Immunology of chronic viral hepatitis and HCC

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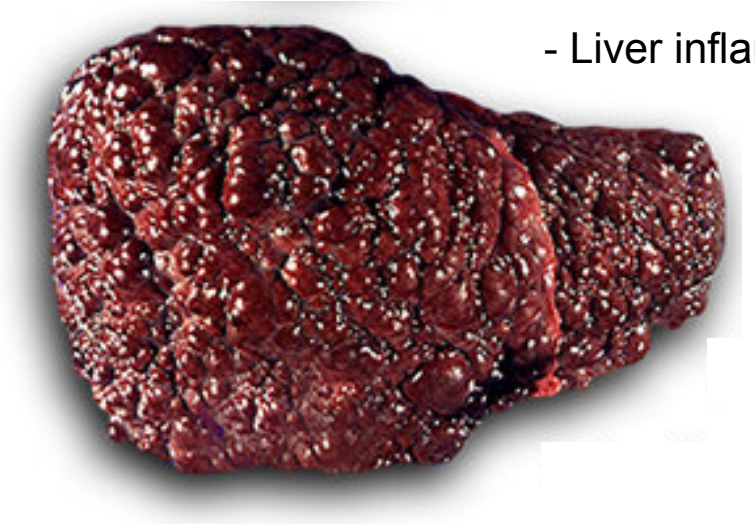
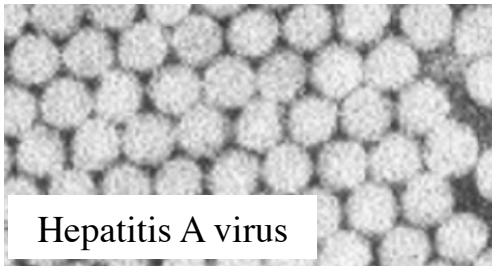
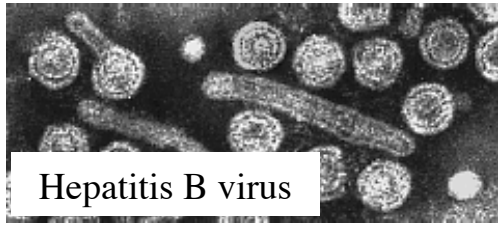
Viral hepatitis



- Most common liver disease
- Liver inflammation due to virus infection



Hepatitis A virus



Hepatitis B virus

	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis Delta	Hepatitis E
Virus family	Picornavirus	Hepadnavirus	Flavivirus	Circular RNA similar to plant viroid	Similar to Calicivirus
Nucleic acid	RNA (+ sense)	DNA (partially double strand)	RNA (+ sense)	RNA (- sense)	RNA (+ sense)
Disease caused	Infectious hepatitis	Serum hepatitis	Non-A, non-B hepatitis		Enteric non-A, non-B hepatitis
Size	~ 28nm	~40nm	30 - 60nm	~ 40nm	30 - 35 nm
Envelope	No	Yes	Yes	Yes	No

Chronic viral hepatitis: a global burden

HBV: discovered in 1970
360 million people chronically infected
1,000,000 deaths/year

HCV: discovered in 1989
170 million people chronically infected
leading cause of liver transplantation
500,000 deaths/year

Chronic viral hepatitis: a global burden

HBV: route of transmission

blood-blood contact

blood transfusion, contaminated needles,
mother to child (vertical transmission)

chronicity at young age: very high (90%)

chronicity at adult age: very low (10%)

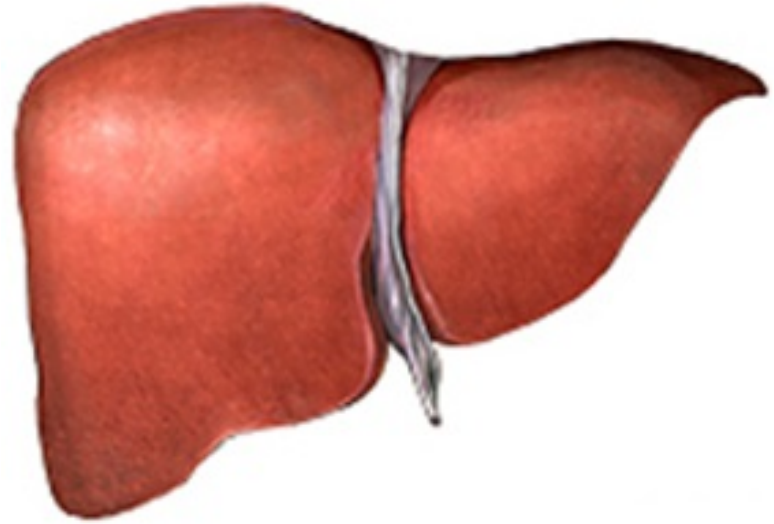
HCV: route of transmission

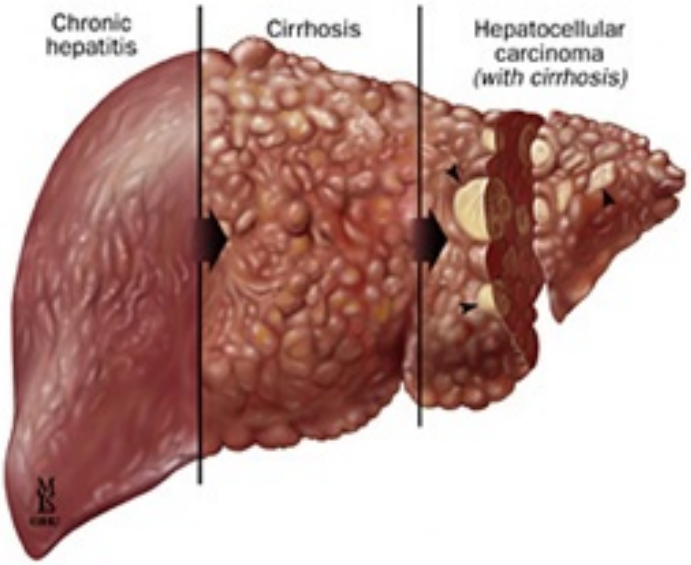
blood-blood contact

blood transfusion, contaminated needles,
sexual contact

chronicity at adult age: 70%

Viral hepatitis -- disease progression

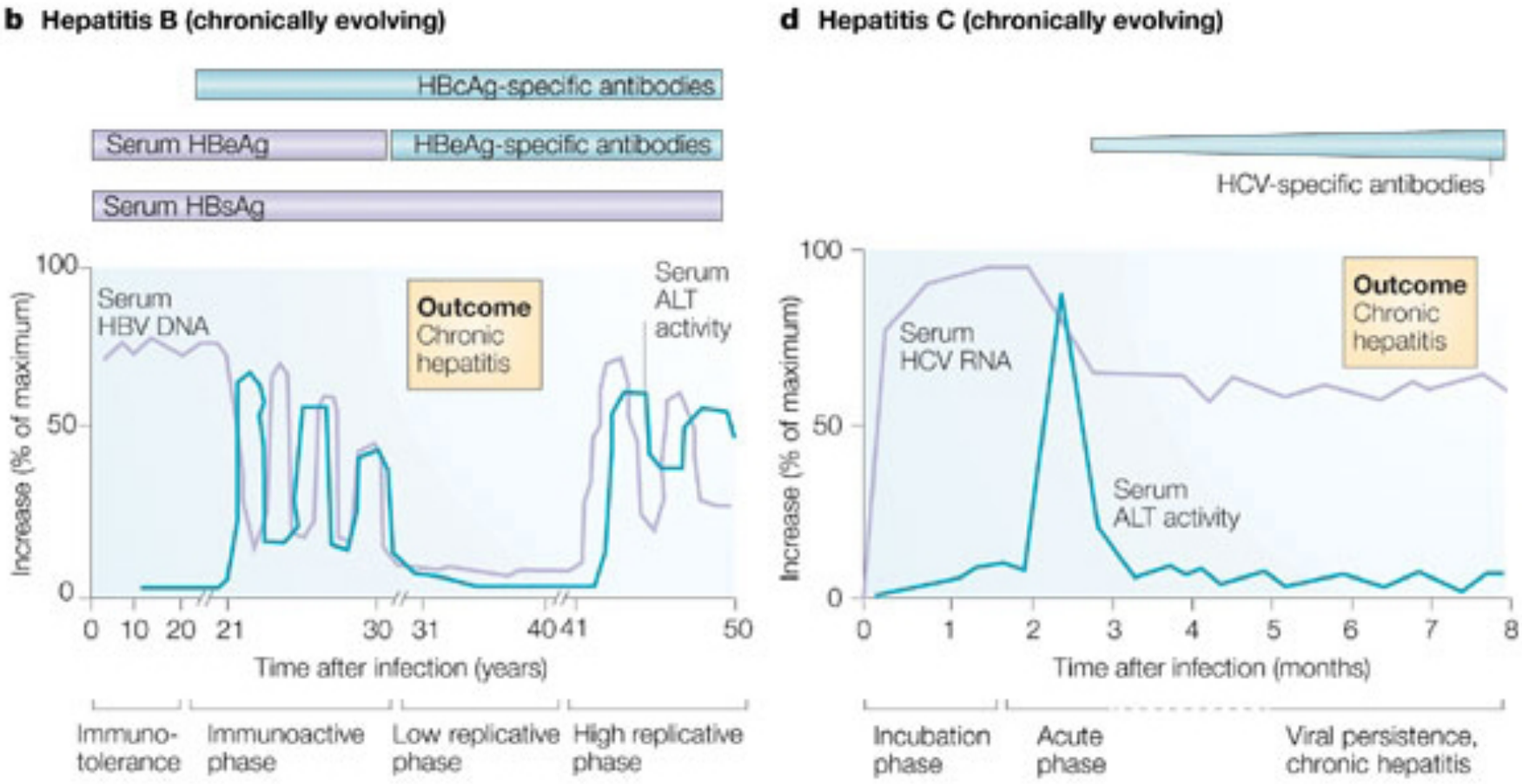


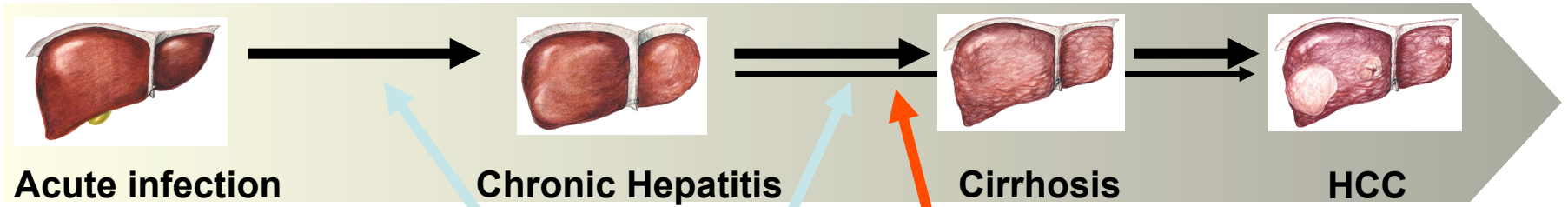


Decompensation
Transplantation
Death

30–50 years

Clinical characteristics of infections with HBV and HCV



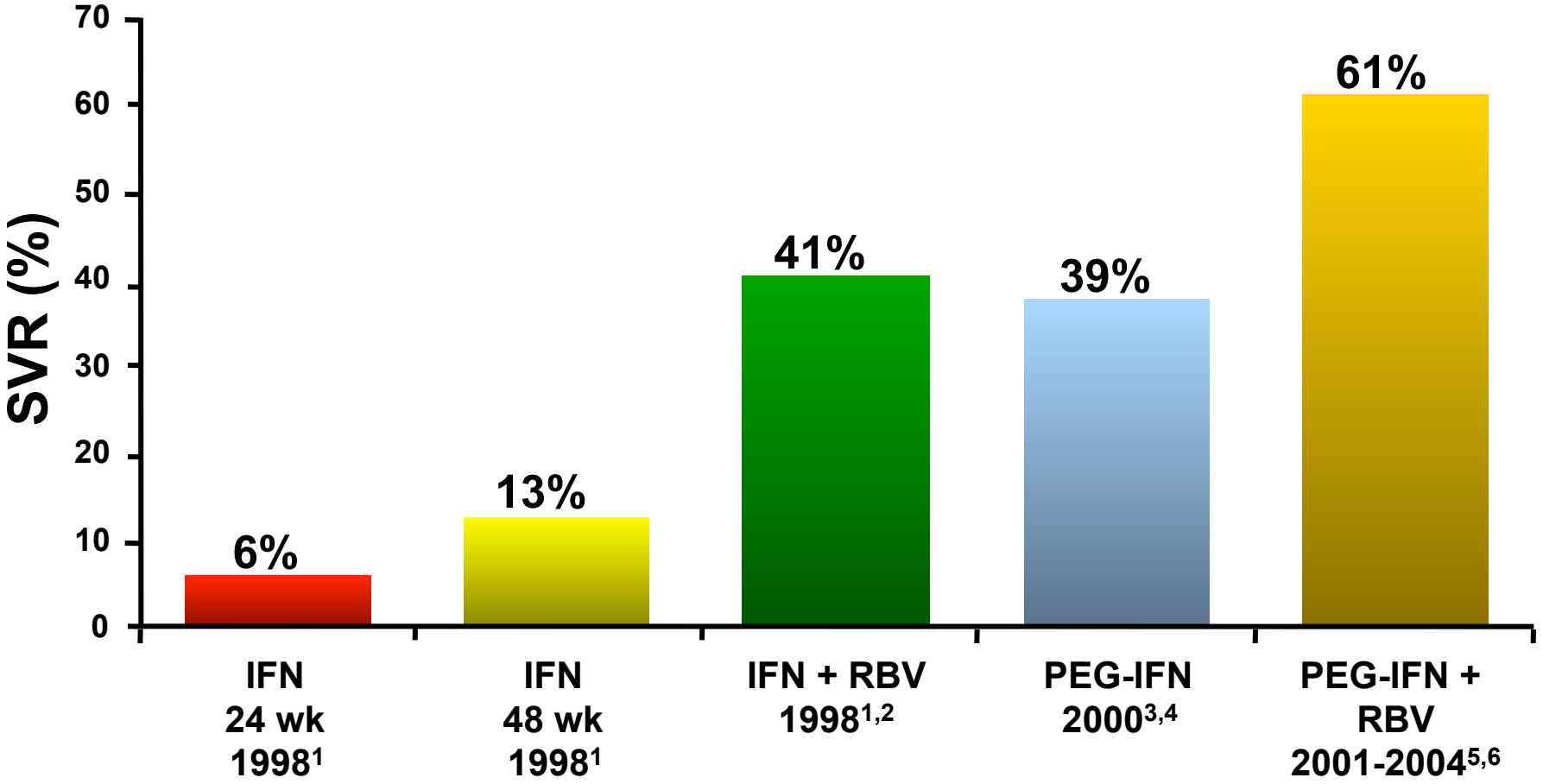


Develop a novel effective immune modulatory therapy to treat chronic hepatitis

Identify immunological parameters / biomarkers to predict/improve:
- Response to therapy
- Disease progression

Focus on the liver

Hepatitis C: Treatment



[1]McHutchison, NEJM 1998; [2]Poynard, Lancet 1998; [3]Zeuzem, NEJM 2000; [4]Lindsay, Hepatology 2001; [5]Manns, Lancet 2001; [6]Hadziyannis, Ann Intern Med 2004

Scarcity of suitable *in vitro* and *in vivo* models

- Scientific progress in the field of human viral hepatitis severely hampered
- Restricted species and cell tropism of most human hepatitis viruses
- Robust replication of HBV and HCV has only been documented in hepatocytes
  - Extremely hard to culture
- Animal models are essential for pathogenesis studies as well as preclinical antiviral efficacy and toxicity studies

