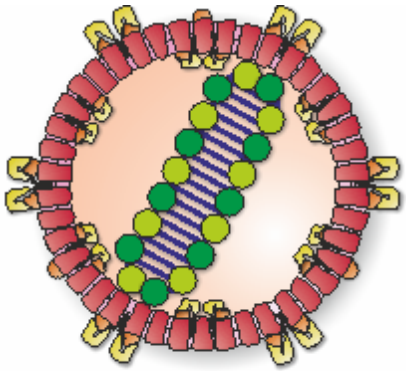
SVPs are synthesized in huge excess over virions ⇒ HBsAg = SVPs (mostly spherical particles)

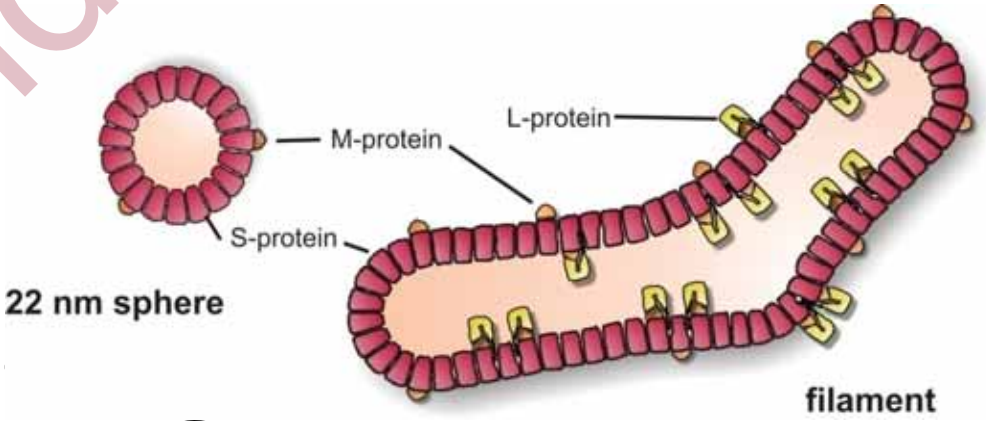
Hepatitis B Virus Genome organization and particle morphology

- HBV encodes seven proteins in a partially double stranded highly overlapping genome (“zip-genome”)
- efficacy of transcription depends on liver-specific transcription factors (contributes to liver specificity)
- HBx, a regulatory viral protein controls transcription of viral mRNAs from cccDNA

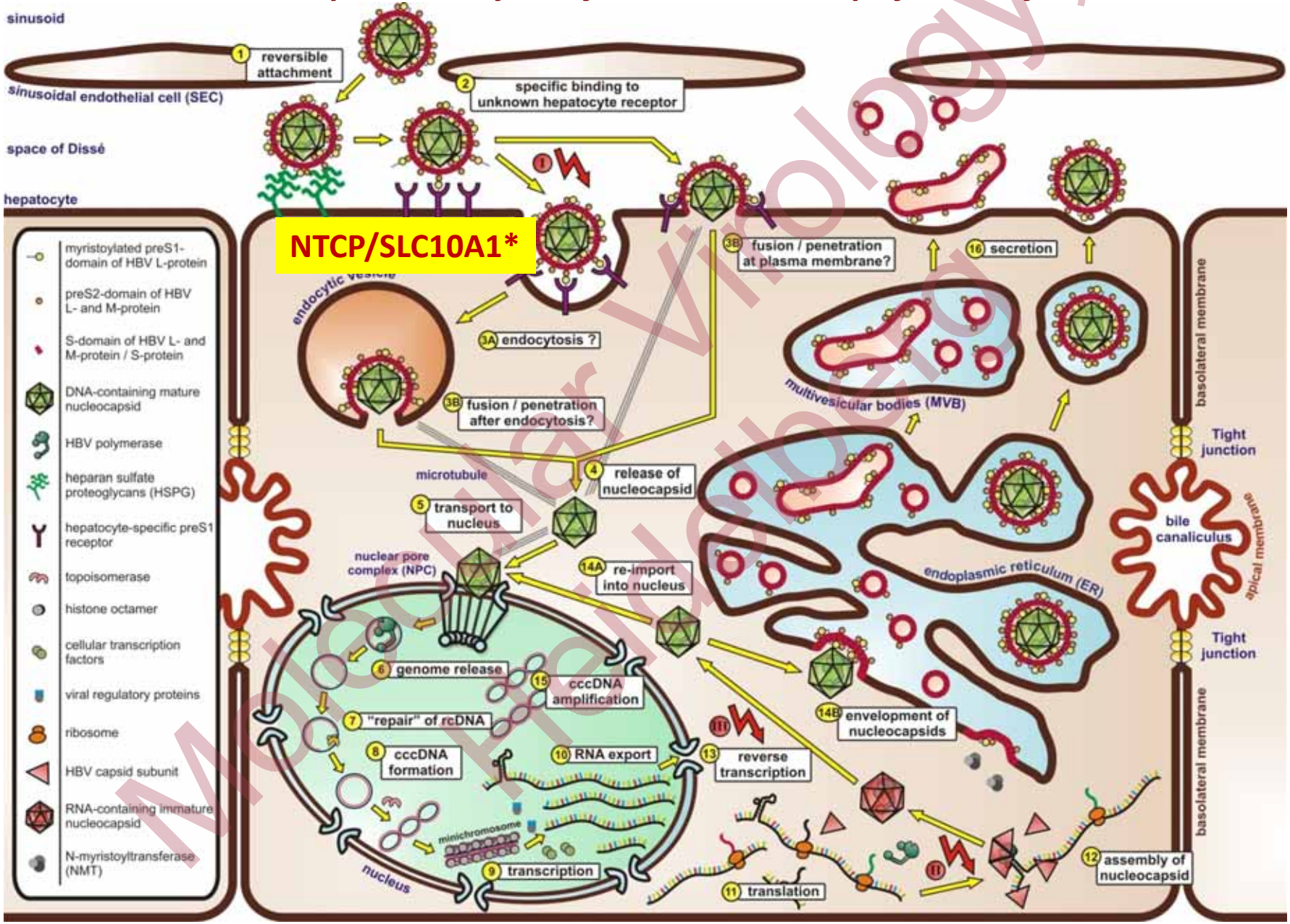








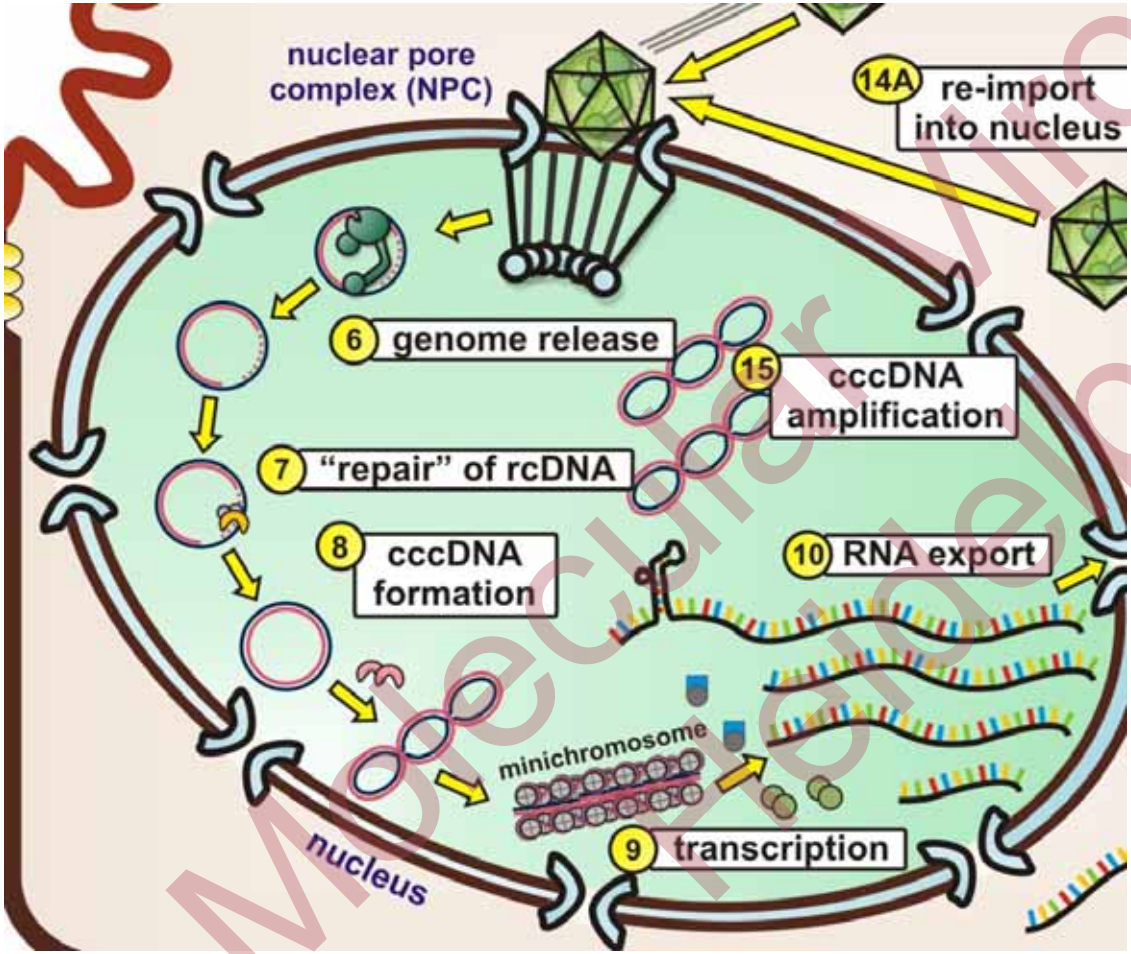
The replication cycle of HBV; crucial steps for interference



