# New models for studying HBV replication and innate immunity

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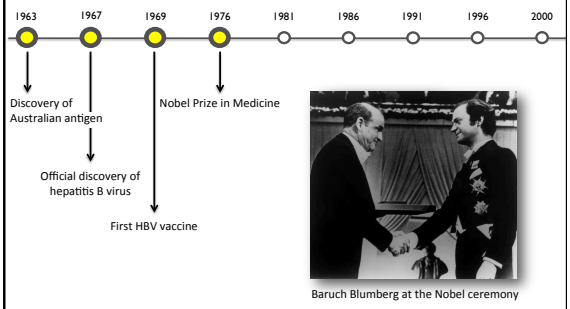
Lausanne, Switzerland
18 January 2018

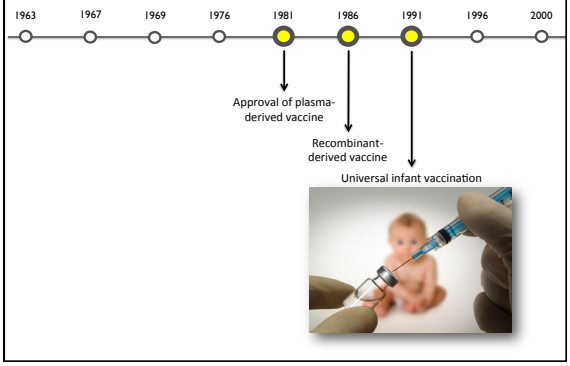
## Discovery of HBV and a prophylactic vaccine



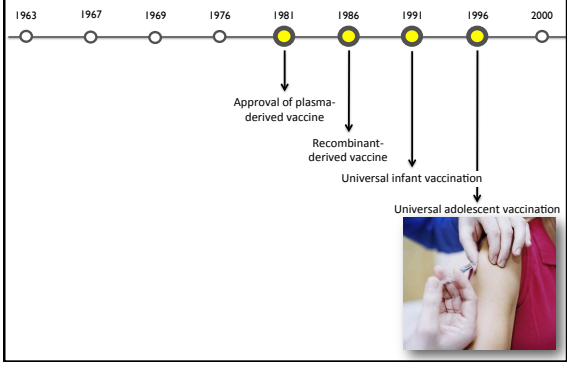



Baruch Blumberg at the Nobel ceremony

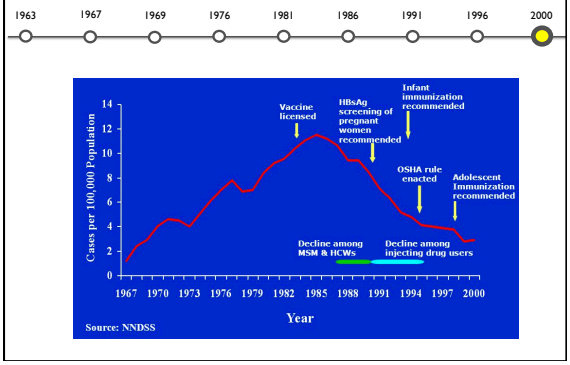
## Discovery of HBV and a prophylactic vaccine



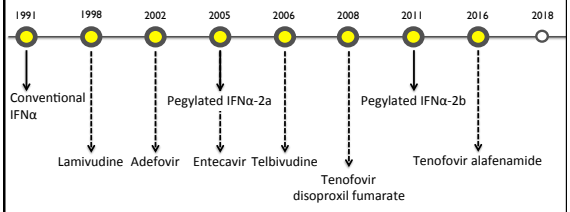

## Discovery of HBV and a prophylactic vaccine




## Hepatitis B in the United States (1966 – 2000)




## HBV therapies: past and present

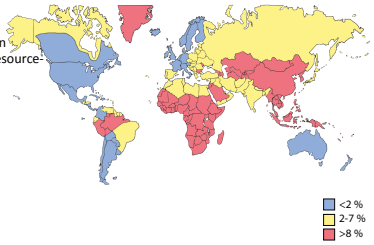



- Interferons
- NUCs

## Challenges in the HBV field

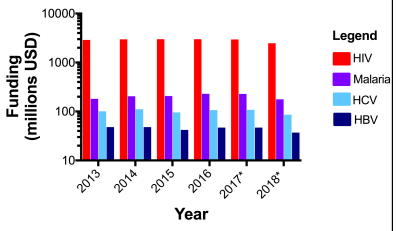
**Clinical picture:**
- Lifelong treatment
- Incomplete HCC prevention
- Ongoing transmission in resource-poor regions