Boonstra, Hepatology 2009

Experimental models for HBV/HCV infection

In vitro

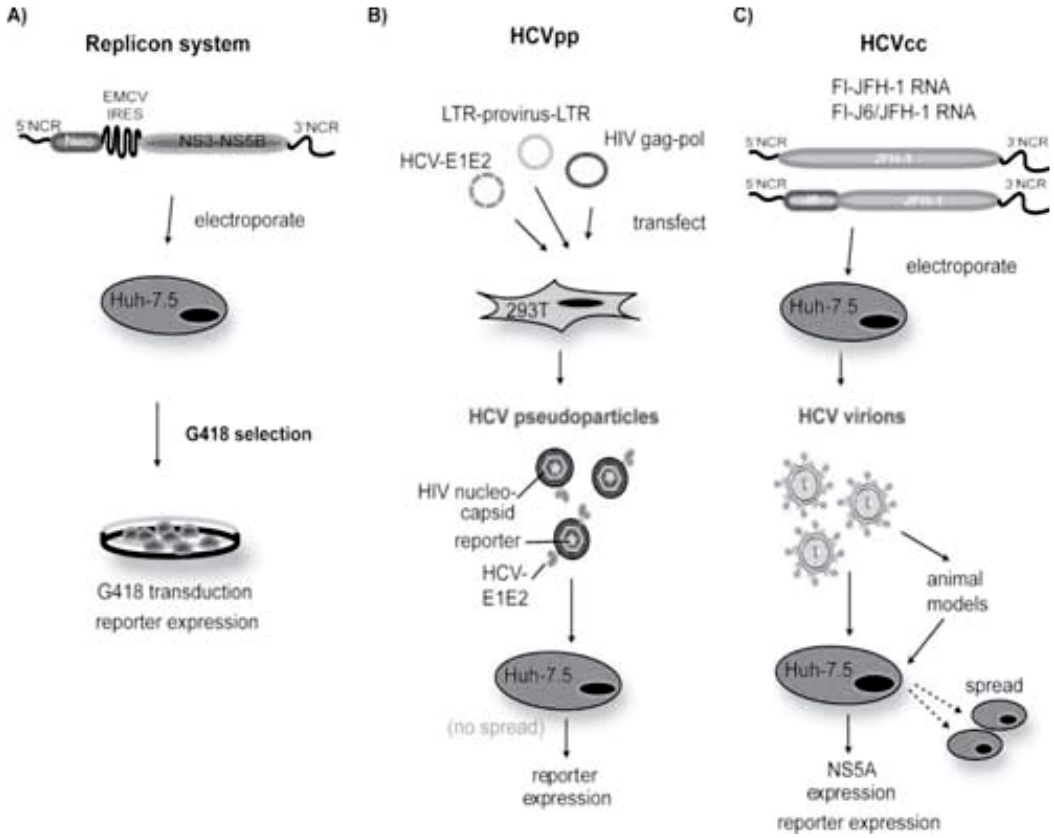
Mostly used in HBV in vitro studies

HepG2.2.15
-Stably transfected hepatoblastoma cell line
-Complete HBV genome

↓

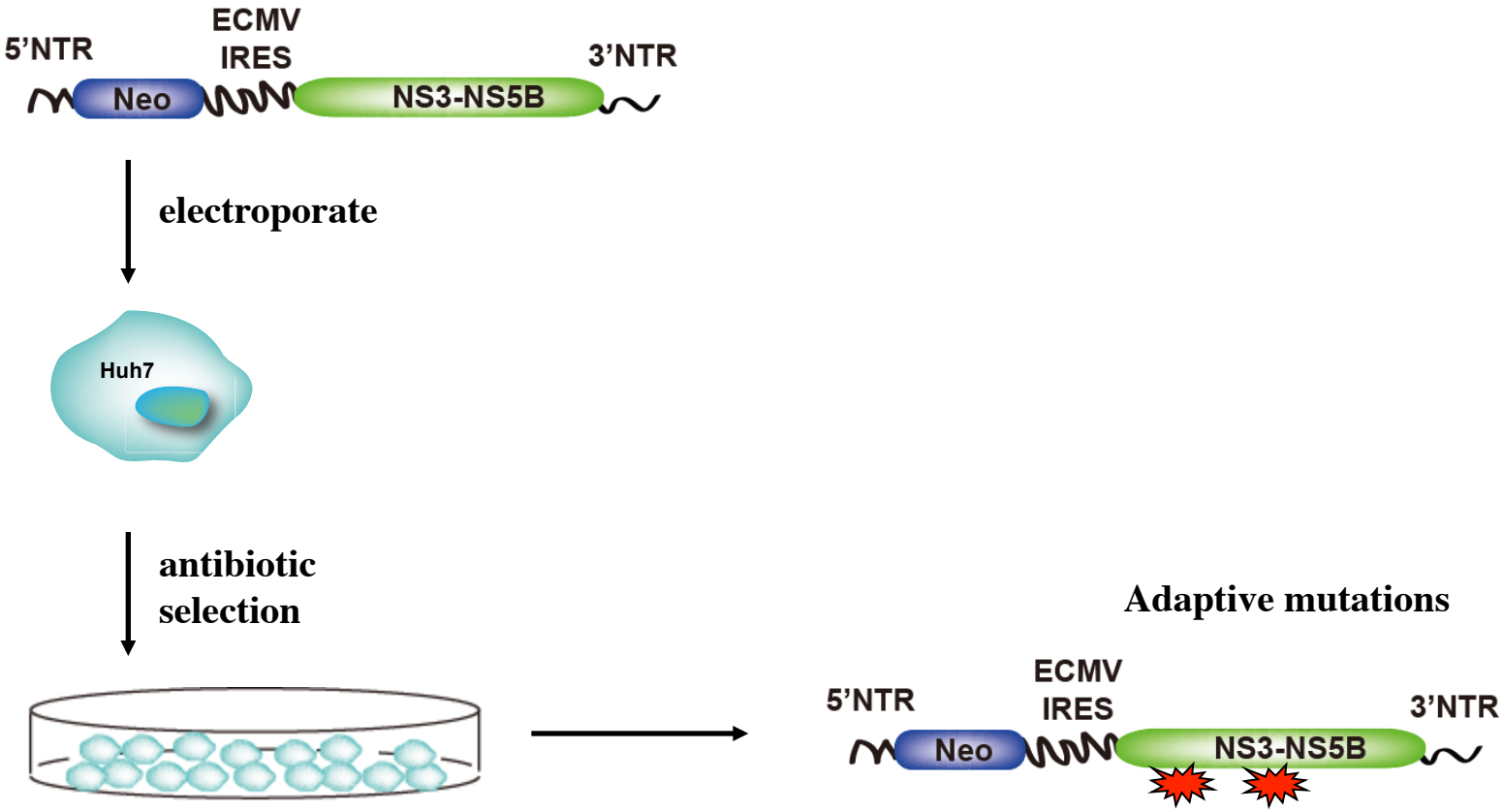
- All viral RNAs and proteins
- Produce viral genomes
- Secrete infectious particles
- Secrete virus-like particles

Systems used in HCV in vitro studies



Note:
Hepatoma cell lines are difficult to infect
Primary hepatocytes are difficult to obtain/culture

Subgenomics replicons

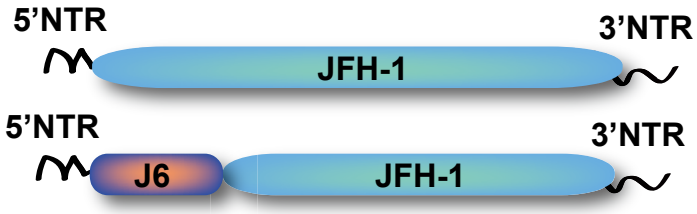


Lohmann, Science 1999

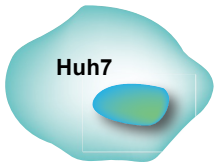
Blight, Science 2000
Lohmann, JVI 2001

Improvement by using Huh7.5 (Blight, JVI 2002)