*Yan et al, eLife, November 2012

Urban *et al.*, J. Hepatology, 2010

Formation of cccDNA in the establishment of HBV infection



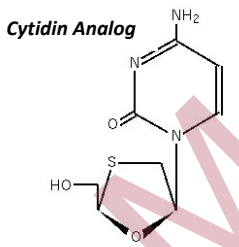
- ***cccDNA is synthesized de novo from incoming virus….(this pathway is not blocked by RT-inhibitors)***
- ***….or after reimport of newly formed mature (!) rcDNA-containing nucleocapsids ("amplification")***
- ***cccDNA formation depends on the activity of cellular DNA-repair enzymes (most are unknown)***
- ***There are no drugs that target and destroy cccDNA directly (innovative approach; site specific nucleases)***
- ***cccDNA stability is assumed to be stable in resting (!) hepatocytes***
- ***the ultimate goal of therapy is cccDNA „eliminiation" and HBsAg seroconversion.***

Approved therapies with nucleoside analogues are efficient in the suppression of viremia but generally non-curative – Why?

Nucleoside analoges: (Lamivudine, Adefovir, Entecavir, Telbivudine, Tenofovir)

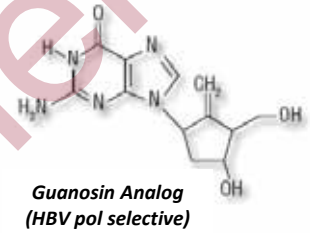
⇒ fast and efficient suppression of viral titers in the serum, preventing disease progression **but:**
⇒ only slow reduction of HBsAg titers during therapy
⇒ development of resistant mutants with some drugs (e.g. Lamivudine, Adefovir, Entecavir)

⇒ naiive hepatocytes establish cccDNA in the presence of NUCs
⇒ undetectable serum levels don´t necessarily mean complete virus suppression
⇒ More than 5x10e6 receptor molecules/hepatocyte (high virus binding capacity of the liver)
⇒ Novel intrahepatic infections of hepatocytes might occur even under strong virus suppression



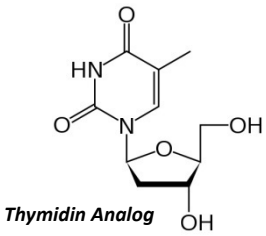
Lamivudine/LMV/3TC/Zeffix (GSK, approved 1998)

Adefovir/ADV/Hepsera (Gilead, approved 2002)



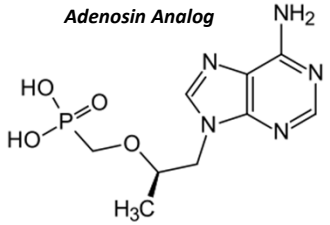
Guanosin Analog (HBV pol selective)

Entecavir/ETV/Baraclude (BMS, approved 2006)



Thymidin Analog

Telbivudine/L-Thymidin/Sebivo (Novartis, approved 2007)

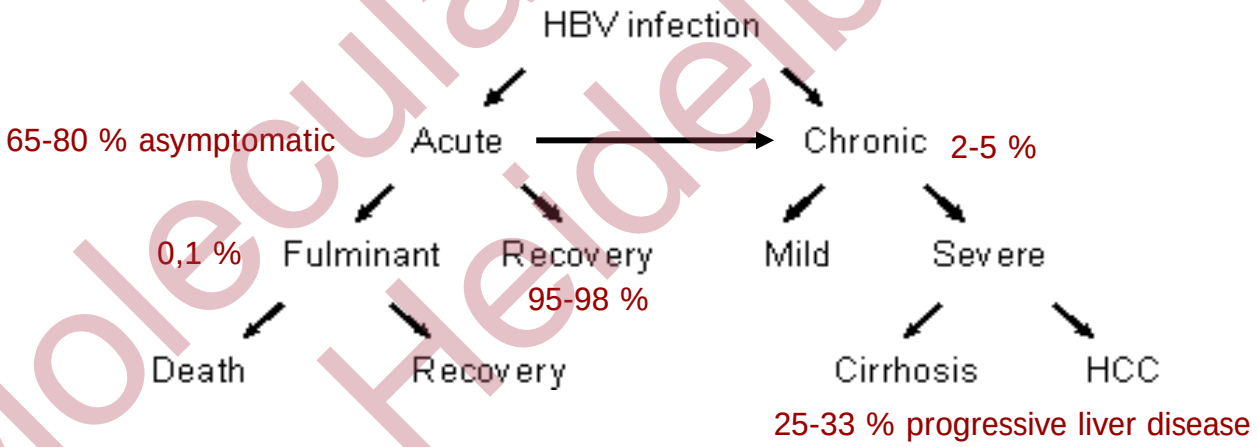


Tenofovir/TDF/Viread (Gilead, approved 2002/2008)

cccDNA clearance in the course of a natural infection

cccDNA is regularly cleared following acute infection of immun-competent individuals

⇒ ***In contrast to retroviral infections where genome integration is mandatory for virus replication and inherited to daughter cells the episomal cccDNA can become eliminated from hepatocytes***

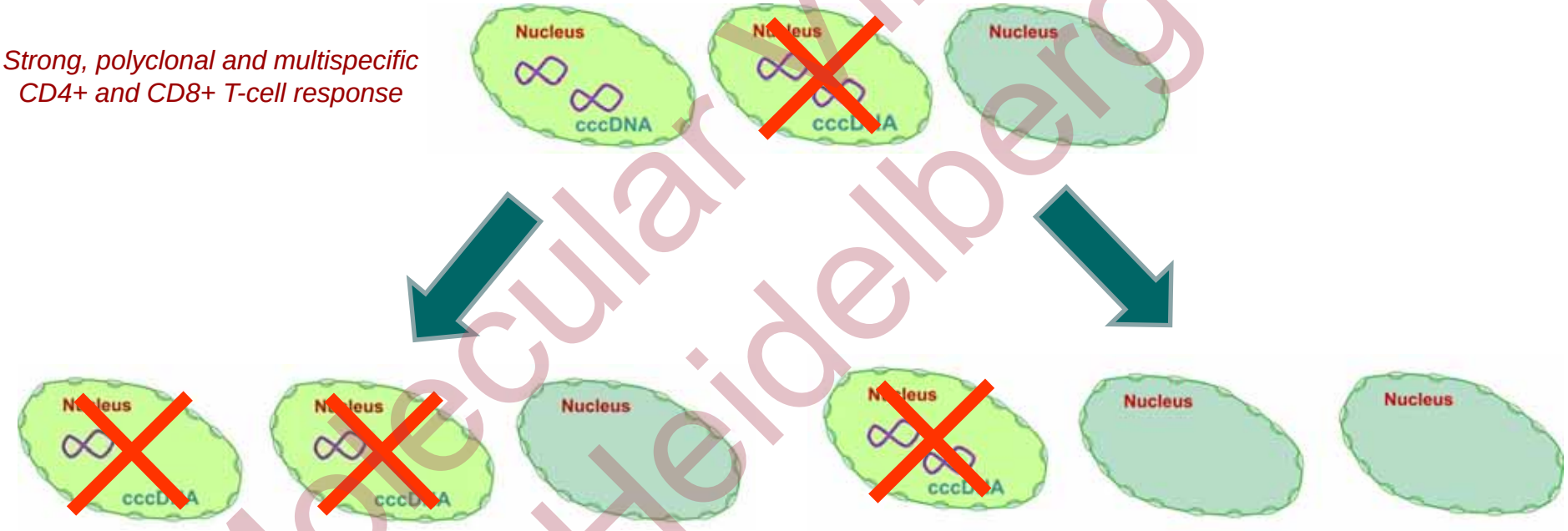


How can cccDNA be cleared in the course of a natural infection?