~257 million people are chronically infected



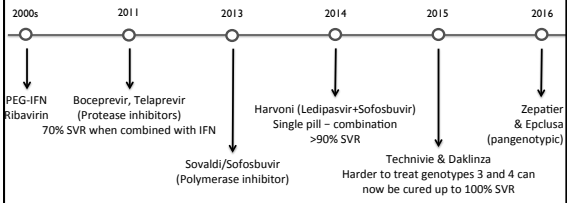

## Challenges in the HBV field

**Poor funding due to:**
- Effective HBV vaccine
- Potent HBV antivirals
- HCV in the spotlight

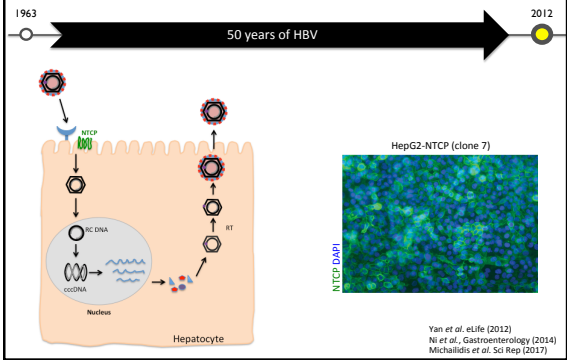



Geraldine A. O'Hara *et al.* PloS Negl Trop Dis (2017)

## The HCV success story: Role model for an HBV cure?

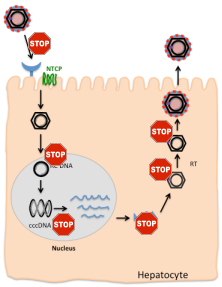



## The breakthrough: NTCP as a receptor for HBV

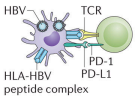



Yan *et al.* eLife (2012)
Ni *et al.*, Gastroenterology (2014)
Michailidis *et al.* Sci Rep (2017)

## The search for new HBV therapies and a functional cure



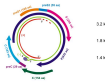

- NUCs block RT but do not eradicate the cccDNA
- New targets include entry, capsid, RNase H, cccDNA…
- Immunotherapies

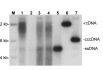



## Challenges in HBV research

**Poor in vitro systems**
- High titers required for efficient infection
- No virus spread/cannot recapitulate the complete viral life cycle
- Hepatoma cells have impaired innate immune functions - not ideal for virus-host interactions
- More physiological systems are based on cryopreserved primary human hepatocytes (PHH)
  - Donor-to-donor variability
  - Non-renewable
  - Cannot be genetically modified
  - Expensive

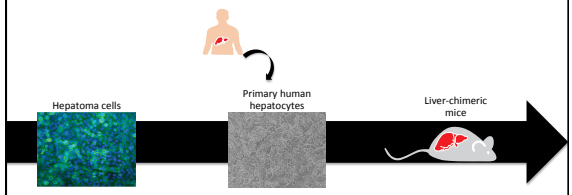
**Lack of molecular tools**
- cccDNA detection methods
- Lack of reporter virus





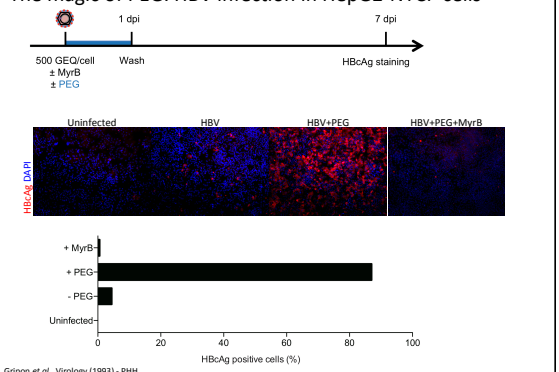
**Limitations in animal models**
- Restrictions on chimpanzee infections
- Immunocompromised mouse models