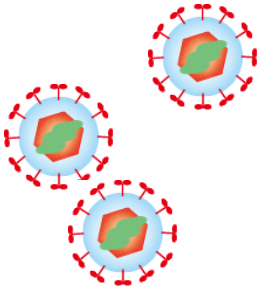
JFH-1- culture of infectious virus



electroporate



HCV virions

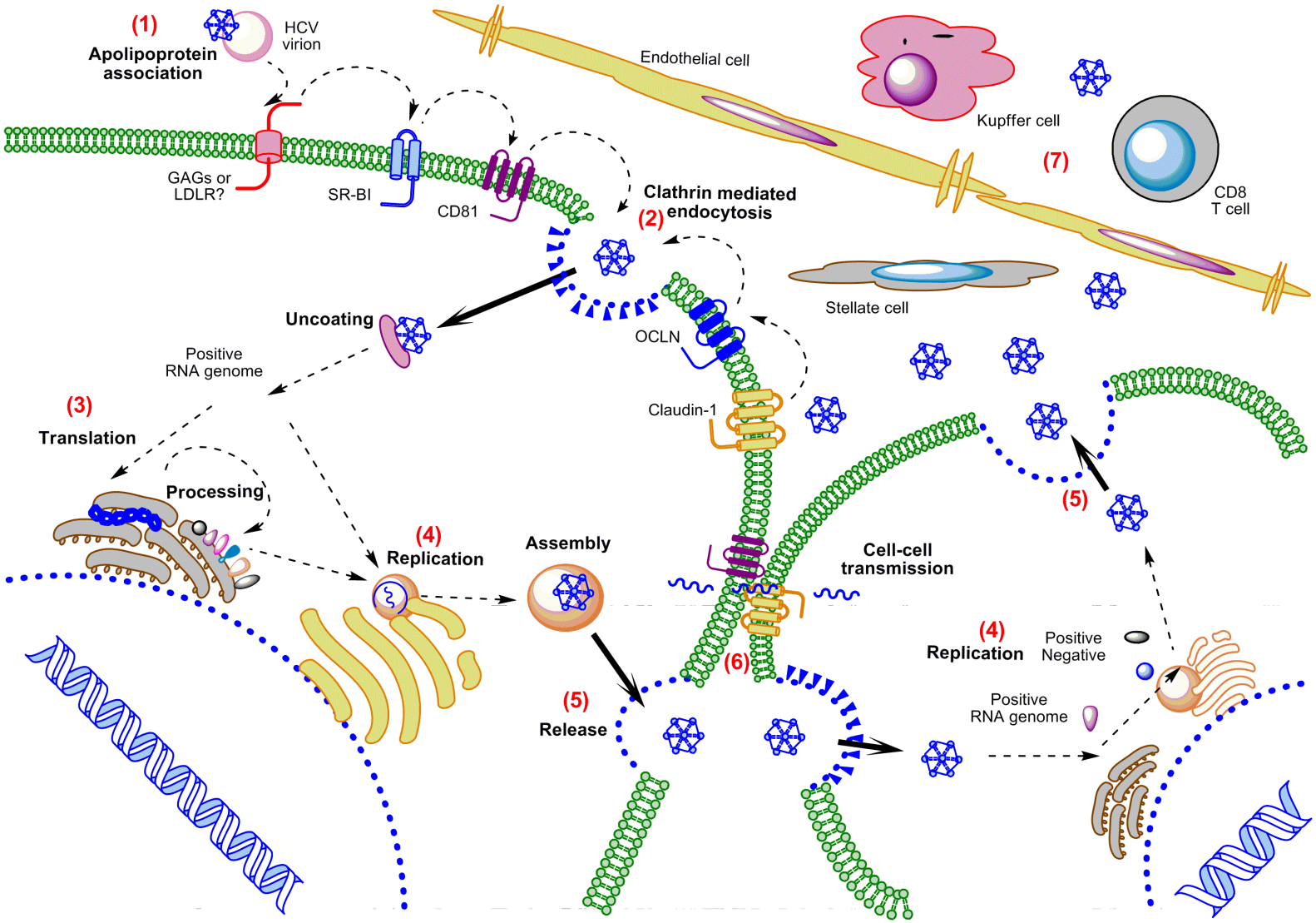


naïve cells

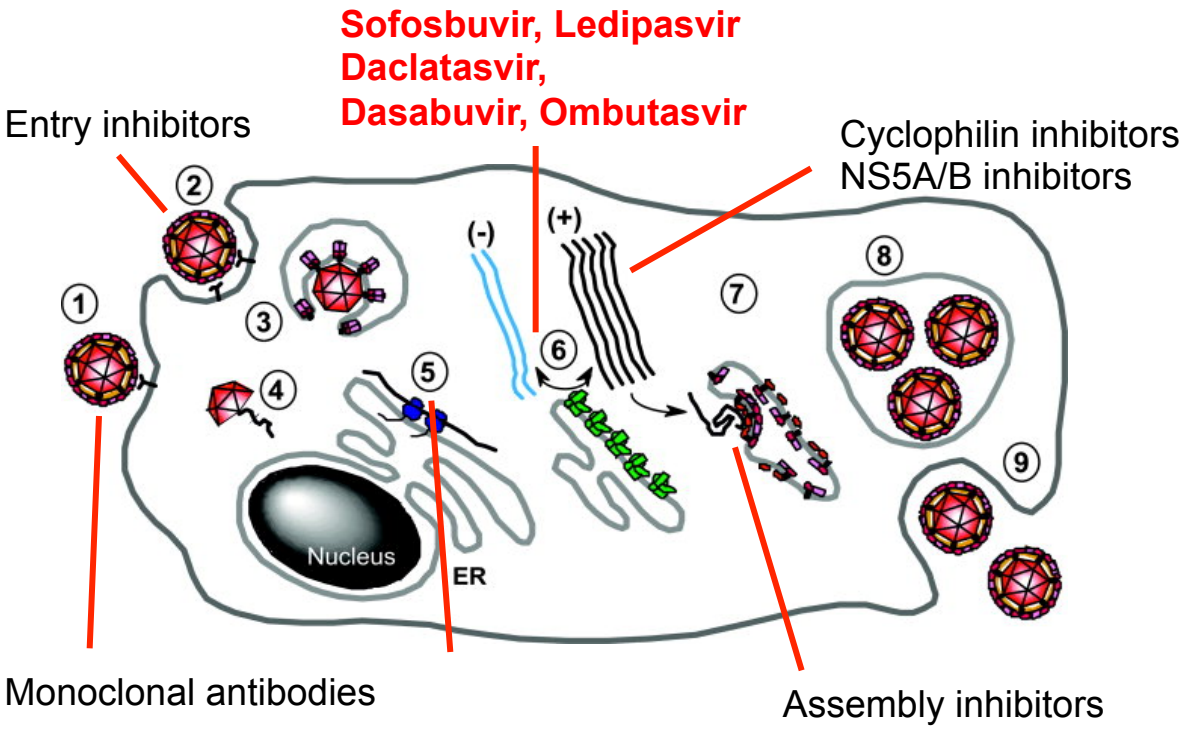
chimpanzee

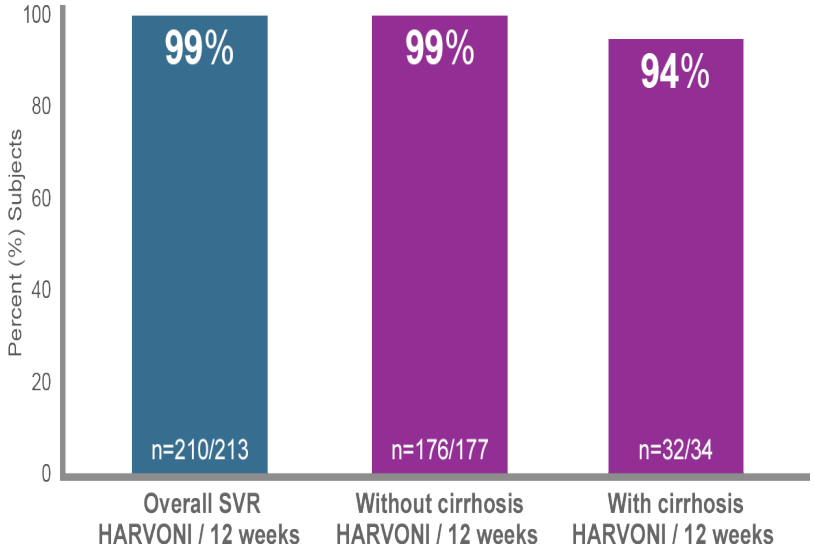
chimeric mice

Wakita, 2005
Lindenbach, 2005
Zhong, 2005



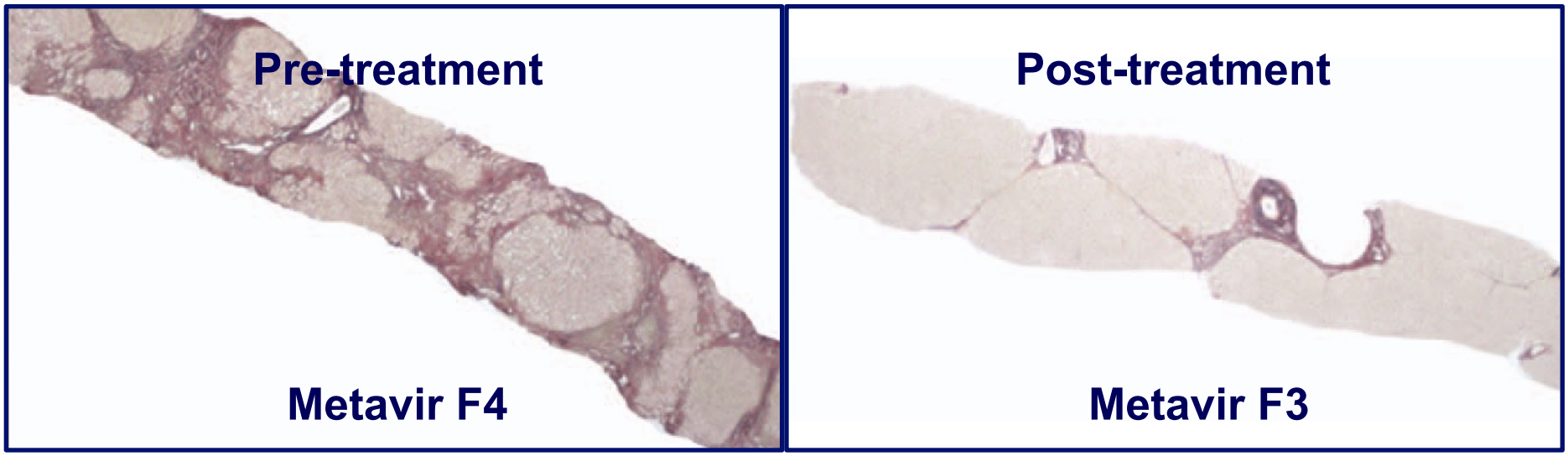
Boonstra, Hepatology 2009


Sofosbuvir, Ledipasvir
Daclatasvir,
Dasabuvir, Ombutasvir
Entry inhibitors
Cyclophilin inhibitors
NS5A/B inhibitors
1
2
3
4
5
6
7
8
9
(-)
(+)
Nucleus
ER
Monoclonal antibodies
Assembly inhibitors

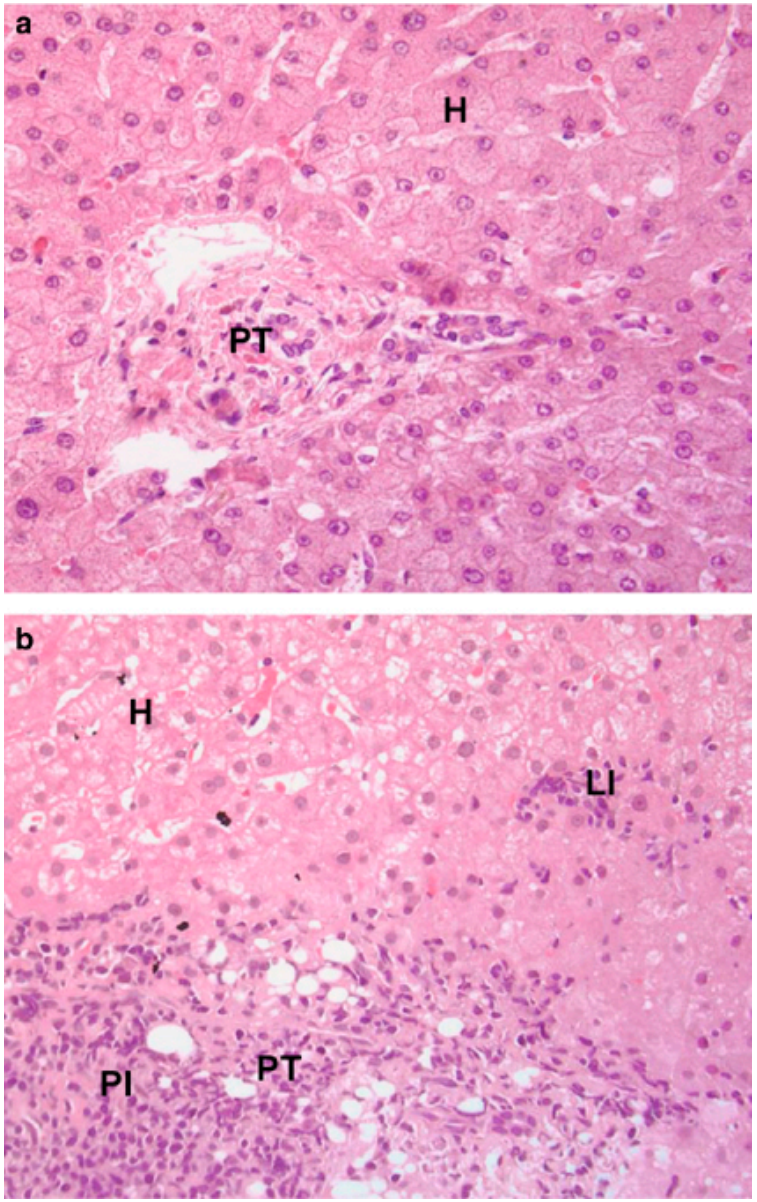

100
80
60
40
20
0
Percent (%) Subjects
99%
99%
94%
n=210/213
n=176/177
n=32/34
Overall SVR
HARVONI / 12 weeks
Without cirrhosis
HARVONI / 12 weeks
With cirrhosis
HARVONI / 12 weeks

Regression of hepatic fibrosis

- Paired liver biopsy in a single patient with HCV-induced cirrhosis who attained SVR



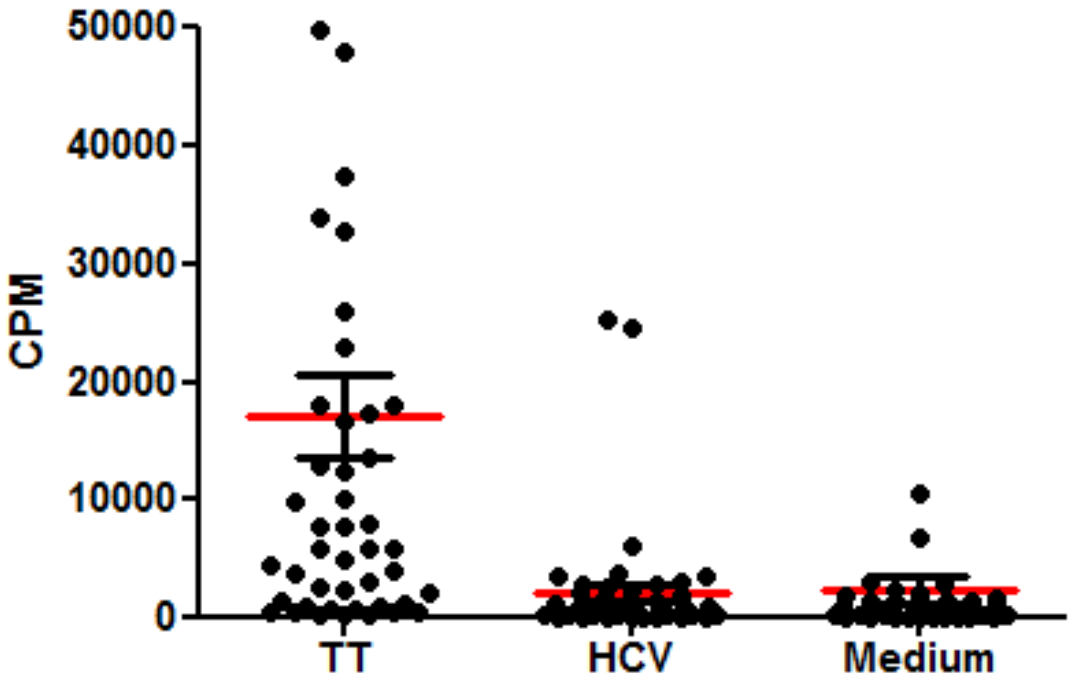
Histological appearance of HCV infection in the liver



Healthy

HCV

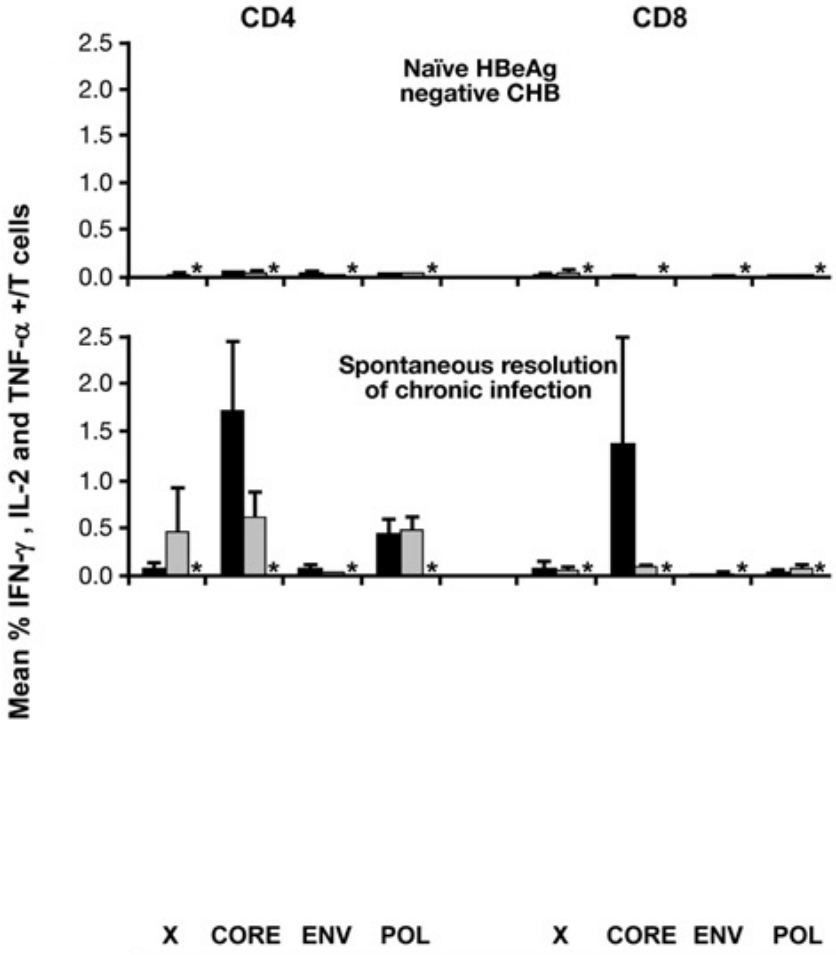
Most chronic HCV patients have a weak or absent HCV-specific CD4+ T cell response



n=48 patients
HCV protein mix: Core, NS3, NS4, NS5a
Day 5-6 overnight 3H-thymidine incorporation