The current view:

(1) Killing (apoptosis) of infected hepatocytes

Strong, polyclonal and multispecific CD4+ and CD8+ T-cell response

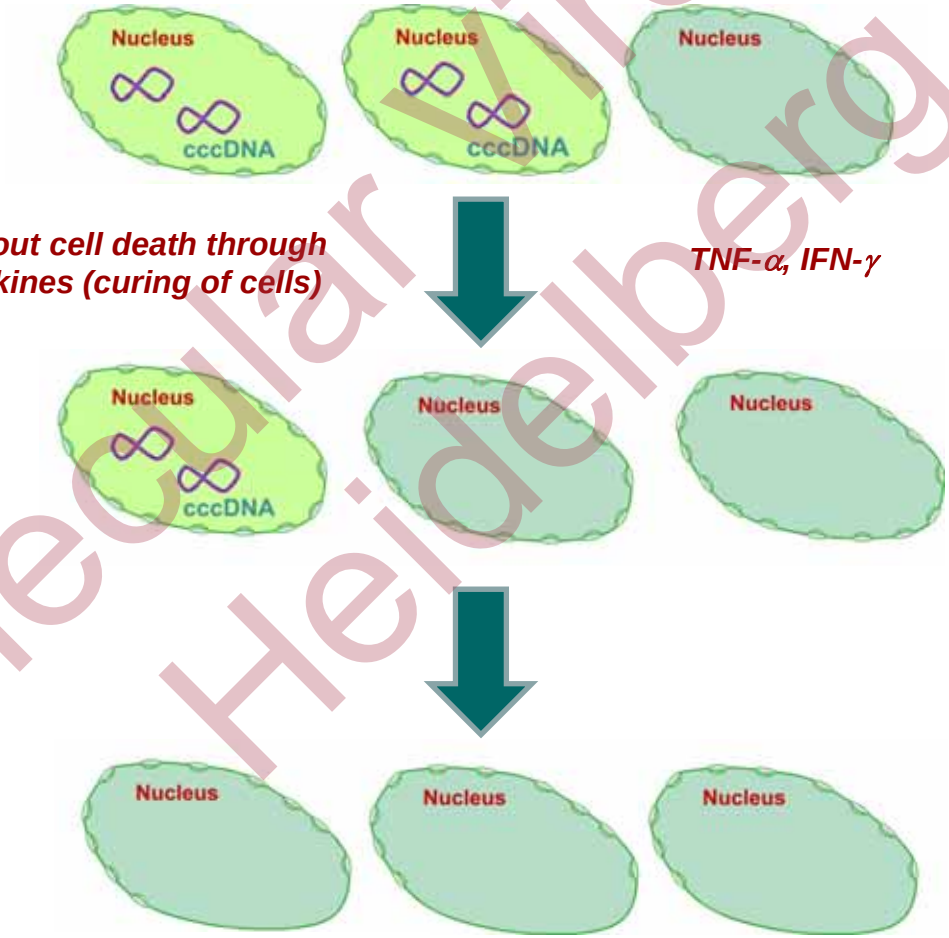


Division of infected cells, propagation and dilution of cccDNA

Division of healty cells

adopted from Anna S.F. Lok, EASL Monothematic conference 2005

2. Non-cytolytic mechanism by „curation“ of cells



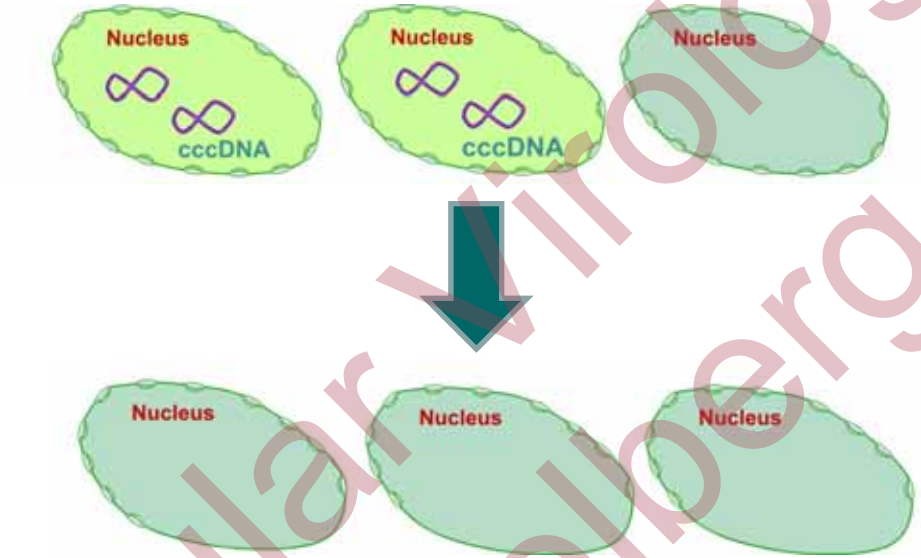
cccDNA elimination without cell death through e.g. direct action of cytokines (curing of cells)

TNF-α, IFN-γ

?

adopted from Anna S.F. Lok, EASL Monothematic conference 2005

If this holds true……



cccDNA depletion under NT-therapy depends on the number of cccDNA molecules/cell..

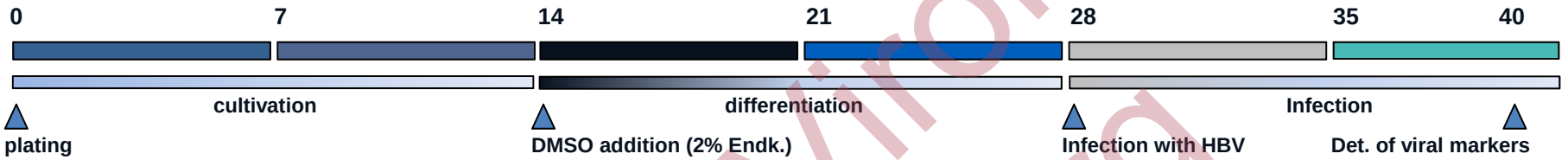
the half-life time of infected hepatocytes….

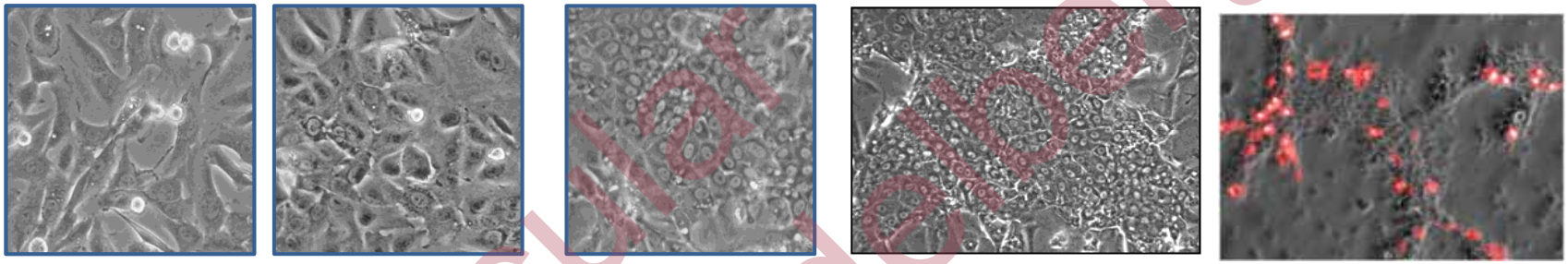
the rate of de-novo infection of naive or cleared hepatocytes under therapy……

…and would probably take decades……

Hypothesis based on the assumption that cccDNA survives cell division !

HepaRG cells to study the authentic cccDNA-based HBV replication cycle in vitro

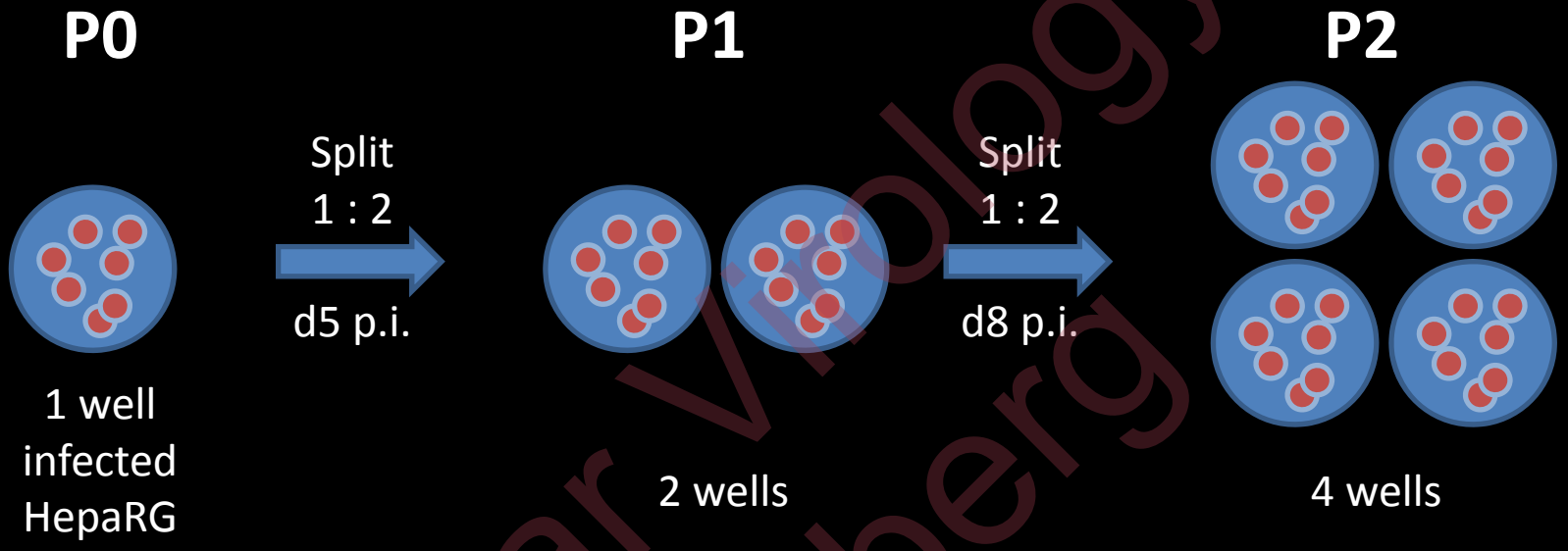




day 1 post plating | day 5 post plating | day 9 post differentiation | completely differentiated | HBV infected