## Model systems for HBV: towards more relevant systems



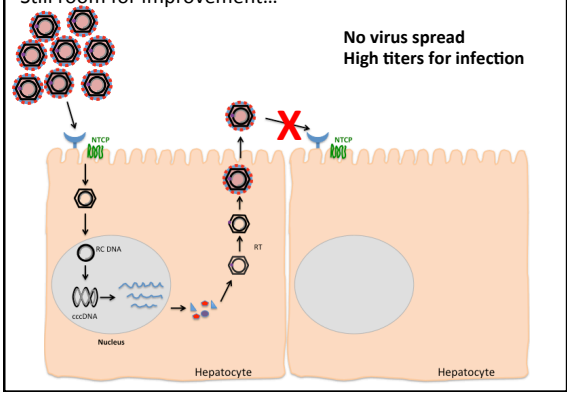

## Model systems for HBV: towards more relevant systems


## The magic of PEG: HBV infection in HepG2-NTCP cells

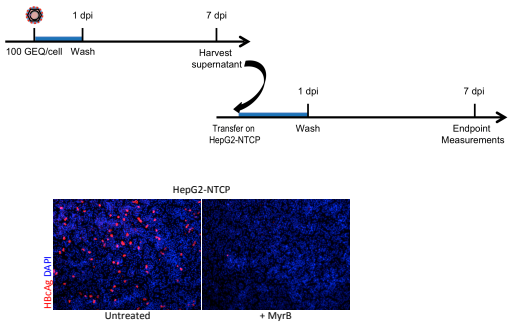



Gripon *et al.*, Virology (1993) - PHH
Michailidis *et al.* Sci Rep (2017)

## Still room for improvement…



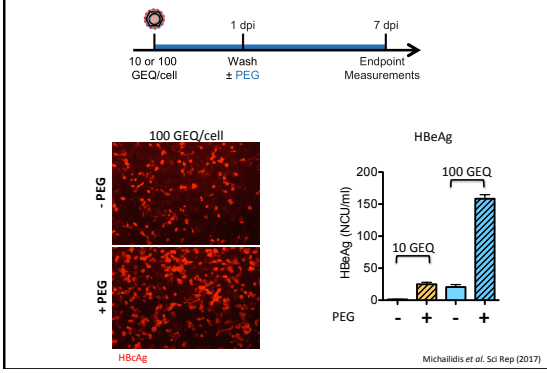

## HepG2-NTCP does produce infectious HBV




…but there is no spread

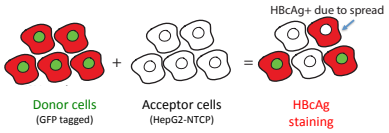
Michailidis *et al.* Sci Rep (2017)

## The magic of PEG: Continuous addition, HBV spread!




Michailidis *et al.* Sci Rep (2017)

## Co-cultures prove HBV spread from donor to acceptor cells

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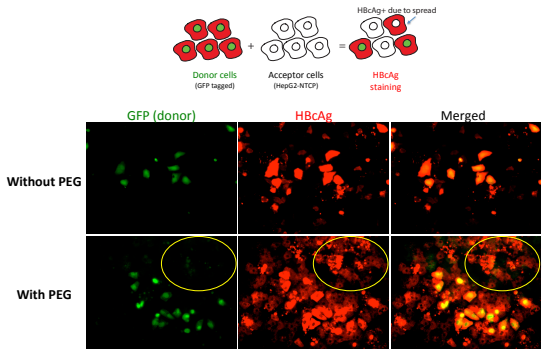
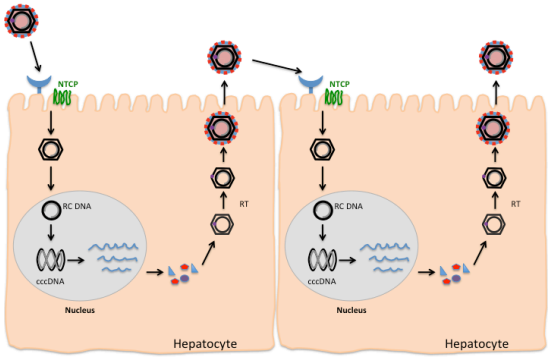



## Finally, the complete HBV life cycle in cell culture




## HBV and IFN: Possible Clue to a Cure?

- HBV is considered a stealth virus that does not induce IFN

JOURNAL OF VIROLOGY, Aug. 2005, p. 9369–9380
0022-538X/05/$08.00+0 doi:10.1128/JVI.79.15.9369–9380.2005


Stealth and Cunning: Hepatitis B and Hepatitis C Viruses

Stefan F. Wieland and Francis V. Chisari*

- IFN treatment can cure HBV

**Interferon Treatment for Hepatitis B**

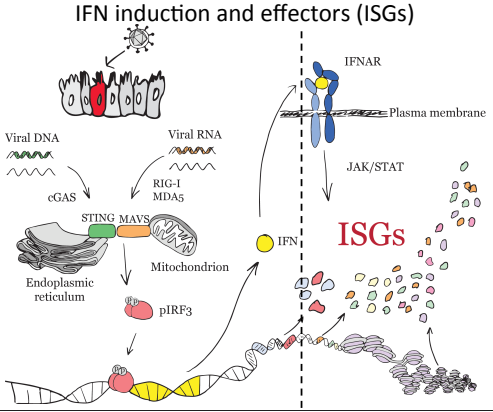
Monica A. Konerman, MD, MSc, Anna S. Lok, MD*