Claassen, Boonstra. Curr Opin Virol 2014

T-cell responses in HBV infection are weak or absent

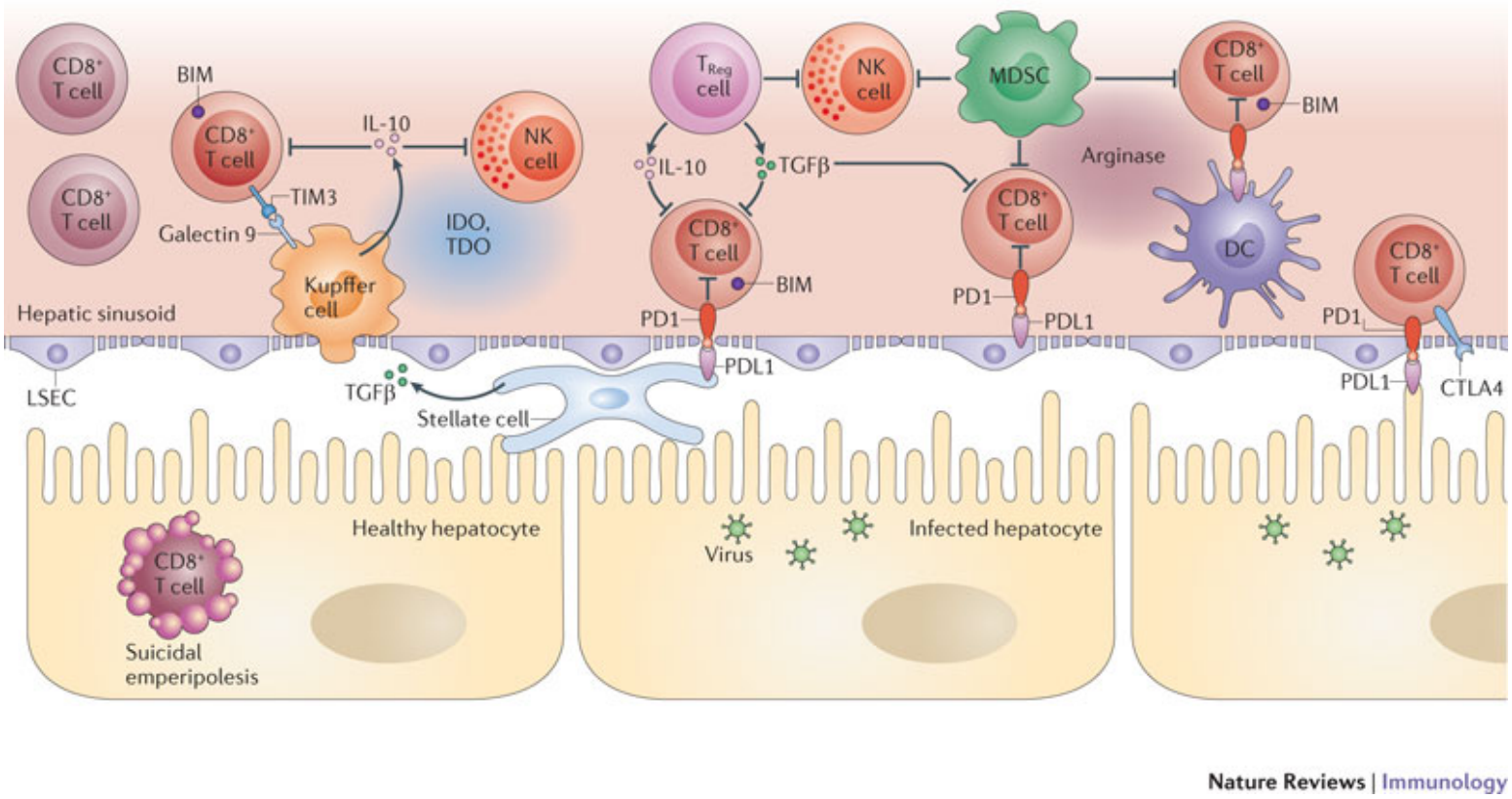


Chronic

Spontaneous resolution

Boni et al, Gastroenterology 2012

Host mechanisms that promote persistence of HBV in the liver



Resides in the liver in an immunosuppressive environment

Protzer, Nature Rev Immunol 2012

Host mechanisms that promote persistence of HBV in the liver

Regulatory T cells

IL-10 and/or TGF-β

Regulatory B cells

Active elimination of T cells

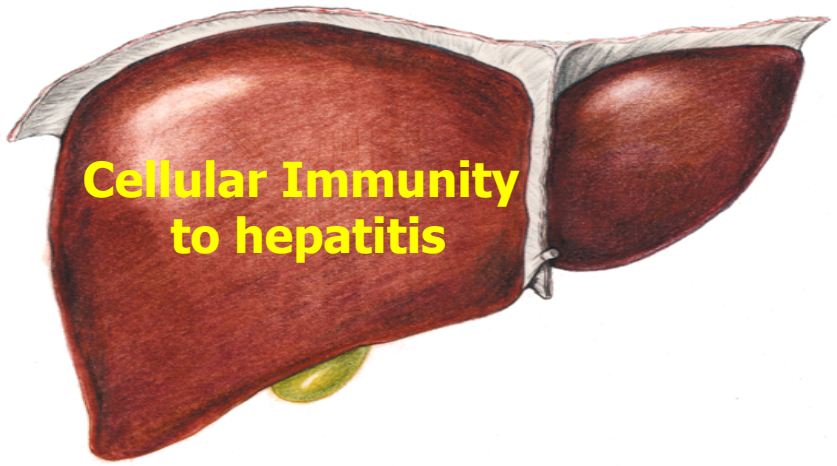
Impaired NK cells

Myeloid derived suppressor cells

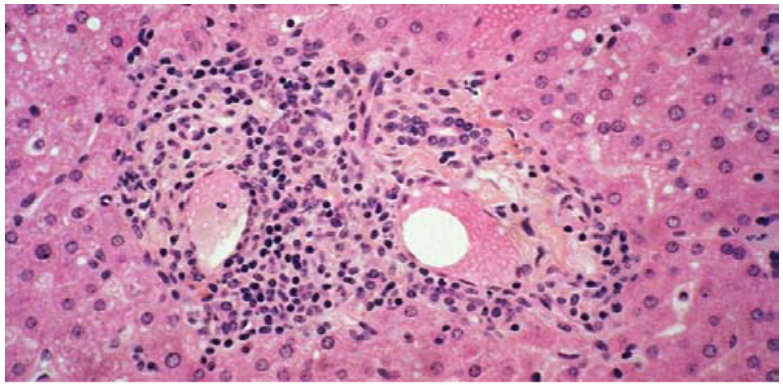
Impaired dendritic cells

High viral load leading to T cell exhaustion

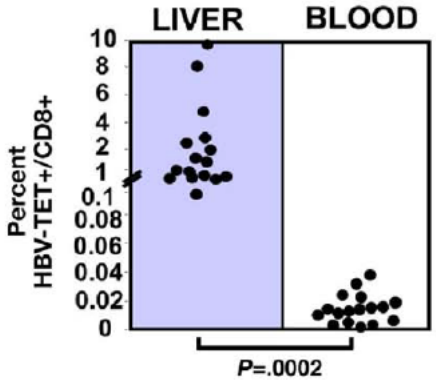
Mitochondrial dysfunction

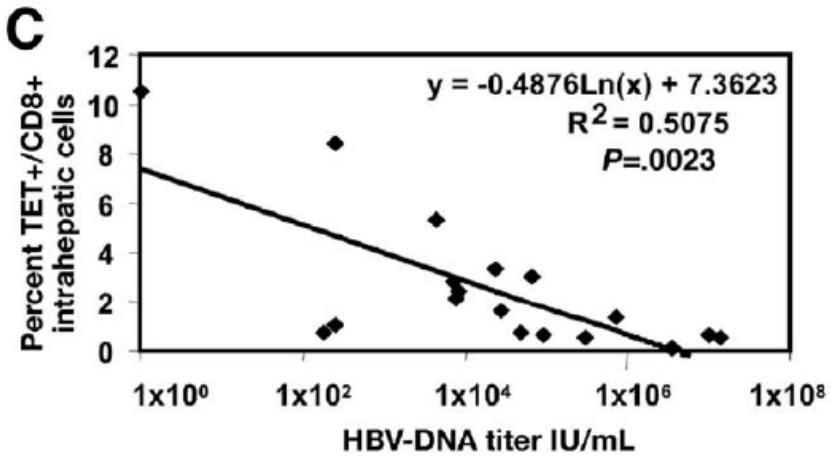


Relative high numbers of HBV-specific T cells in liver inverse correlation with HBV DNA load

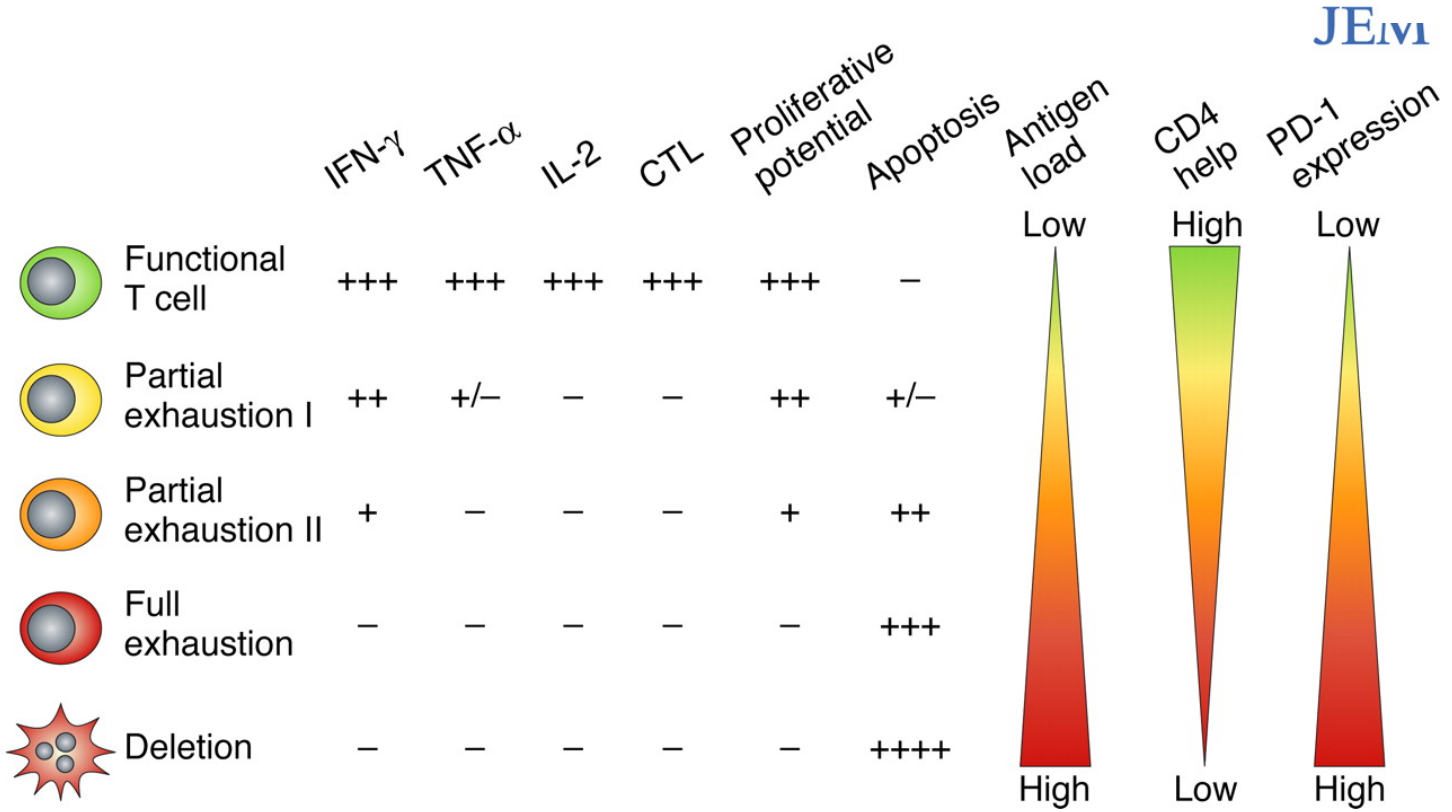


Photomicrograph of the liver with chronic hepatitis B.



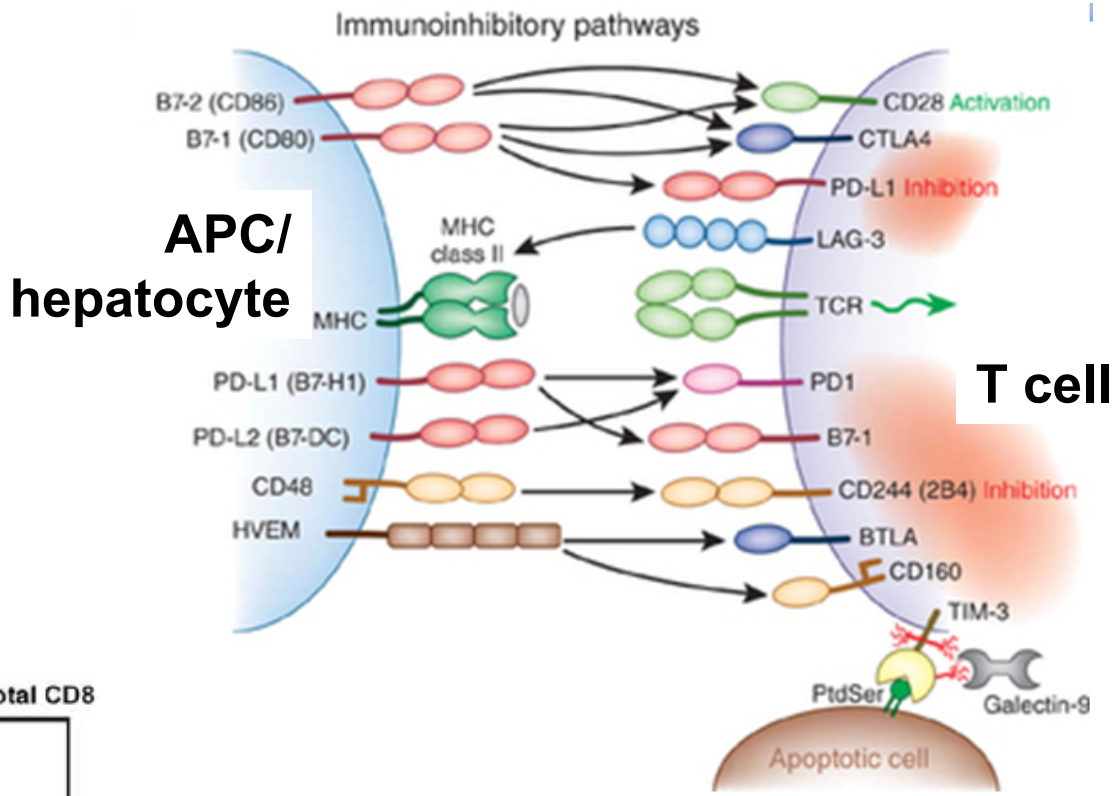


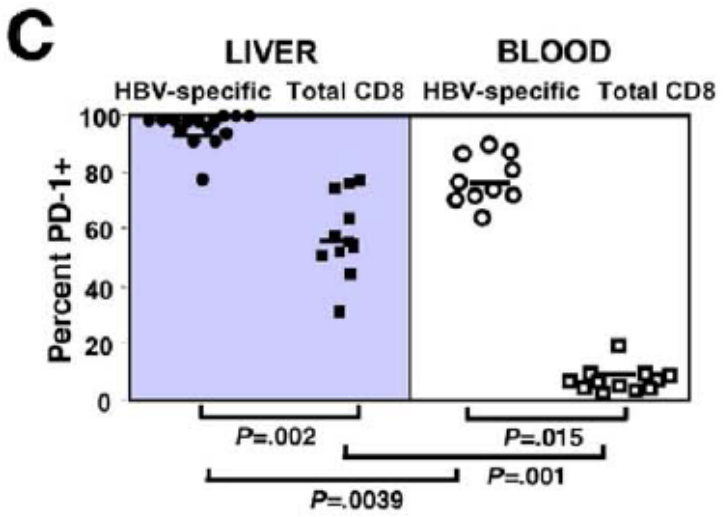
T cell exhaustion during chronic viral infections