Gripon, Rumin, Urban et al., PNAS, 99, 15655-15660 (2002)

Rapid loss of intracellular HbcAg-expression following cell splitting……



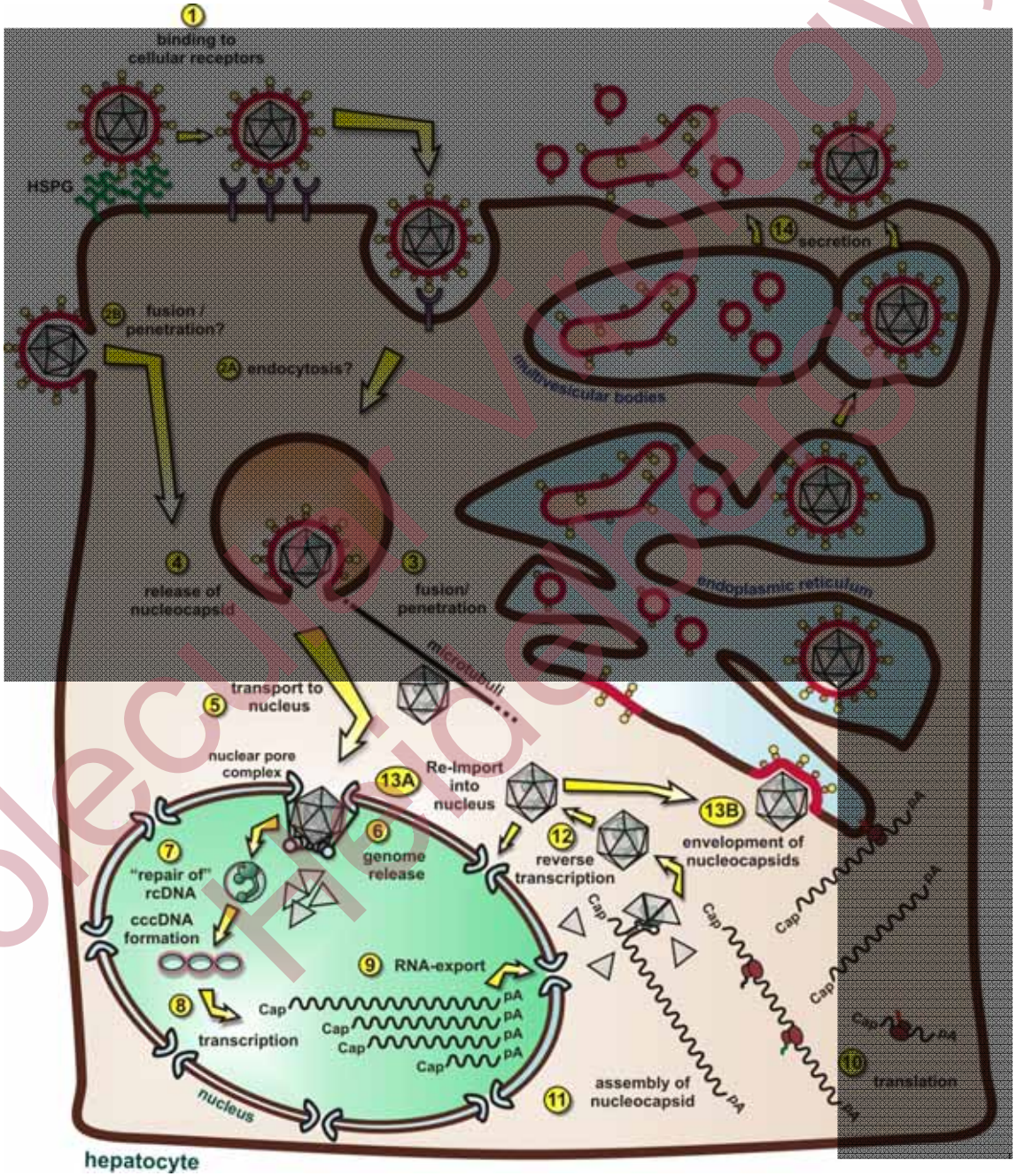
Anti-Core

P0 at d8 | P1 at d8 | P2 at d13

existing cccDNA gets lost during cell division (no immune system)

Ineffective replenisment of cccDNA by nucleocapsids reimport from the cytosol

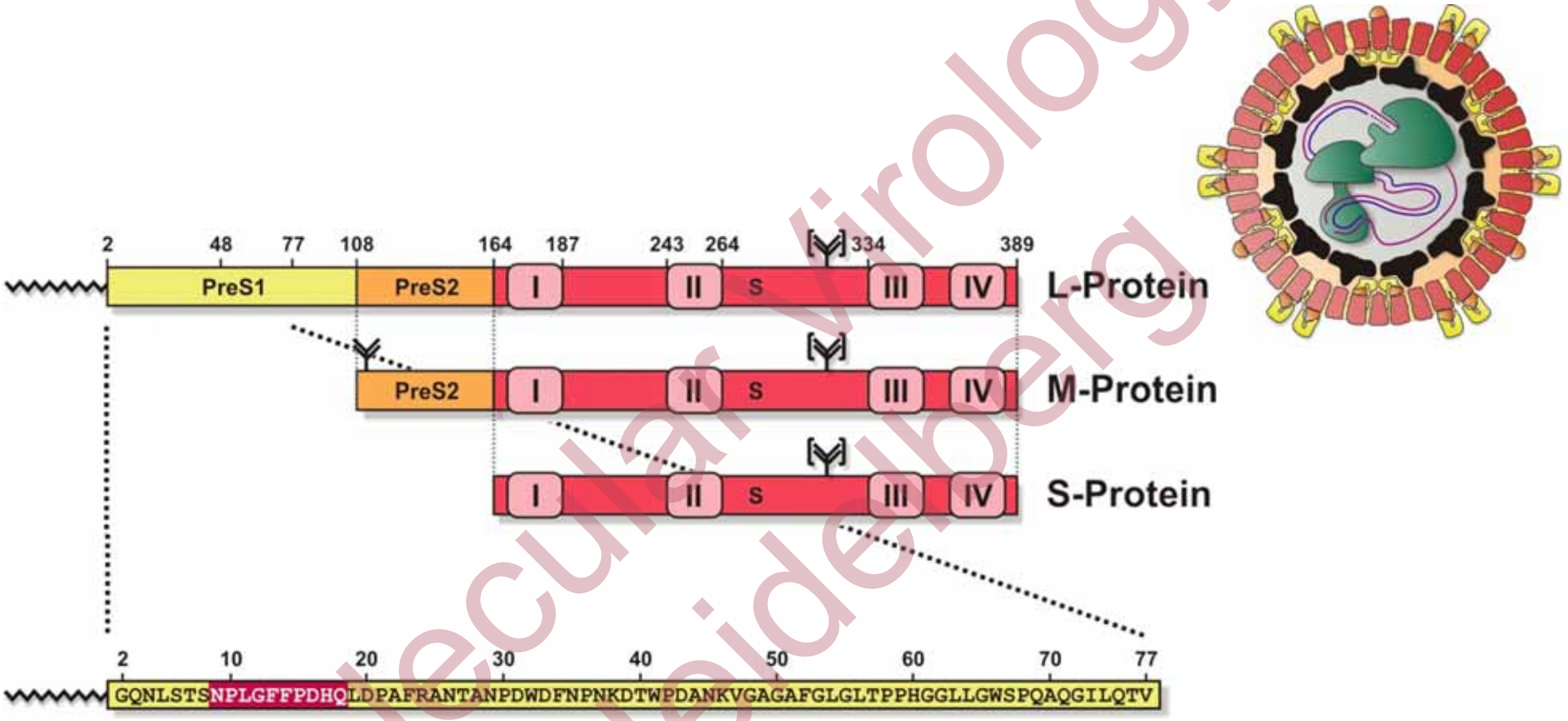
How is reimport of nucleocapsids regulated in HBV infected cells?