KEYWORDS
• Chronic hepatitis B • Cirrhosis • Hepatocellular carcinoma • Hepatitis B e antigen
• Hepatitis B surface antigen • Hepatitis B virus genotype

KEY POINTS

- Interferon (IFN) therapy offers several benefits over nucleos(t)ide analogs for treatment of chronic hepatitis B including a finite duration of therapy and a higher rate of clearance of hepatitis Be antigen and surface antigen.
- Patients who respond to IFN therapy have been shown to have improvement in clinical outcomes, specifically a decrease in incidence of hepatocellular carcinoma and development of cirrhosis.
- IFN is associated with a broad spectrum of potential adverse effects, including psychiatric effects, bone marrow suppression, and exacerbation of autoimmune diseases.
- Recommendations to use IFN should balance benefits versus risks and decisions should be tailored to individual patient characteristics and preference.

## IFN induction and effectors (ISGs)




## Antiviral ISG screens

LETTER

doi:10.1038/nature09907

**A diverse range of gene products are effectors of the type I interferon antiviral response**

LETTER

doi:10.1038/nature12862

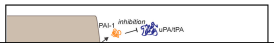
**Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity**

Article

Cell

**A Serpin Shapes the Extracellular Environment to Prevent Influenza A Virus Maturation**

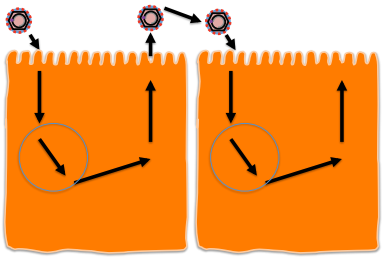
Graphical Abstract

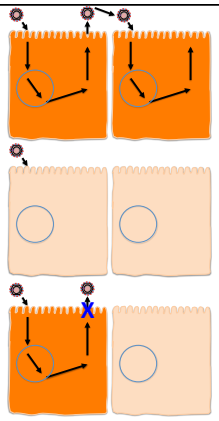


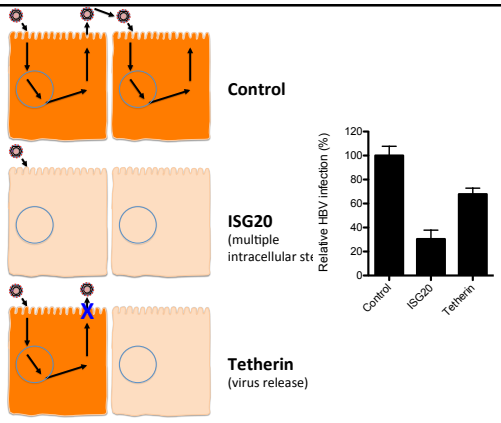

Authors
Meike Dittmann,
Hans-Heinrich Hoffmann, ...,
Paul D. Bieniasz, Charles M. Rice

## What about HBV?

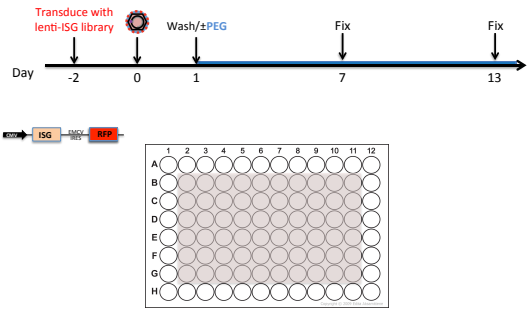
+ PEG






## Anti-HBV ISG screens: In progress

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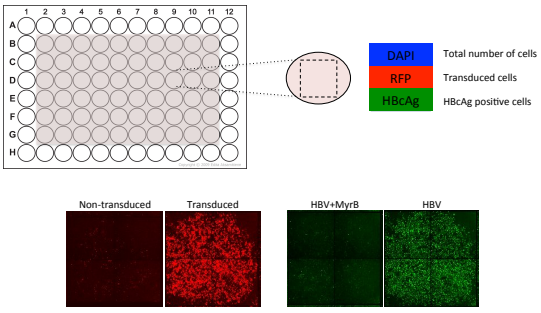