Freeman, J Exp Med 2006

High PD-1 expression on HBV-specific CD8+ T cells

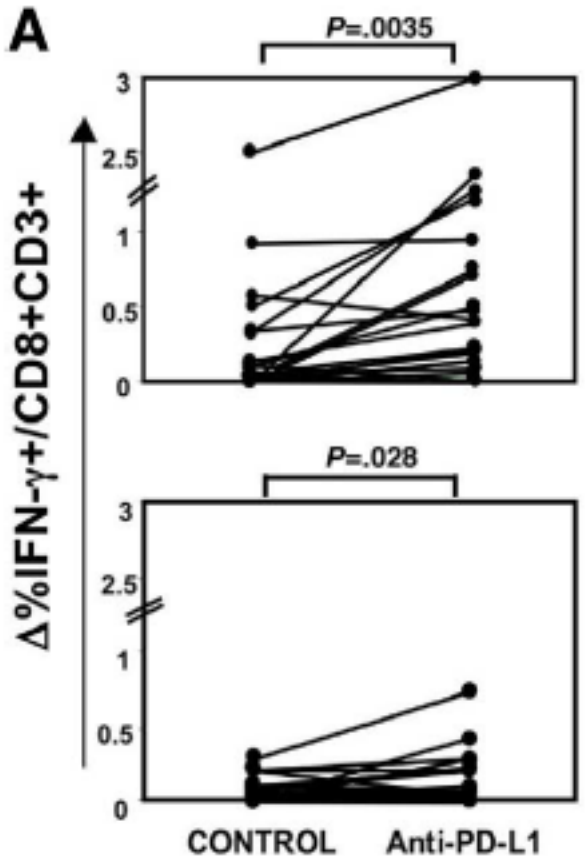


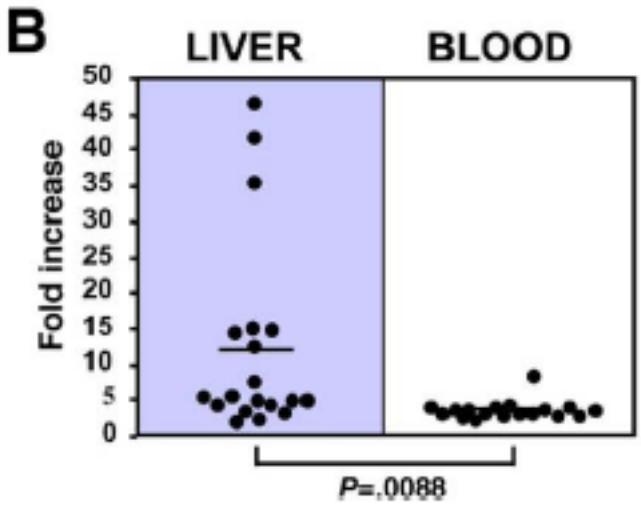


PD-1 blockade enhances HBV-specific T cell function

Liver

Blood

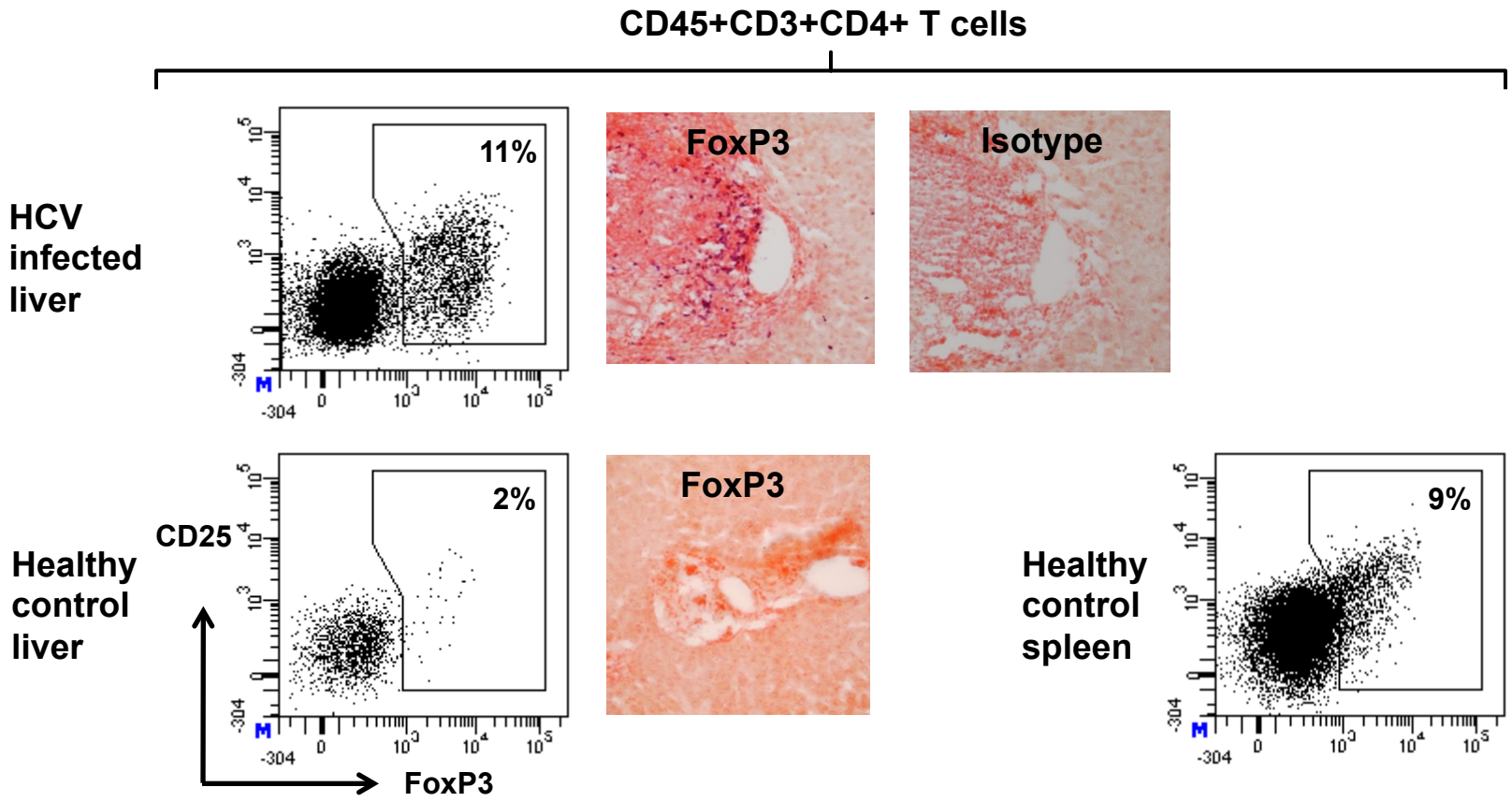




Fisicaro, Gastro 2010

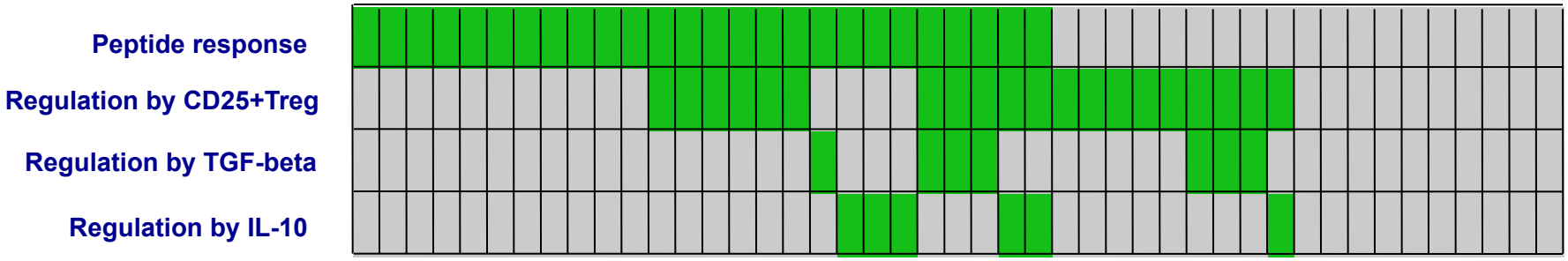
Nivolumab

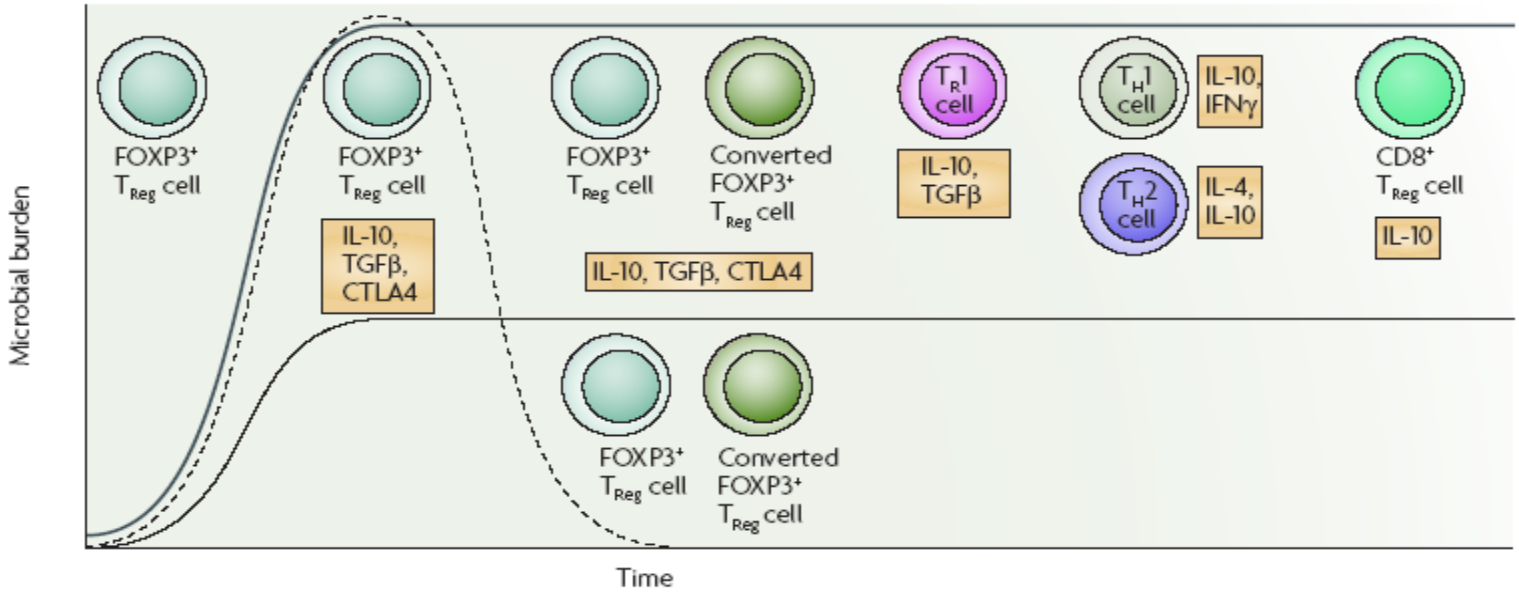
CD4+CD25+FoxP3+ Treg are abundantly present in HCV infected livers, while absent from healthy livers



Claassen, de Knegt, Turgut, Tilanus, Janssen, Boonstra. J.Hepatol. 2010. 52: 315-321

Regulation of HCV-specific CD4+ and CD8+ T cell responses differs between chronic HCV patients

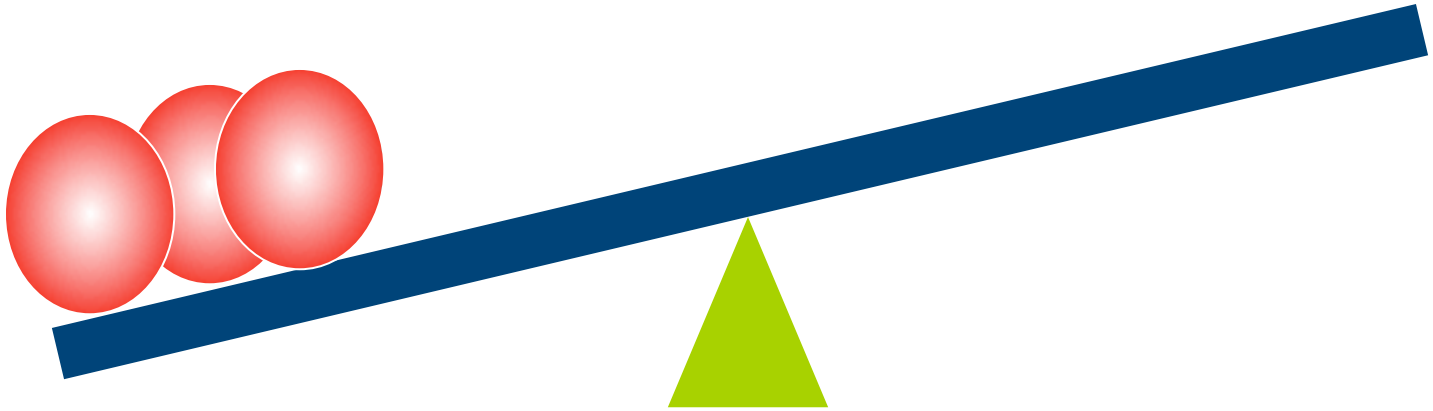




The balance between protective immunity and pathology

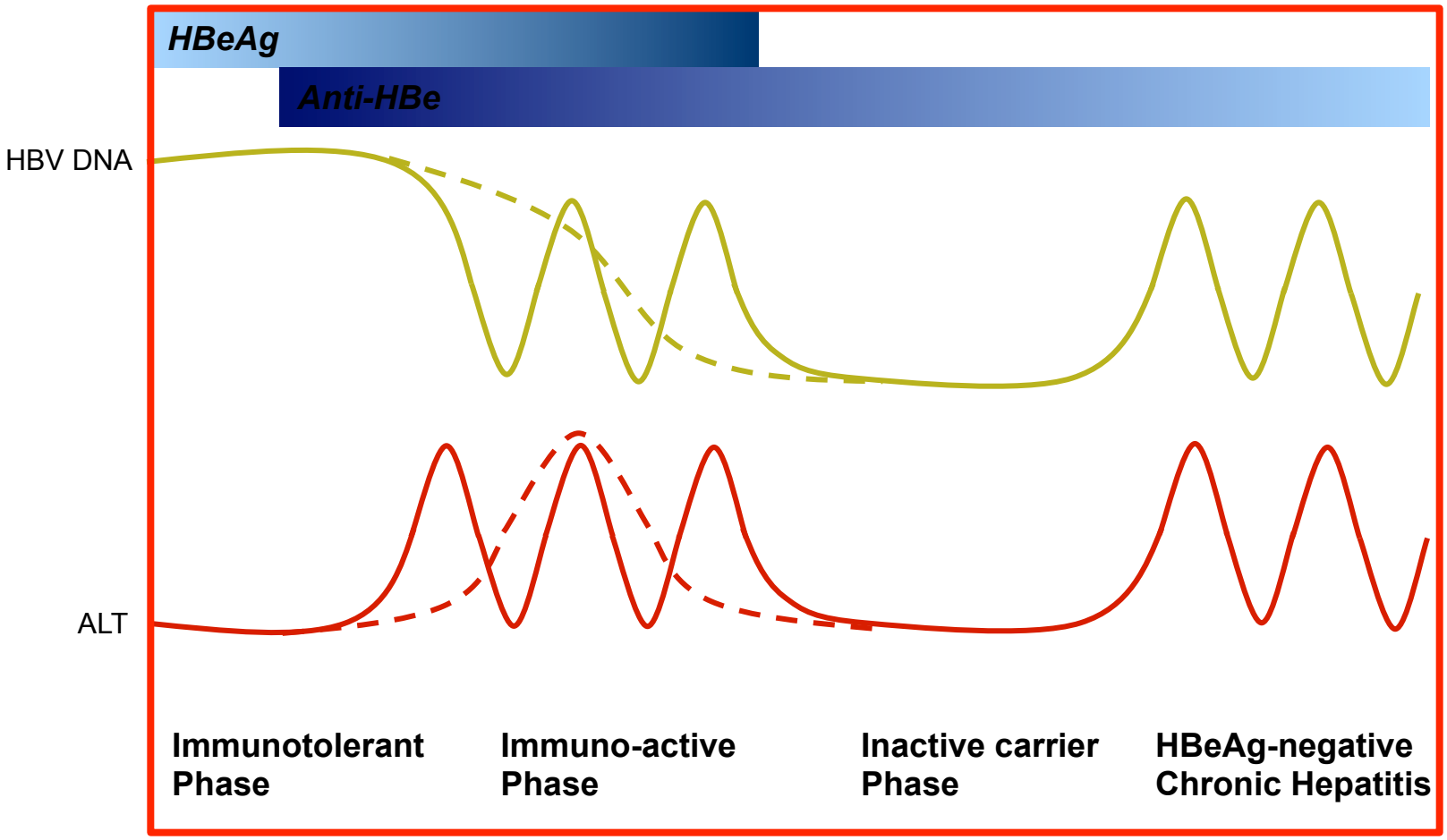
Protective immunity
+ pathology

chronicity
+ mild pathology



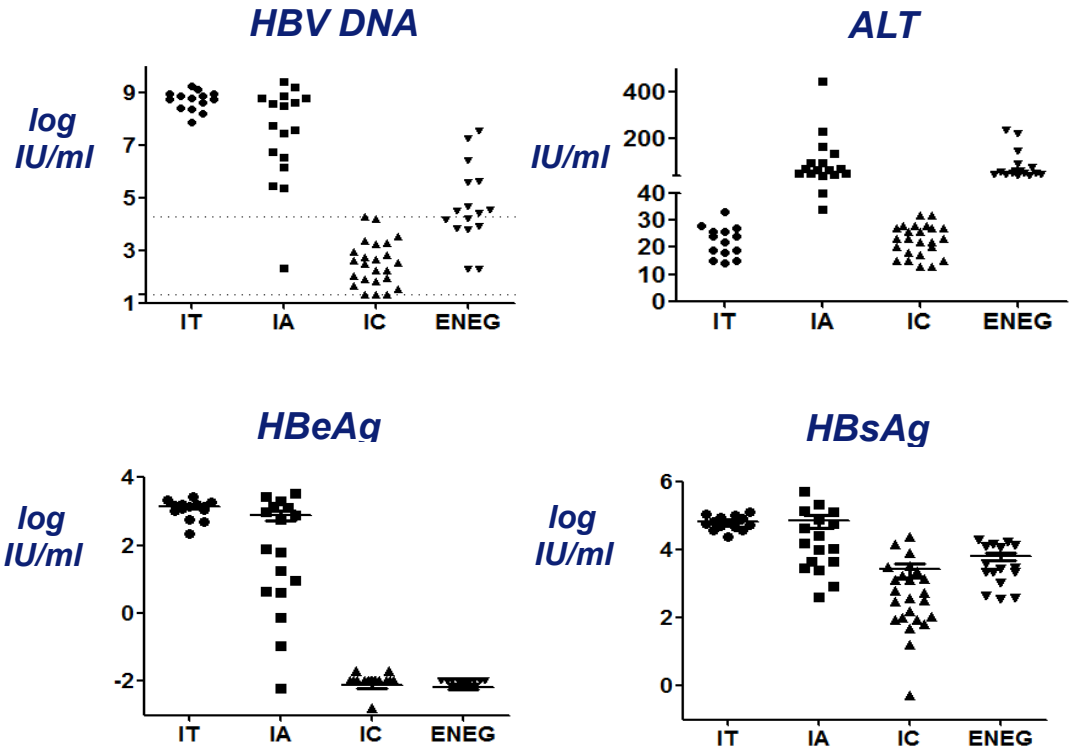
Claassen, Boonstra. Curr Opin Virol 2013