Conclusions and clinical consequences

- **HBV infected hepatocytes support superinfection and replication of HDV (⇒ not surprising).**
- **HBV infected hepatocytes support HBV entry but prevent nucelocapsid import and de novo cccDNA formation (unexpected).**
- **The presence of the L-protein is sufficient to compromise infection.**
- **Hypothesis: reimport of nucleocapsids cannot occur in the presence of L-protein present! ⇒ Replenishment of cccDNA is inefficient**
- **Therapeutical implications: Entry inhibition and the induction of hepatocyte proliferation might be a key for curative therapies.**

A determinant in the preS1 region required for HBV entry

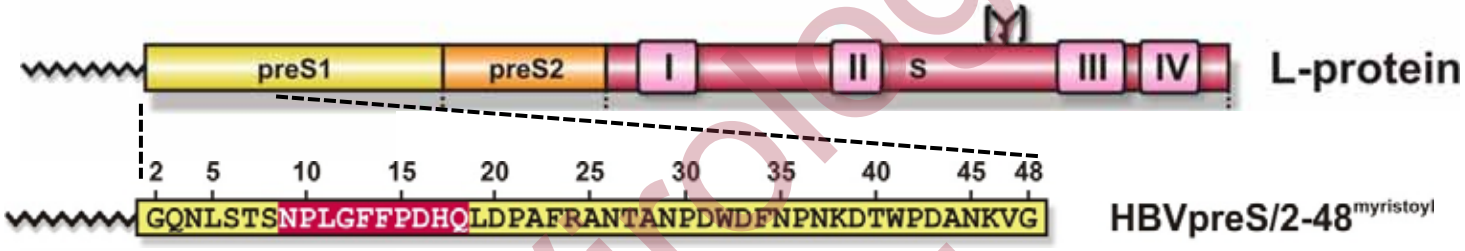


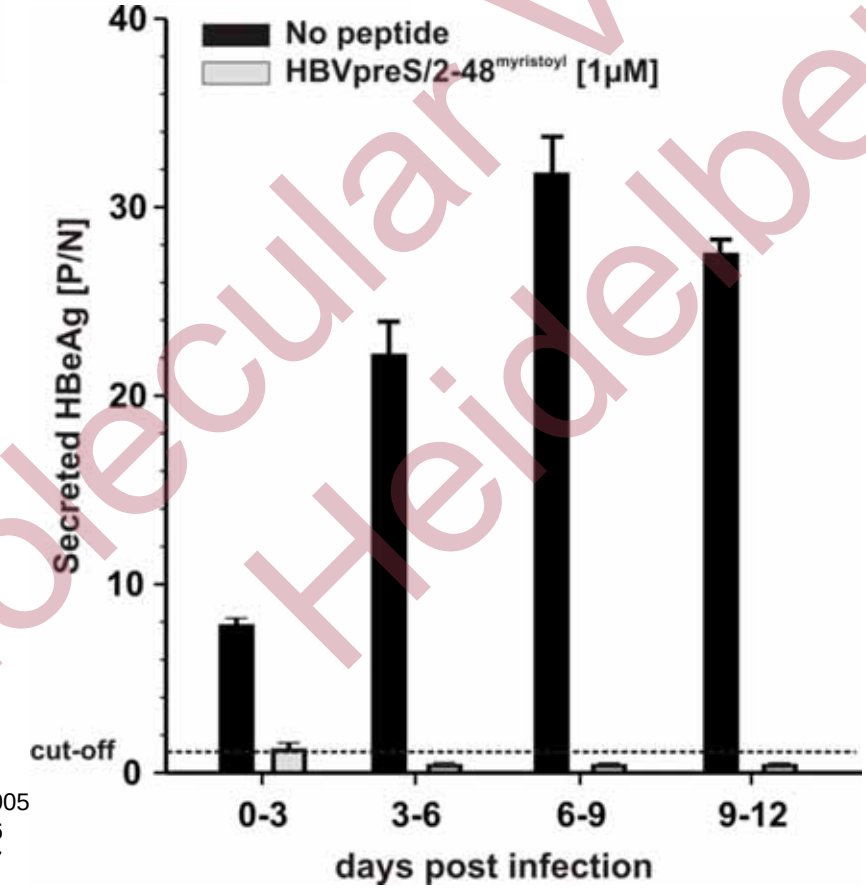
Le Seyec et al. *Infection process of the hepatitis B virus depends on the presence of a defined sequence in the pre-S1 domain.* J Virol. (1999) 73(3):2052-7.

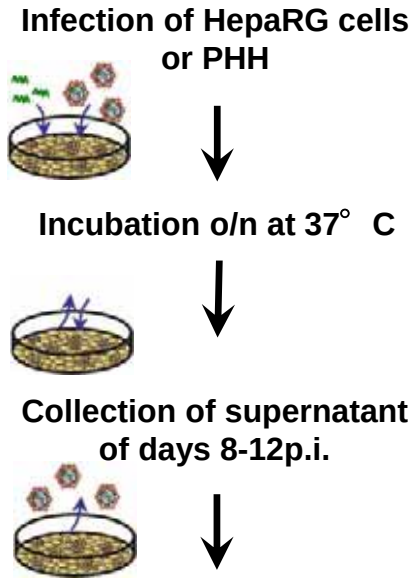
Gripon et al. *Myristoylation of the hepatitis B virus large surface protein is essential for viral infectivity.* Virology (1995) 213(2):292-9.

A synthetic peptide derived from the large envelope protein of HBV blocks HBV infection in HepaRG cell culture….

HBV Dane particle

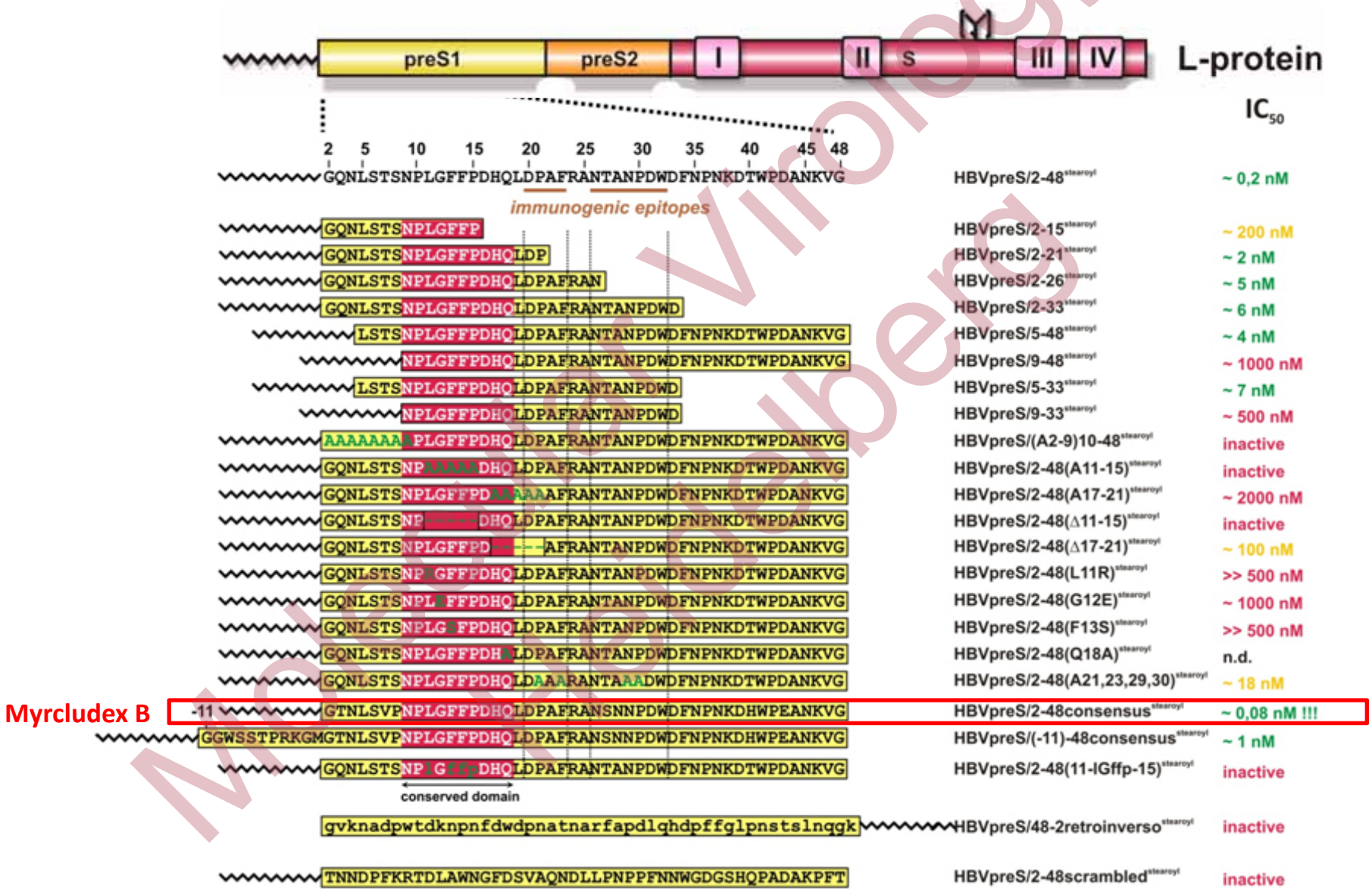






Gripon et al., PNAS, 99 (24) 2002
Urban et al., J. Virol, 79 (3), 2005
Glebe et al., Gastroenterology, 129, 2005
Engelke et al., Hepatology, 43, 2006
Schulze et al., Hepatology, 46, 2007