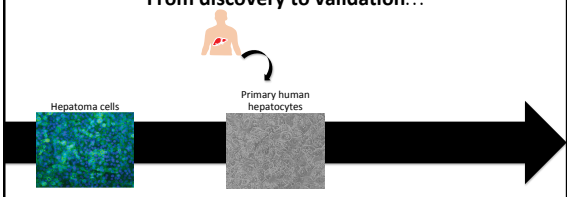







## Model systems for HBV: towards more relevant systems

**From discovery to validation**…

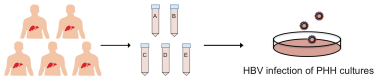


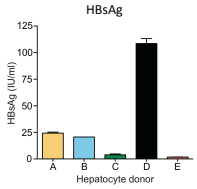
## Limitations of primary hepatocyte cultures

- Donor-to-donor variability
- Limited availability for each donor
- Difficult to maintain in culture
- Cannot be expanded in culture
- Difficult to manipulate genetically

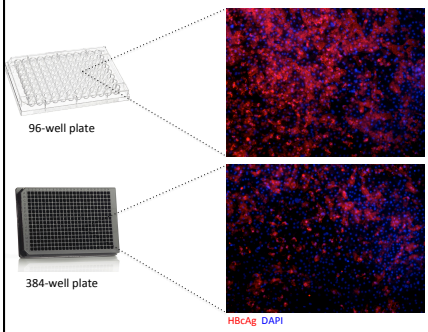
…but remain the gold standard for HBV studies

## Primary human hepatocytes: not all donors are the same

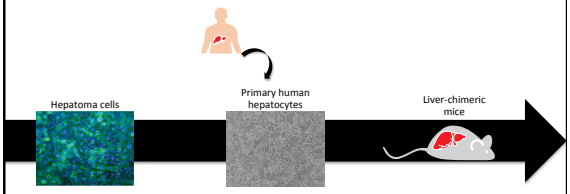


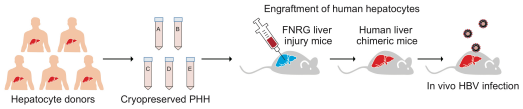

## Scalable for high-throughput screens



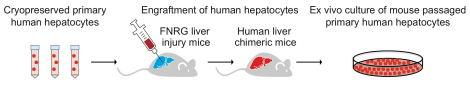

## Model systems for HBV: towards more relevant systems



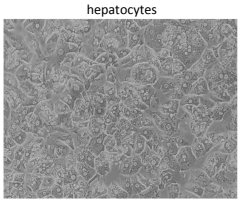

## Human liver chimeric mice support HBV infection

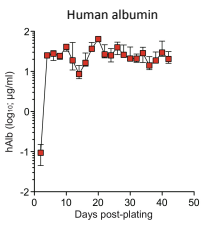



## Primary human hepatocytes can be expanded in mice and used in long term in vitro cultures

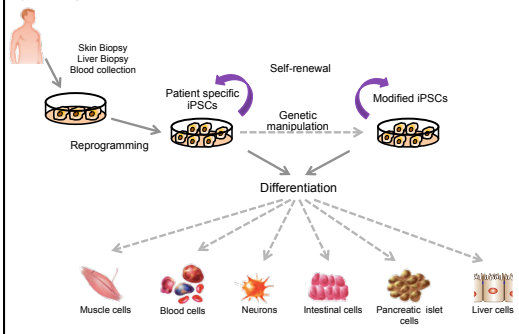



6-week culture of mouse-passaged hepatocytes



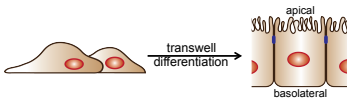


## Stem cell-derived models for studying viral infection and pathogenesis

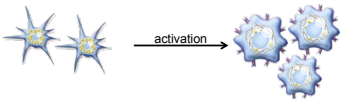



## Applications . . .

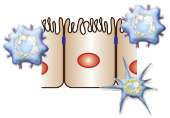
Polarized iHLCs




Stellate cells

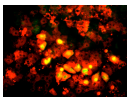



Complex systems

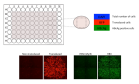


## Summary 1

Continuous PEG treatment facilitates HBV spread in HepG2-NTCP cells



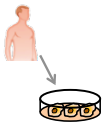
HBV spread system can be efficiently applied in antiviral screens



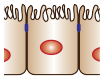
Primary human hepatocytes can be modified in vitro and expanded in liver chimeric mice


## Summary 2

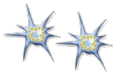
Stem cell derived hepatocytes (HLCs) offer a potential tool for genetic studies and patient derived systems



Polarized HLCs are a suitable system for HEV biology



Stem cells can be used to generate multiple cell types (HLCs, iStellates)



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Jonathan Pabon

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Viet Loan Dao Thi

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Jérôme Gouttenoire
University of Lausanne

Amir Shlomai
Tel Aviv University

Sangeeta N. Bhatia
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Indiana University

Stephan Urban
Heidelberg University