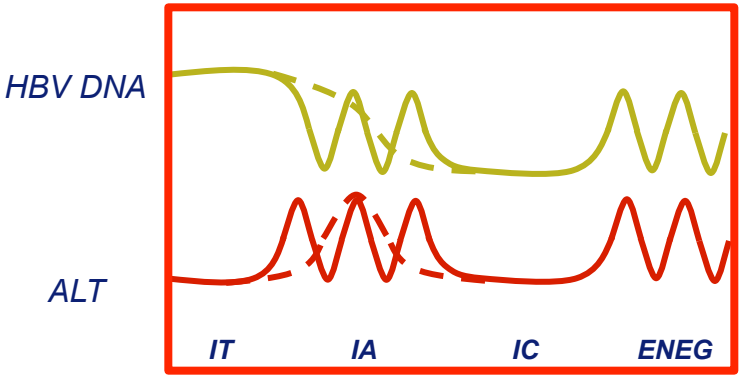
Phases of chronic infection in HBV patients



Erasmus MC

Phases of chronic infection in HBV patients



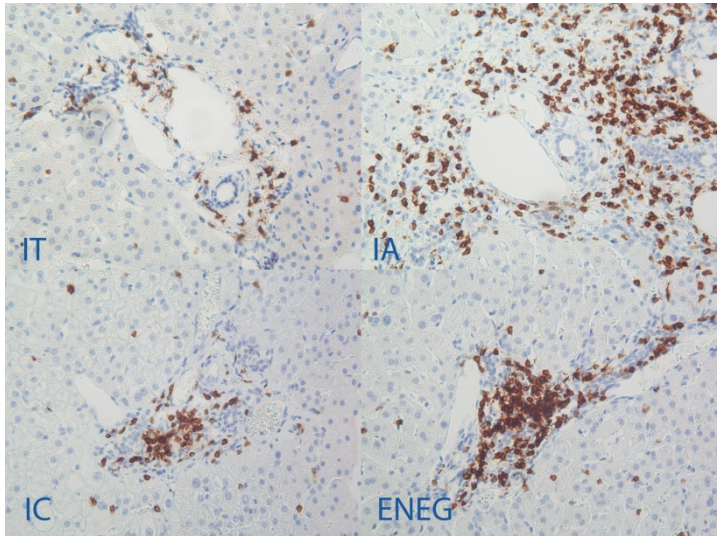


IT	*immunotolerant*
IA	*immuno active*
IC	*inactive carrier*
ENEG	*HBeAg-neg hepatitis*

Quantification of T and B cells in livers from HBV patients at different clinical phases

Intrahepatic T cells

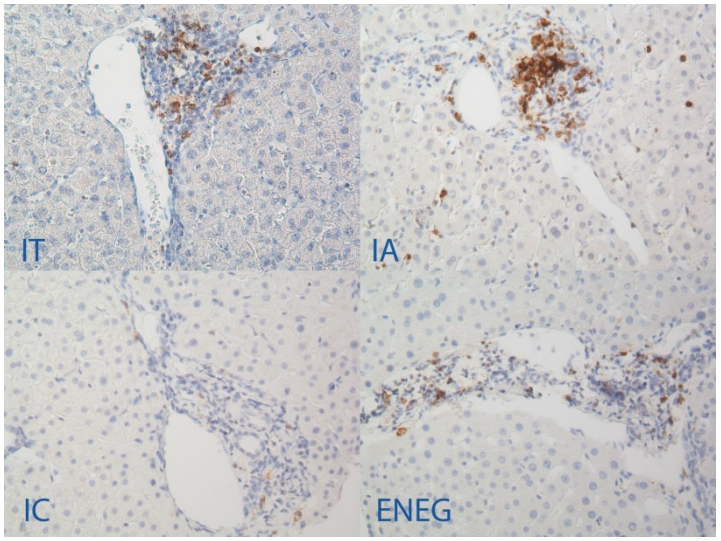
Immunotolerant | Immuno-active



Inactive carrier | HBeAg-neg hepatitis

Intrahepatic B cells

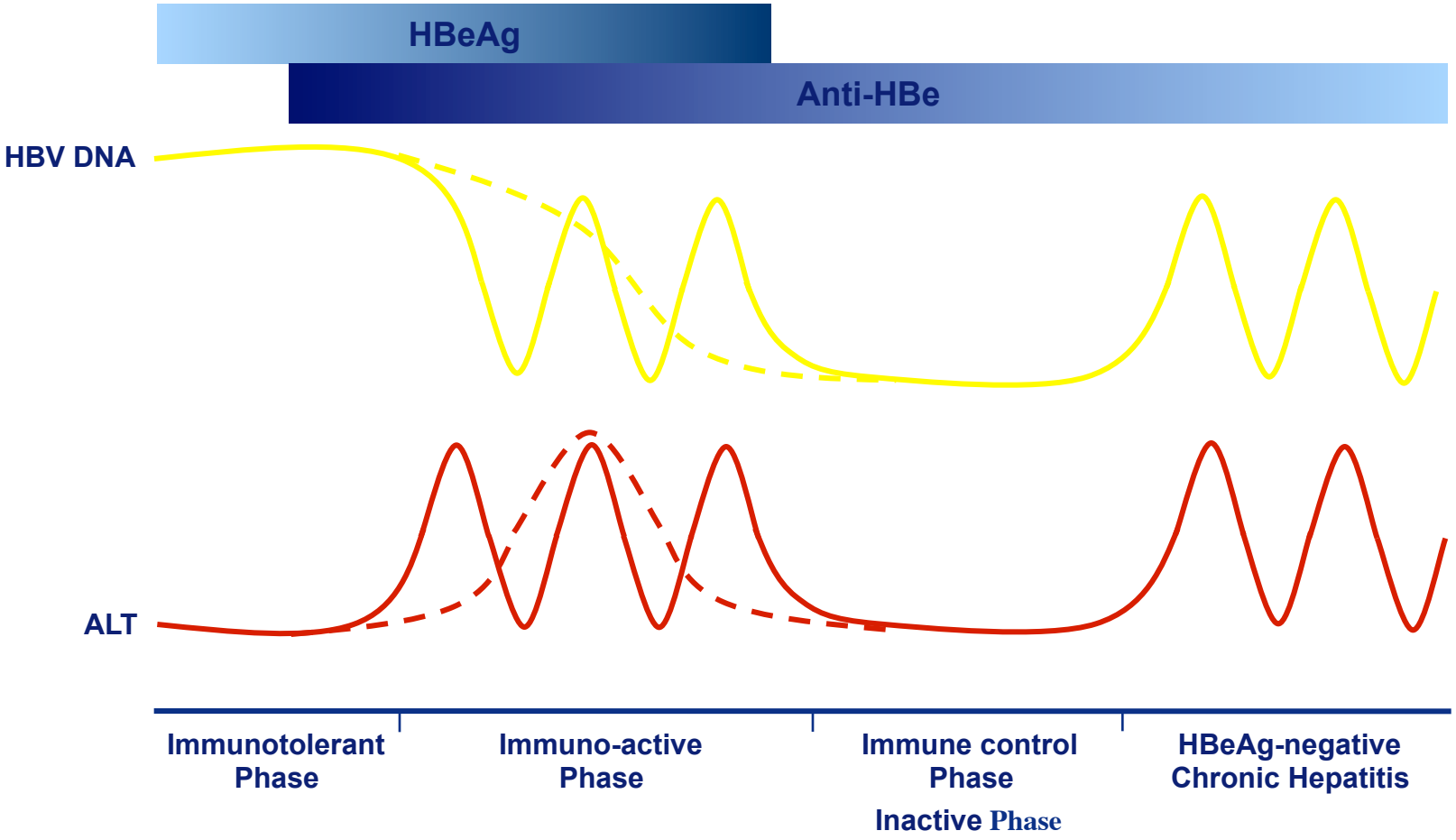
Immunotolerant | Immuno-active



Inactive carrier | HBeAg-neg hepatitis

Erasmus MC

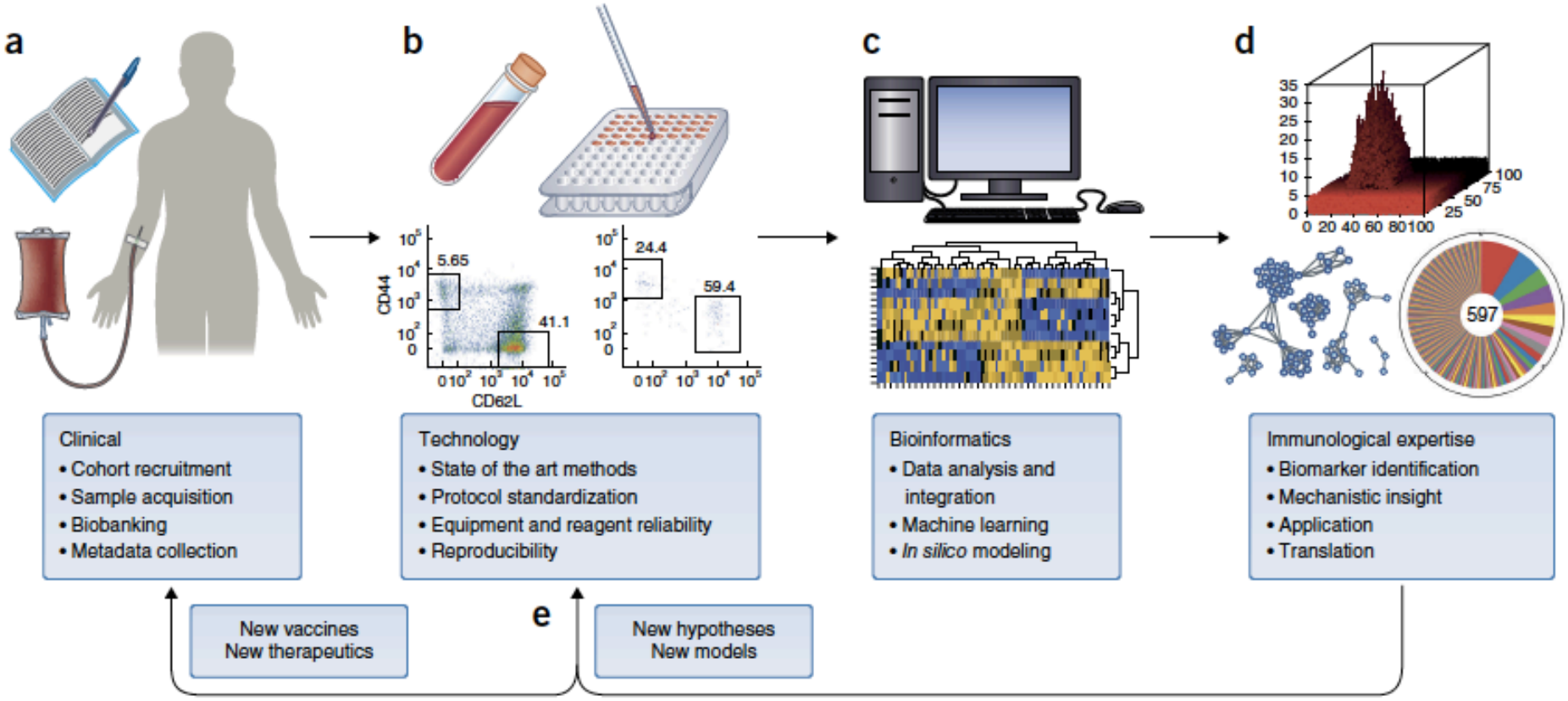
Phases Of Infection



Buster, Neth J Med 2006

Erasmus MC

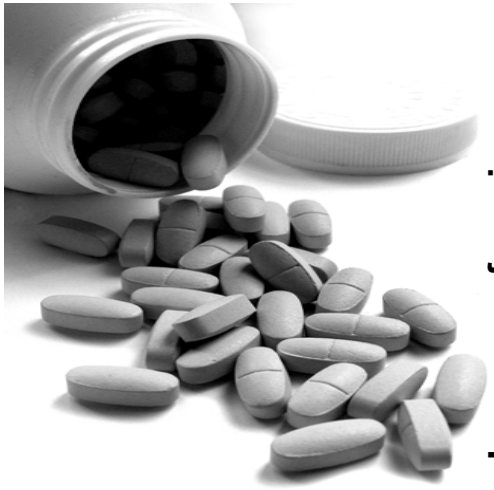
Identify virus or host biomarkers in peripheral blood that distinguish HBV clinical phases

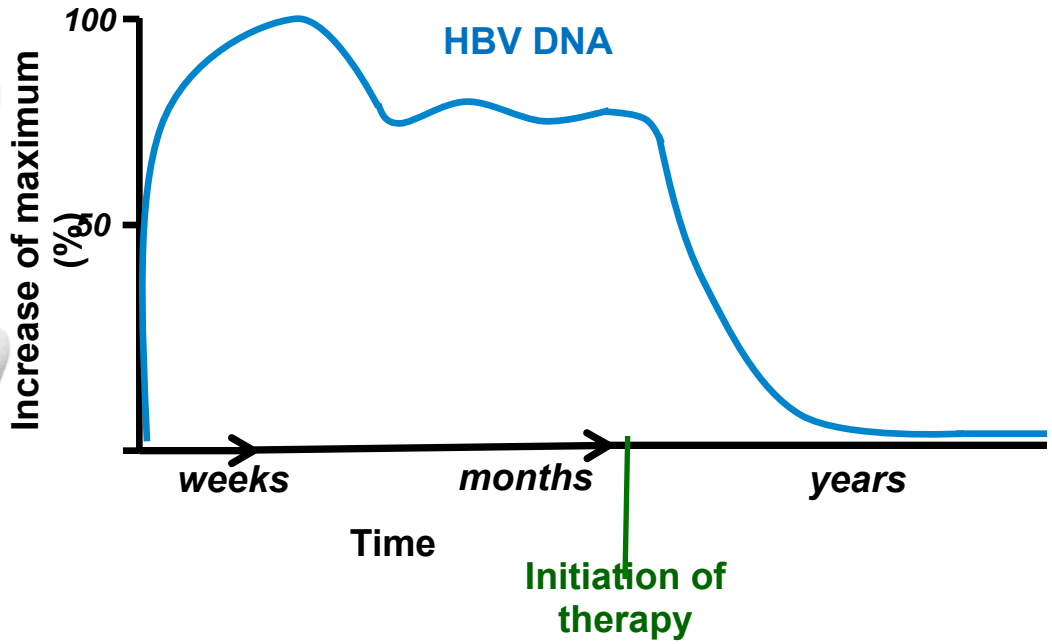


Erasmus MC

Nucleos(t)ide analogues cause chain termination and give viral suppression, but treatment is generally lifelong