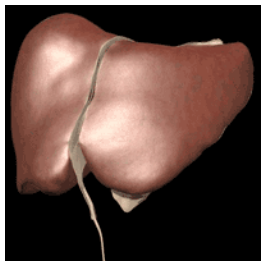
Fine mapping of the sequence requirements for HBV infection inhibition: Definition of Myrcludex B as a lead substance for clinical development



Schulze *et al.*, J. Virol, 2010

A transplanted mouse model to study in vivo infection of HBV

Transplantation of uPA/RAG-2 mice with primary human hepatocytes (PHH)

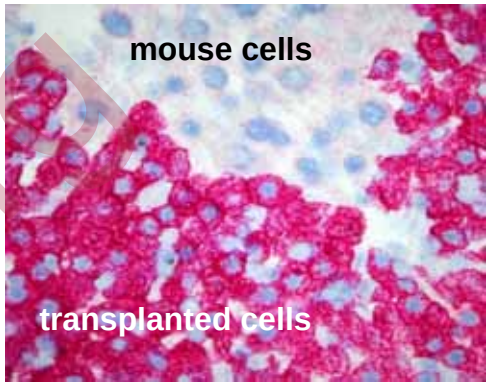


human hepatocytes (HH)

transplantation

immune-deficient uPA-mice

cimeric liver



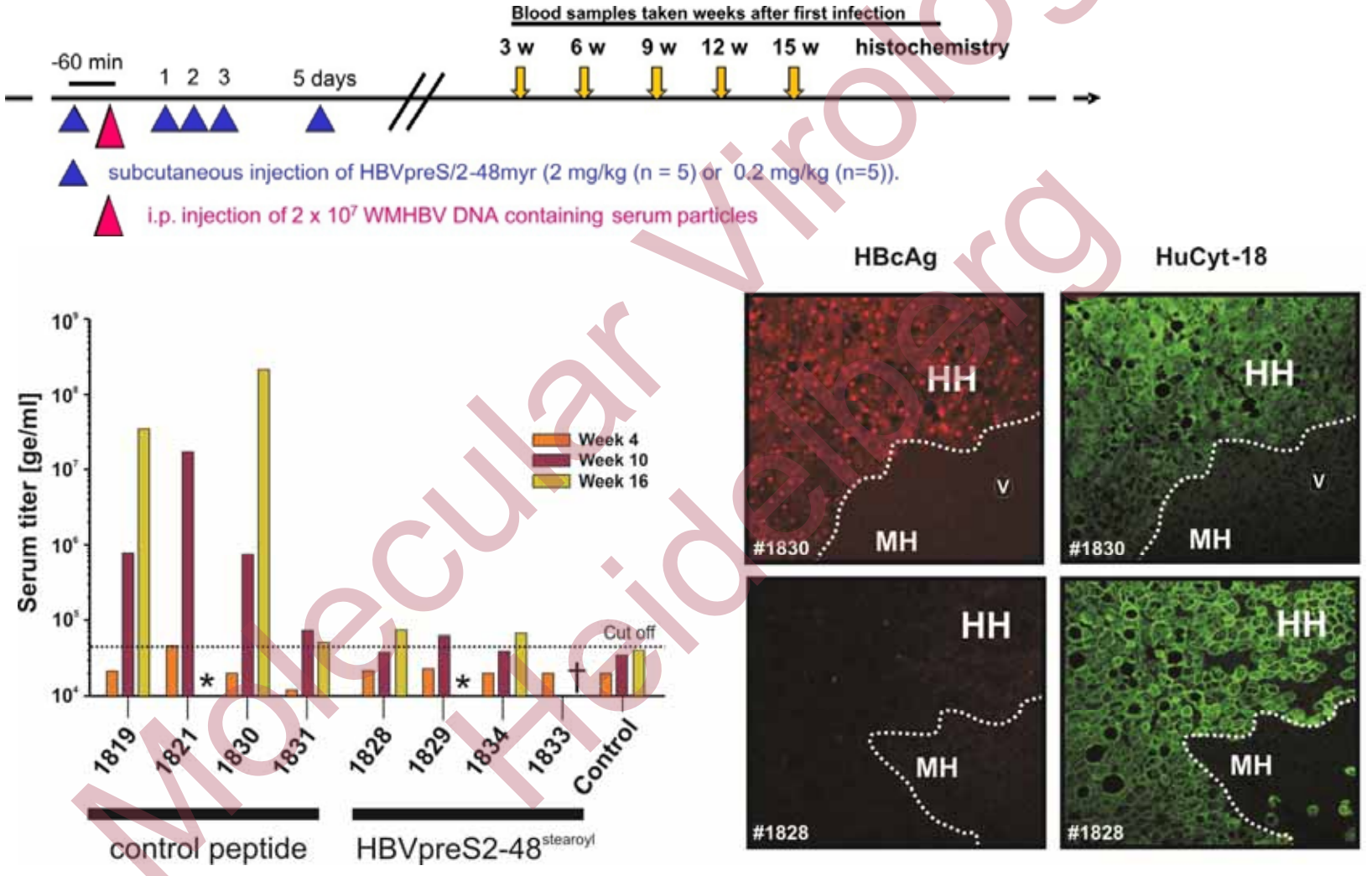
nature biotechnology

Prevention of hepatitis B virus infection *in vivo* by entry inhibitors derived from the large envelope protein

Joerg Petersen[1,7], Maura Dandri[1,7], Walter Mier[2], Marc Lütgehetmann[1], Tassilo Volz[1], Fritz von Weizsäcker[3,6], Uwe Haberkorn[2], Lutz Fischer[4], Joerg-Matthias Pollok[4], Berit Erbes[5], Stefan Seitz[5] & Stephan Urban[5]

Nat Biotechnol., 26:335-341 (2008)

Myrcludex B blocks HBV infection after subcutaneous administration in PHH-transplanted uPA-mice



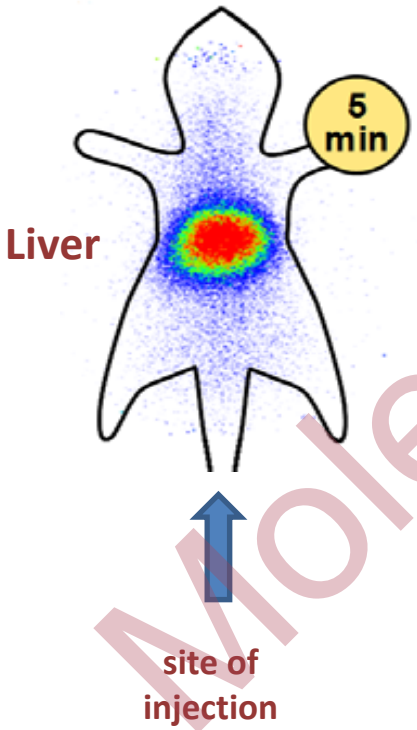
Petersen et al., Nat Biotechnol., 26:335-341 (2008)

Why is s.c. administration of HBVpreS/2-48^{myr} so efficient?

Myrcludex B accumulates in the liver of mice after i.v. injection

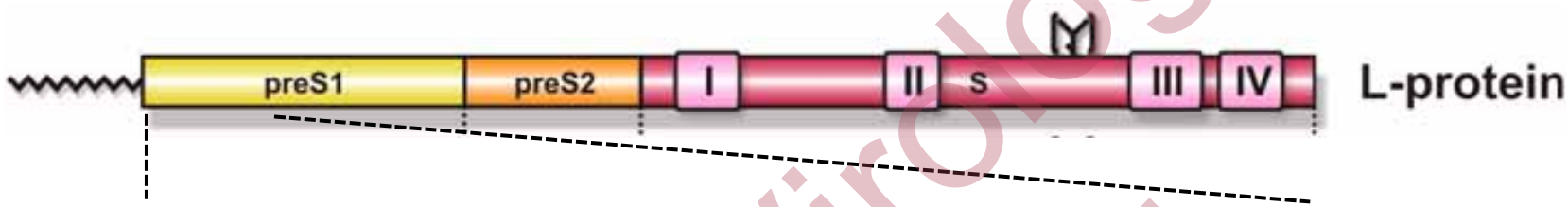
conserved domain

Stearoyl-GQNLSTSNPLGFFPDHQLDPAFRANTANPDWDFNPNKDTWPDANKVGy-I^{125}



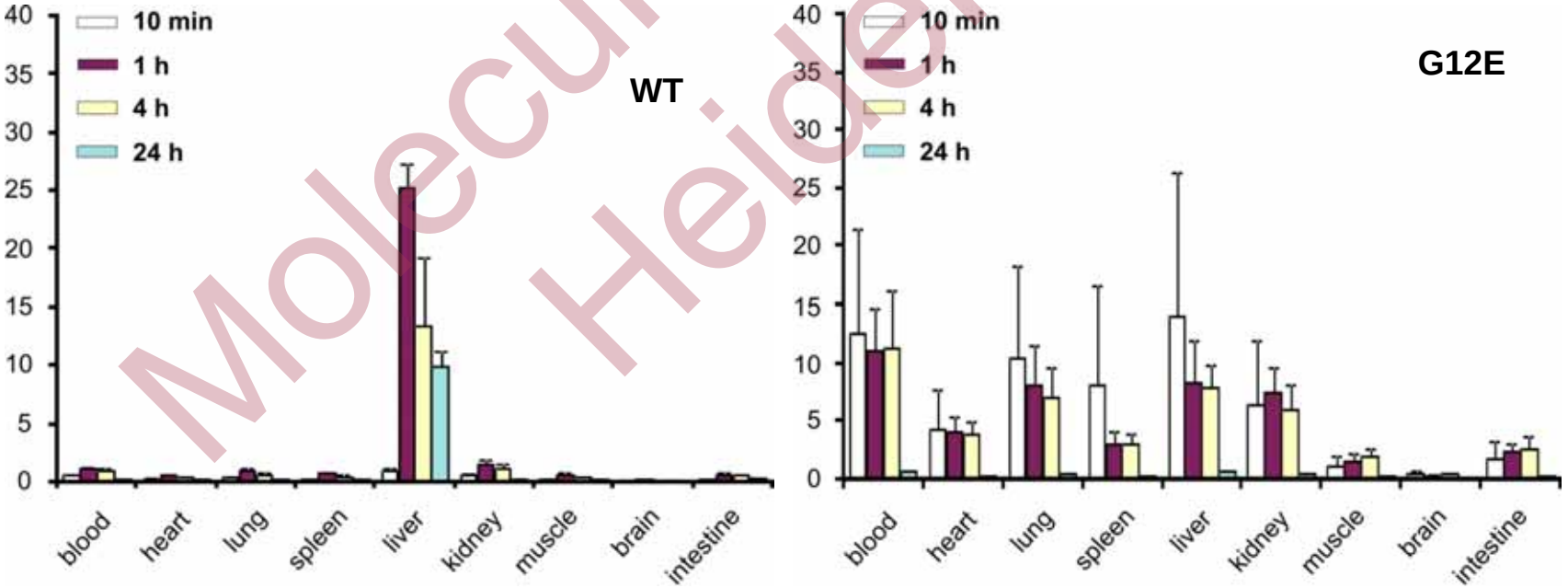
Schieck *et al.*, Hepatology 2013

A single amino acid exchange in the highly conserved receptor binding site abolishes peptide hepatotropism and liver accumulation

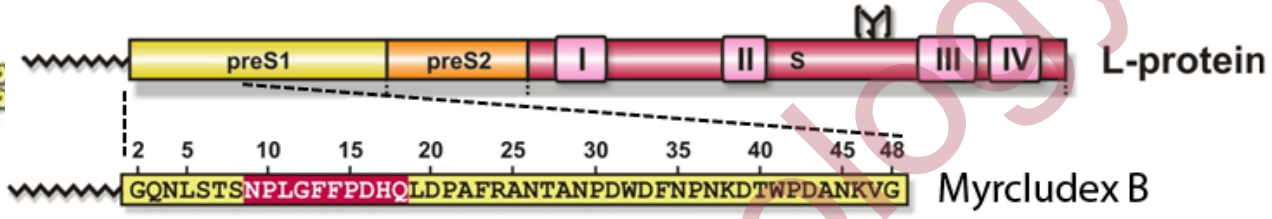


Stearoyl-GQNLSTSNPLGFFPDHQLDPAFRANTANPDWDFNPNKDTWPDANKVGy-I^{125} **WT**

Stearoyl-GQNLSTSNPL**E**FFPDHQLDPAFRANTANPDWDFNPNKDTWPDANKVGy-I^{125} **G12E**



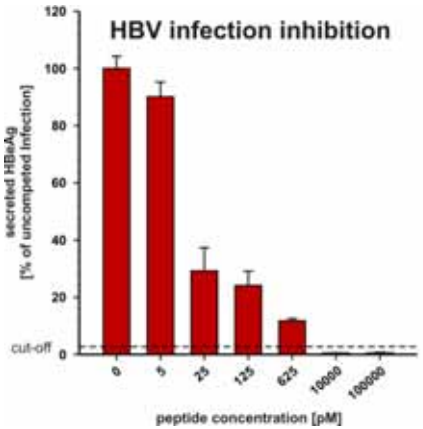
State of Myrcludex B clinical development

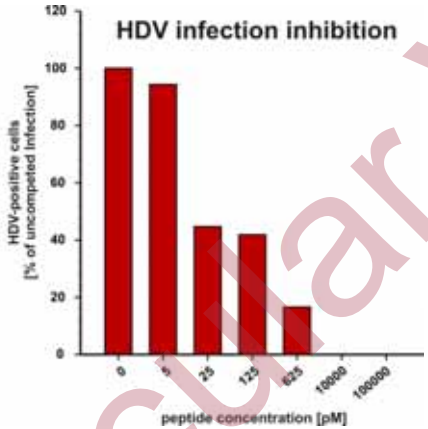


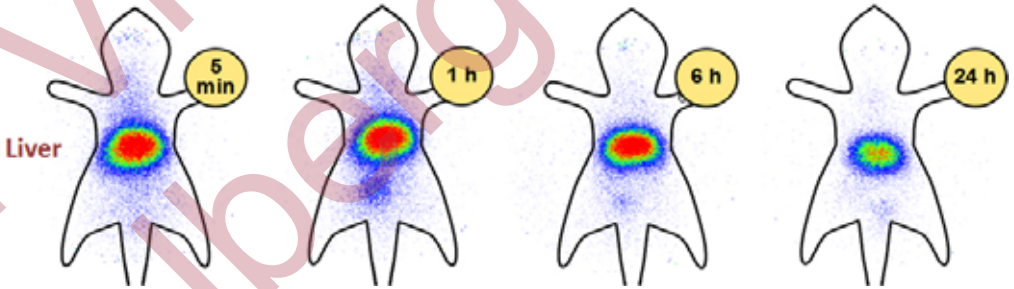
Hepatitis B Virion

Lead Substance: N-terminally-derived lipopeptide of the HBV L-protein

GMP Myrcludex B







Pharmakokinetics: Targeting to the liver within minutes
Long half life time at the hepatocyte

Inhibitory activity: $IC_{50} \approx 90$ pM

- GMP-production of 100g Myrcludex B accomplished (stability studies successful)
- Long term toxicity studies successfully completed without drug related side effects
- Single dose and PK studies in 3 chimpanzees completed (liver targeting confirmed)
- Approval by the BfArM for Phase 1a clinical trial in healthy volunteers (May 2011)
- Successful completion of the single dose escalating phase 1 study (24 individuals) in February 2012
- Start of a multiple dose s.c. Phase Ib study and a phase IIa efficacy study in November 2012.
- Search for a pharmaceutical company for further product development