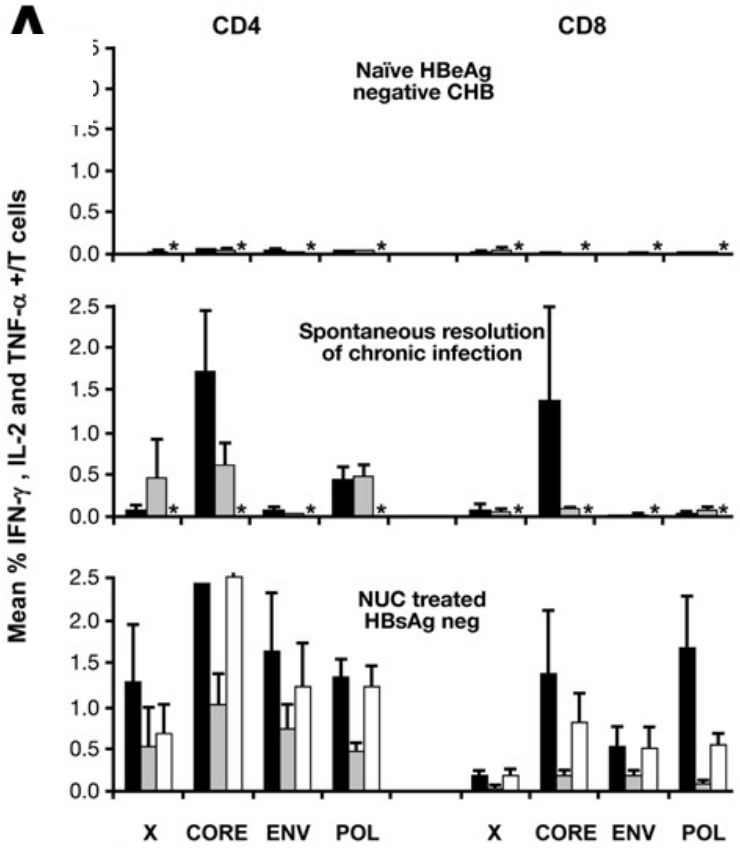
Options:
Lamivudine
Adefovir
Telbivudine
Entecavir
Tenofovir





Peg-interferon –ISG induction

NUC therapy restores T-cell responses in HBV infection



Chronic

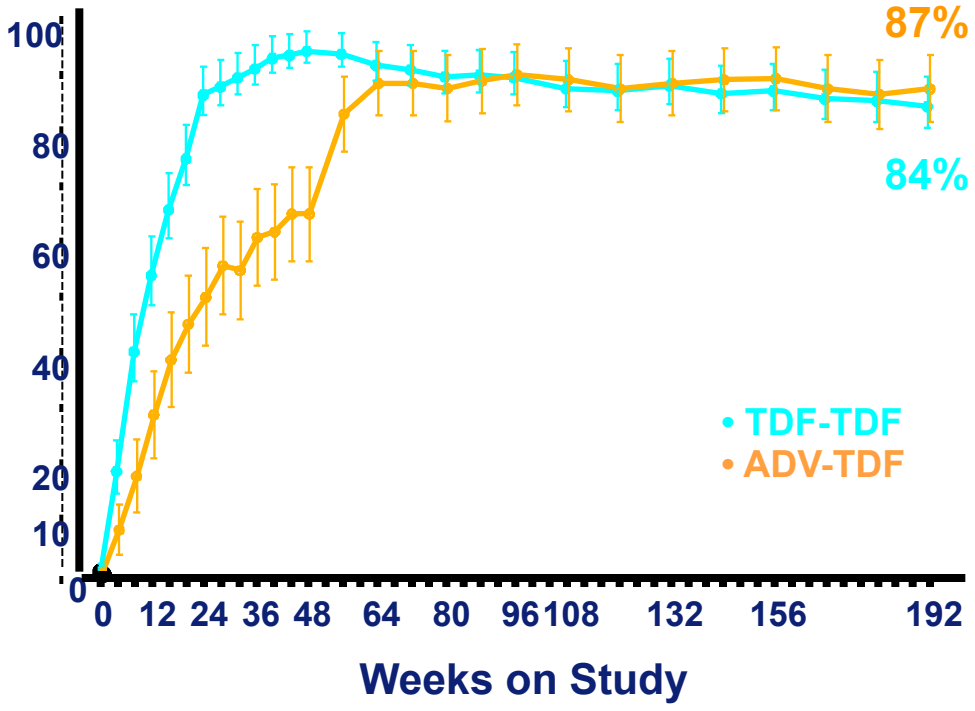
Spontaneous resolution

NUC HBsAg -

Boni et al, Gastroenterology 2012

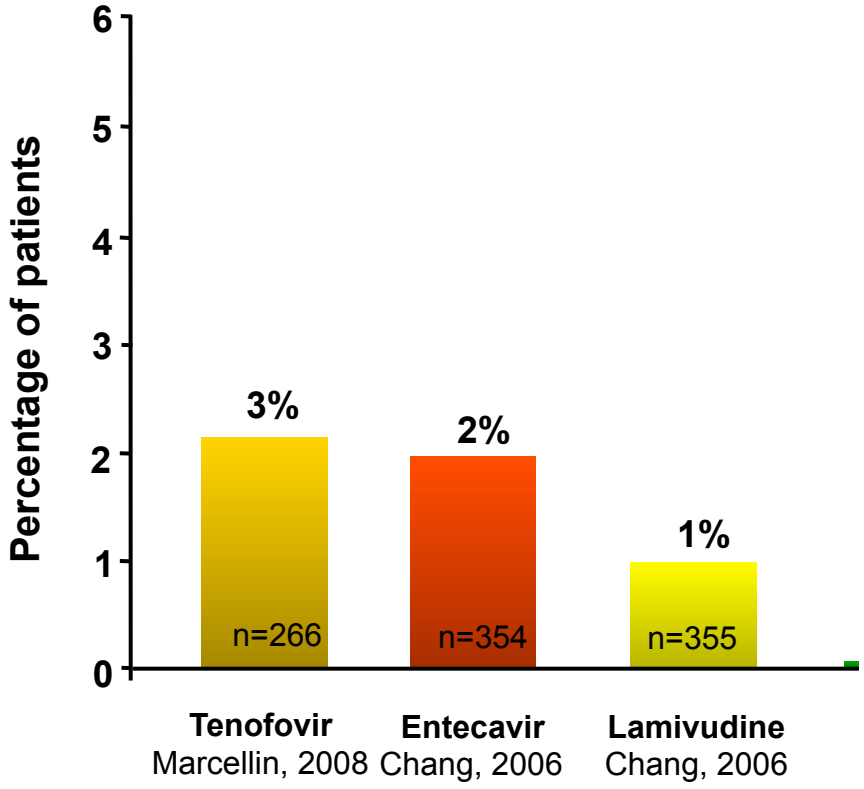
However, NUC therapy generally does not lead to HBV cure or functional cure.
Stopping of NUC therapy leads to reactivation in many patients

Most patients achieve HBV DNA undetectability during antiviral therapy

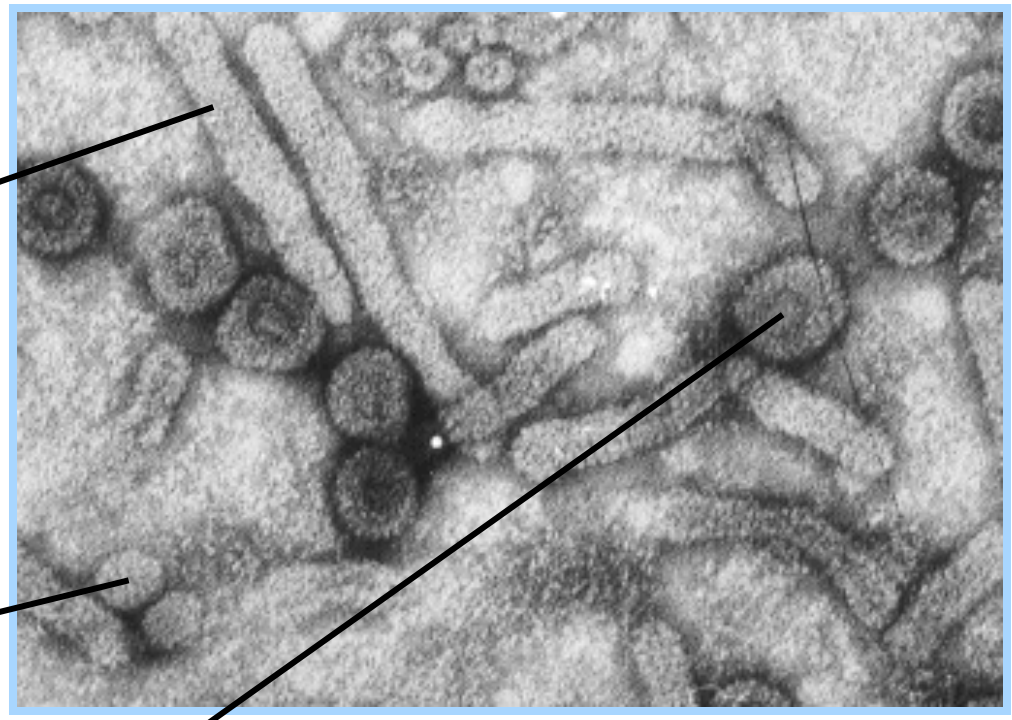


Marcellin, AASLD 2011

Clearance of HBsAg is rare in patients treated with nucleo(s)tide analogues



Hepatitis B Particle Types

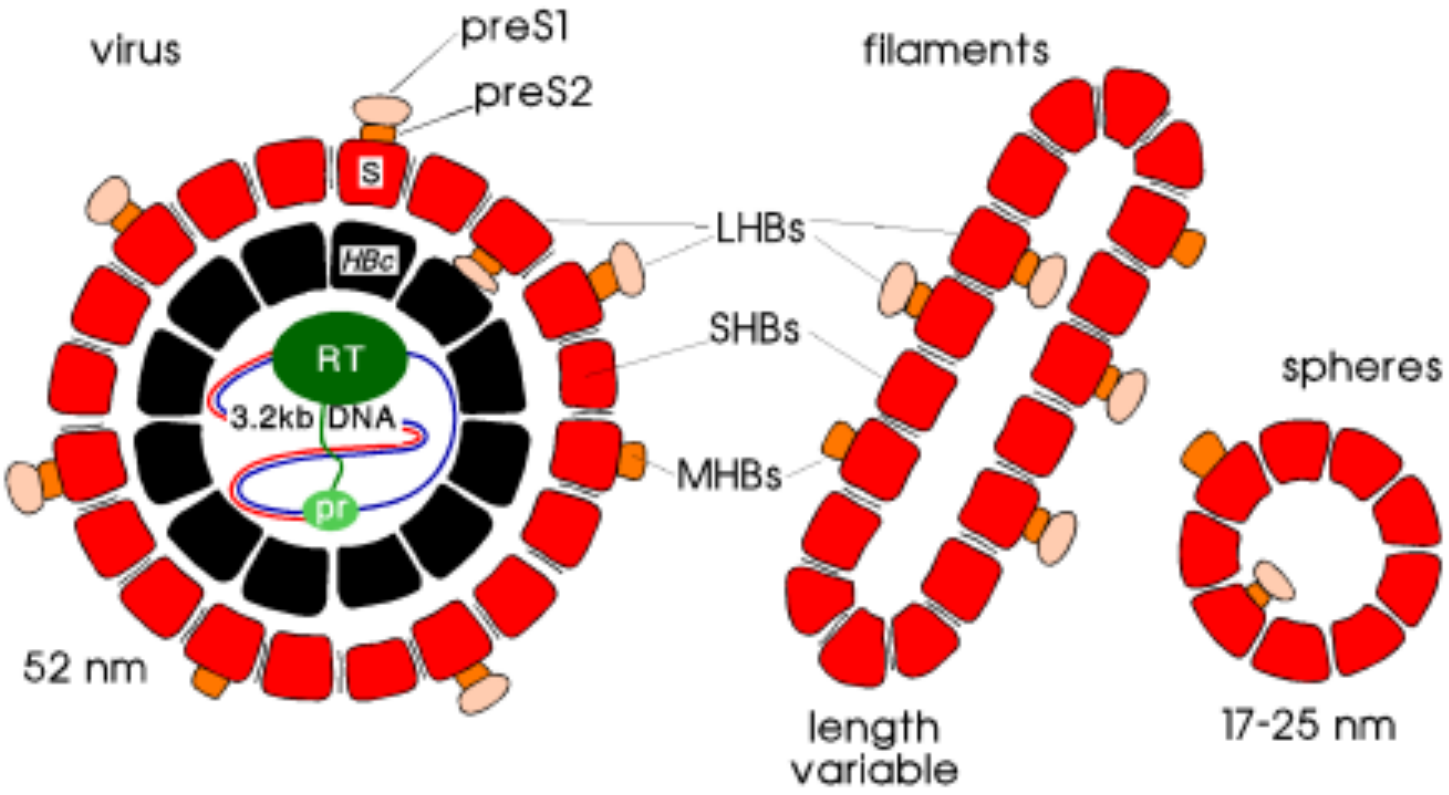


Hepatitis B filament
- Non-infectious
- Consists of HBsAg (small + large)

Hepatitis B sphere
- Non-infectious
- Consists of HBsAg (small only)

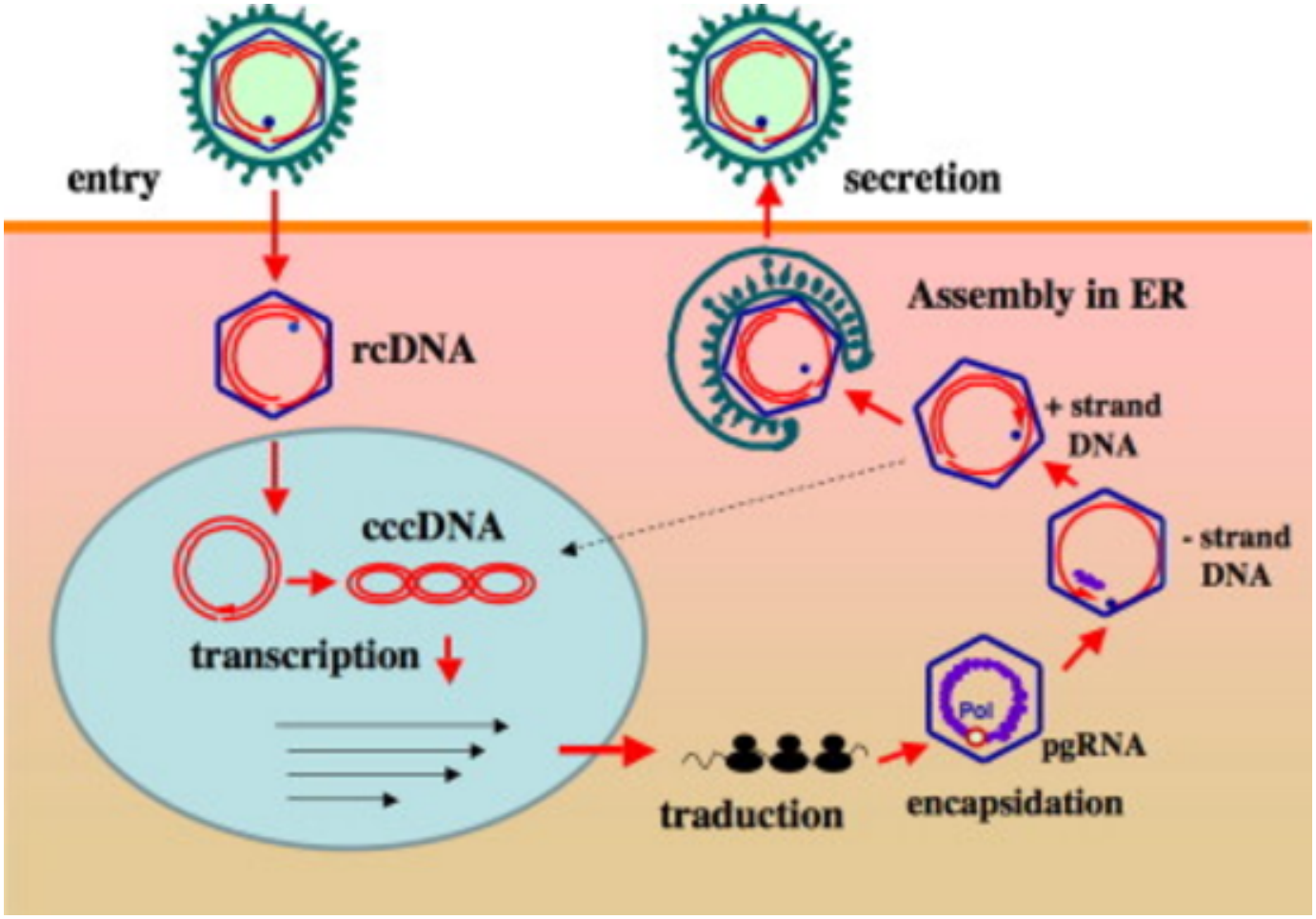
Hepatitis B virion (Dane particle)
- Infectious
- Consists of HBsAg, HBcAg, polymerase and HBV DNA