eLIFE
elife.elifesciences.org

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, November 2012

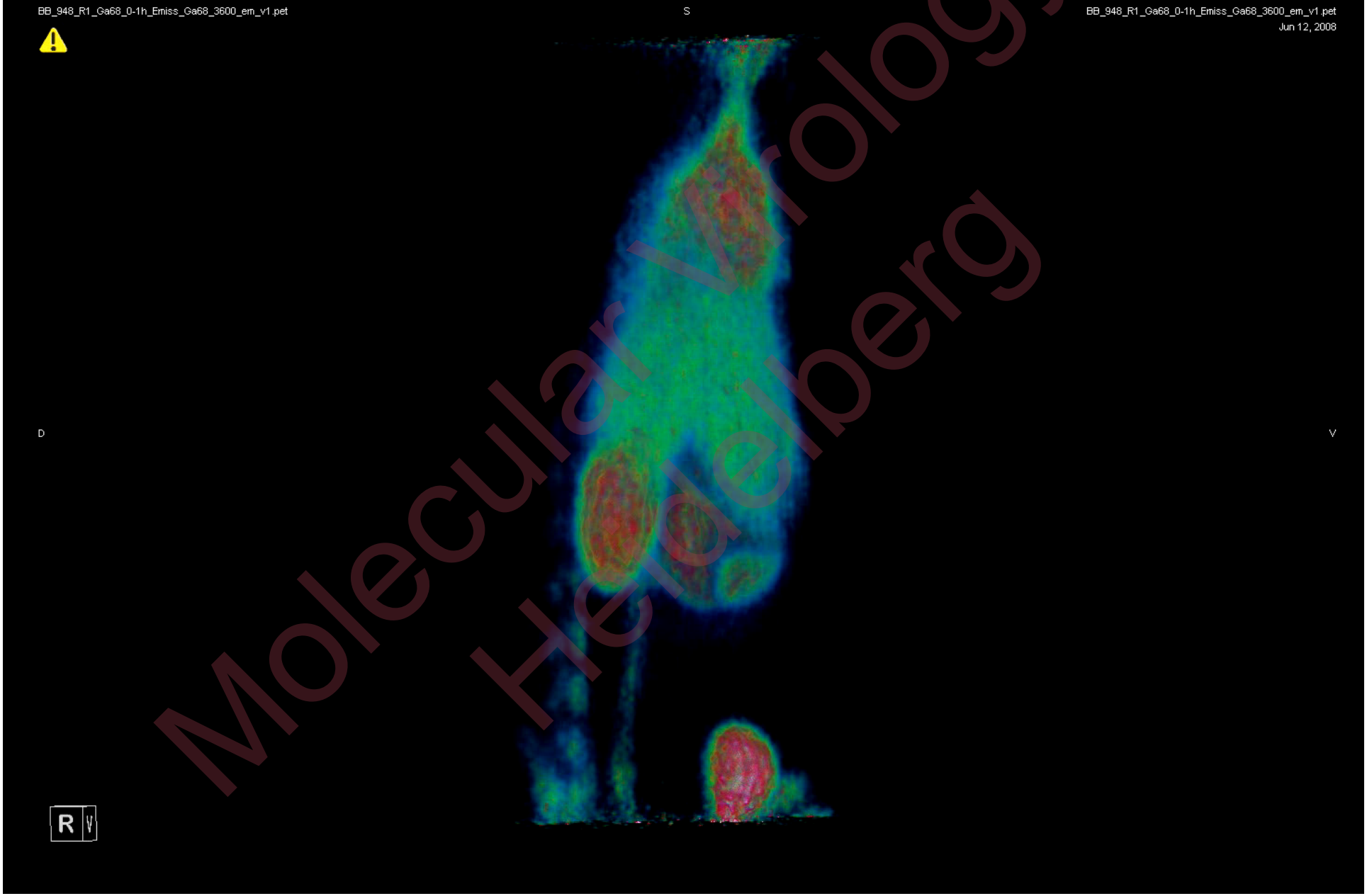
Summary, conclusion and outlook

- **Future therapeutic approaches should aim at eliminating cccDNA, the key regulator of HBV replication**
- **cccDNA clearence is possible and can be achieved by several means**
- **HBV cccDNA is not efficiently propagated to progeny cells after induction of hepatocyte division.**
- **Control of cccDNA might be obtained by a sustained combination of induced cell proliferation and entry inhibition**
- **Myrcludex B a first in class entry inhibitor of HBV/HDV currently in phase IIa trial will help to clinically prove that concept**
- **Novel NTCP-based cell culture systems will accelarate future drug development including those that directly target cccDNA**
- **NTCP-addressing HBVpreS-peptides are useful vehicles for liver specific drug targeting**

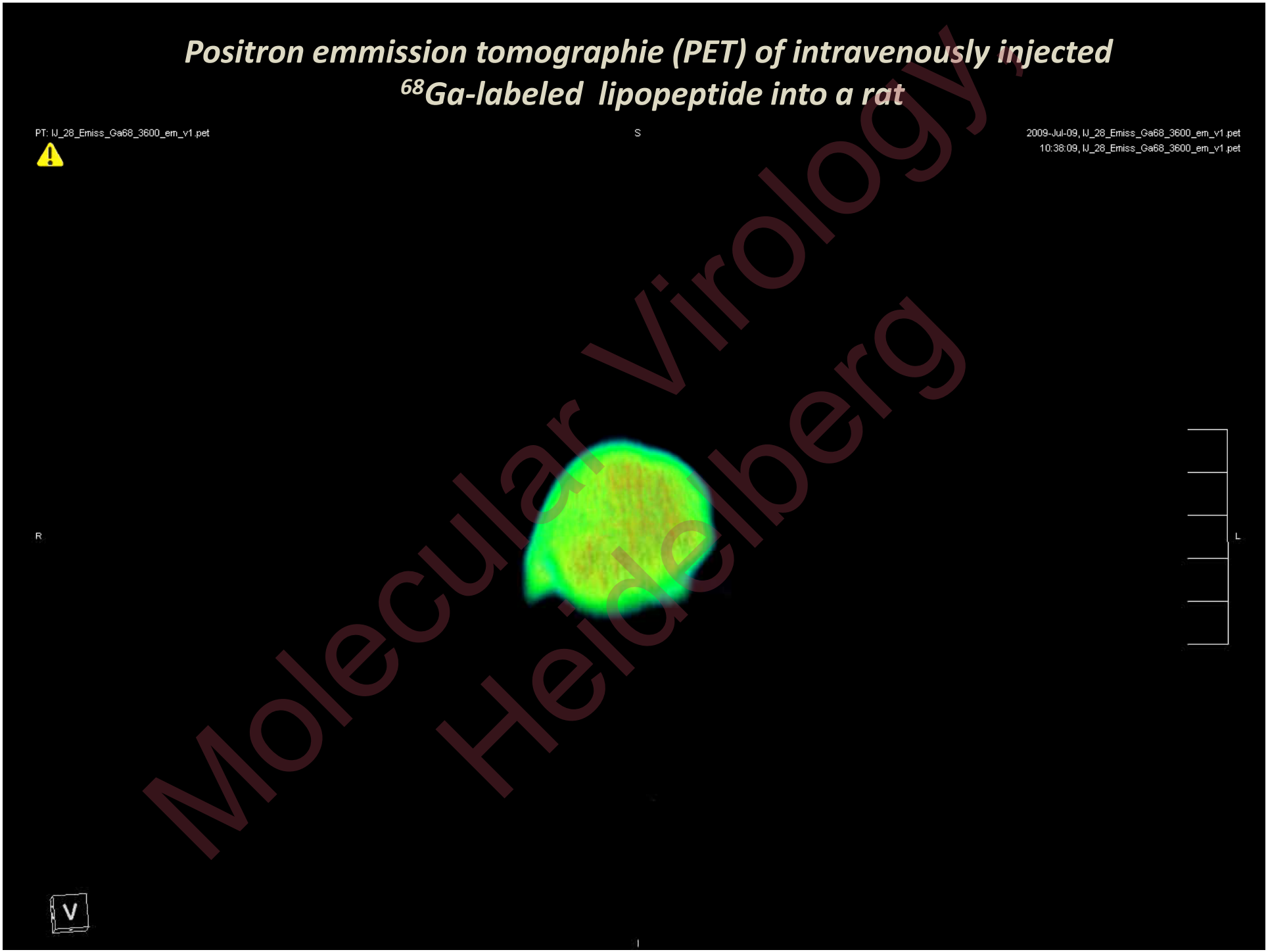
Possible applications for HBV preS-lipopetide-mediated drug delivery

- **imaging of liver diseases**
- **targeted HBV therapies (e.g. Entecavir, Adefovir ...)**
- **targeted HCV therapies (e.g. Protease inhibitors, polymerase inhibitors..)**
- **targeted therapies for hepatocellular carcinoma (HCC) (e.g. apoptosis induction, inhibition of angiogenesis , kinase inhibitors - Sorafenib)**
- **targeted Malaria therapies (e.g. primaquine)**
- **delivery of peptides for hepatocyte-specific antigen presentation**
- **delivery of siRNAs by hepatotropic liposomes or nanoparticles (e.g. metabolic diseases, infections...)**
- **targeted Interferon therapies to avoid side effects (chronic Hepatitis B, C)**

Positron emission tomography (PET) of an intravenously injected HBV unrelated ^{68}Ga-labeled peptide in a rat



Positron emmission tomographie (PET) of intravenously injected ^{68}Ga-labeled lipopeptide into a rat



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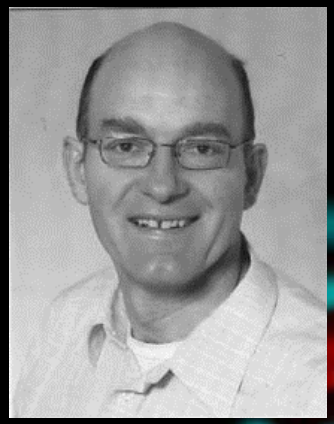

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