Structural Components of HBV and HBsAg Particles



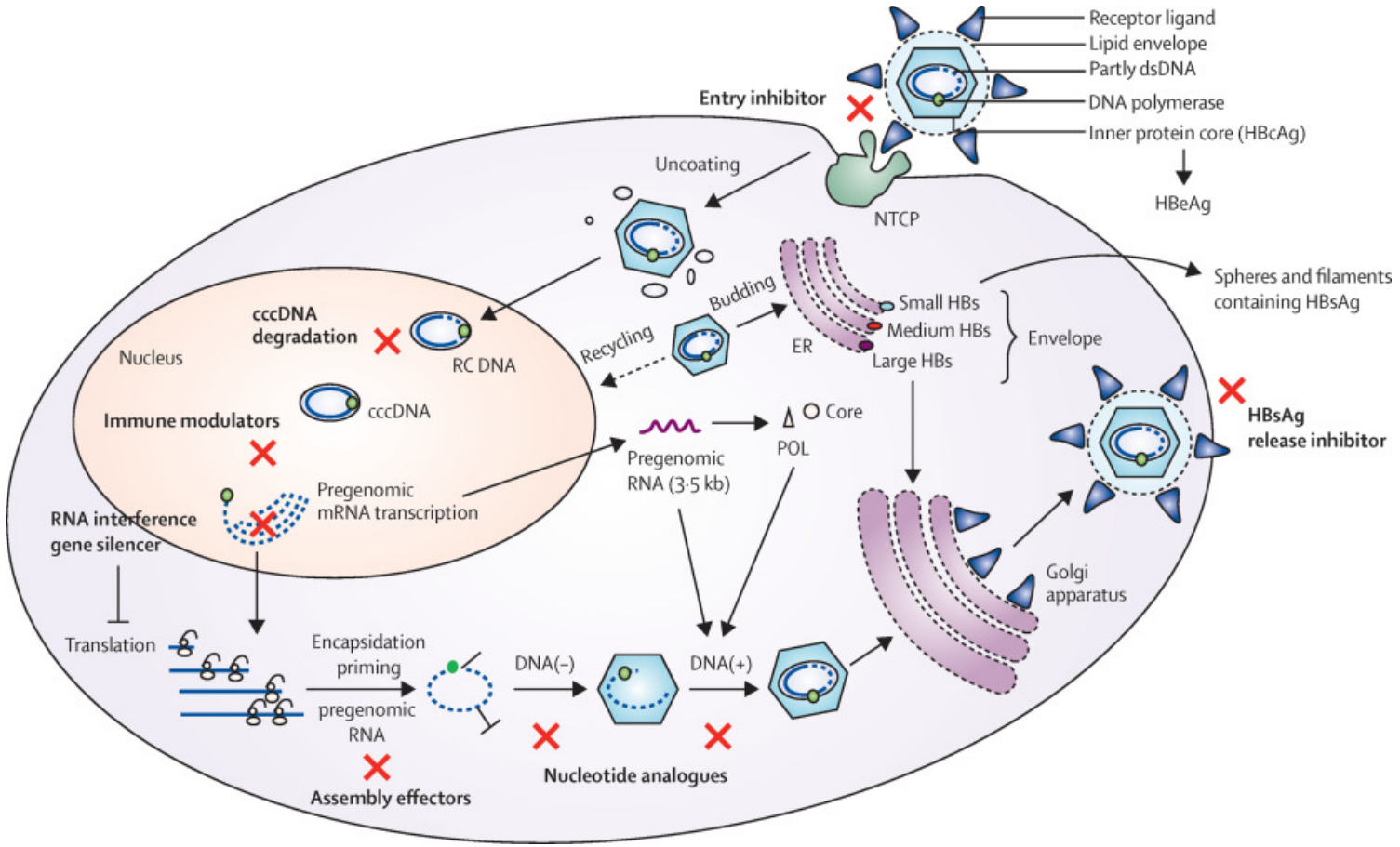
Erasmus MC

Closed coiled circular DNA (cccDNA)



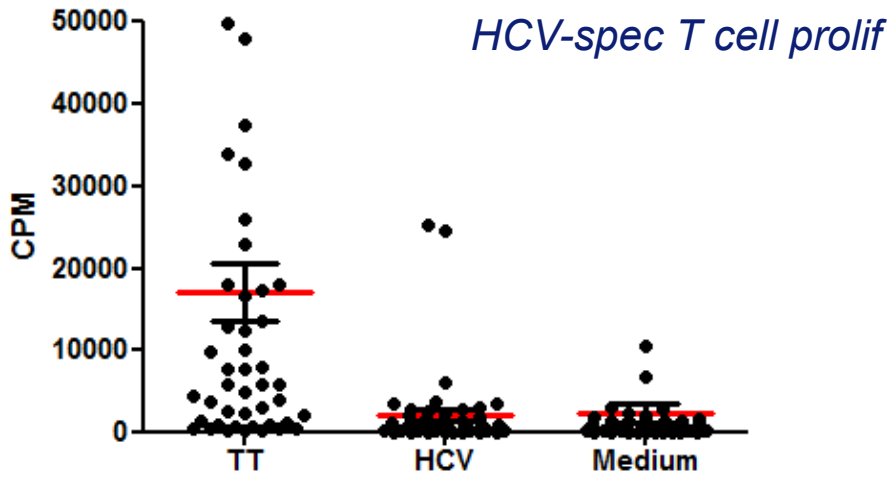
Improvement of current therapy in order to:

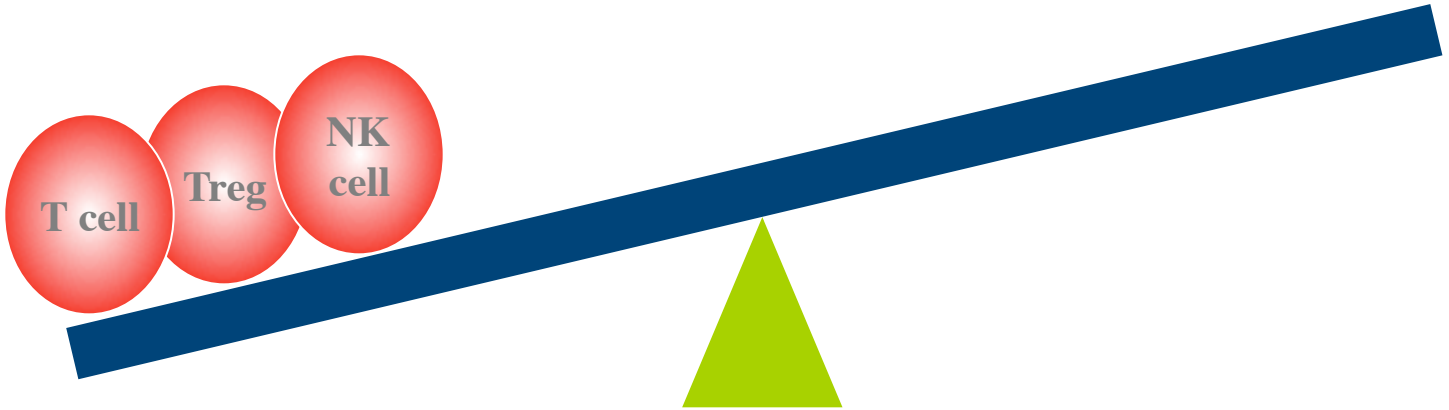
- Get true immune control
- HBsAg seroconversion
- Eradication of cccDNA



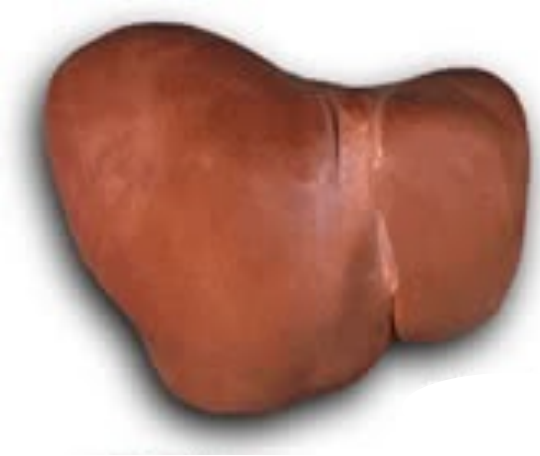
Novel therapeutic strategies for HBV and HCV

Nucleotide analogues and DAA
Peg-IFN lambda
TLR 7 agonists
Interfering RNA's
Entry blockers
Therapeutic vaccines (T cell-based)
Reversal of exhausted phenotype



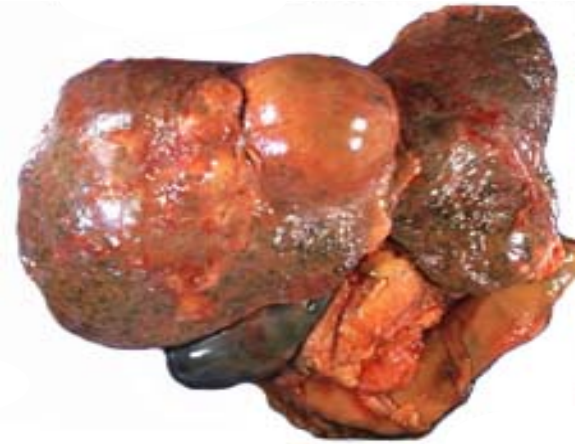


Hepatocellular carcinoma, epidemiology and risk factors

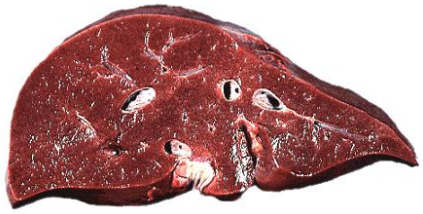


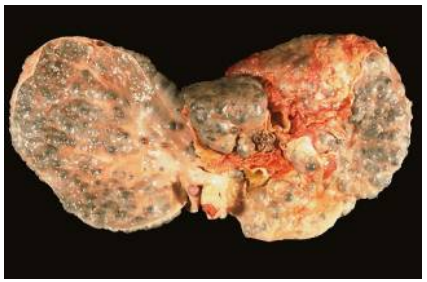
Healthy Liver

Fibrosis/
Cirrhosis

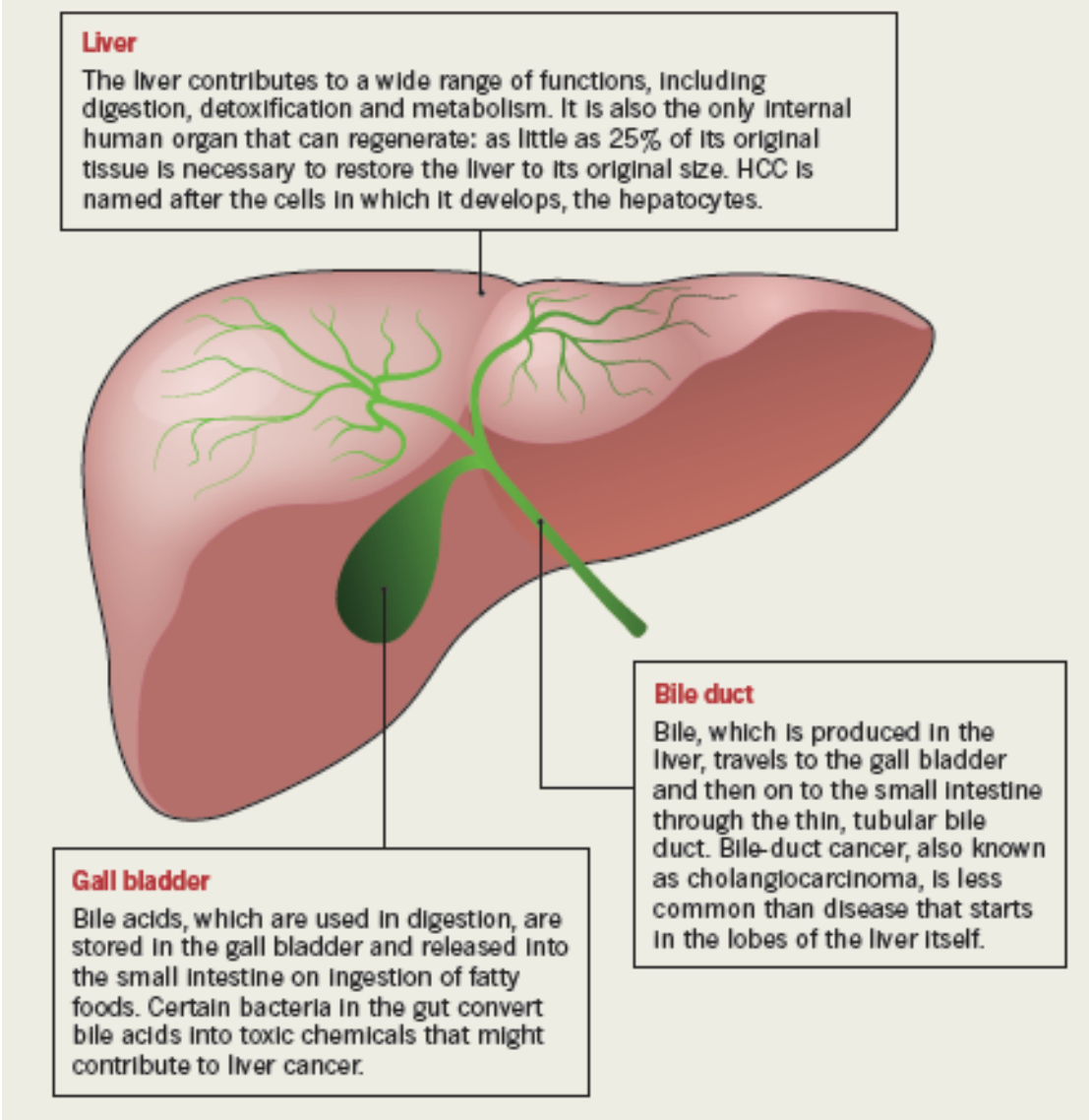


Hepatocellular carcinoma

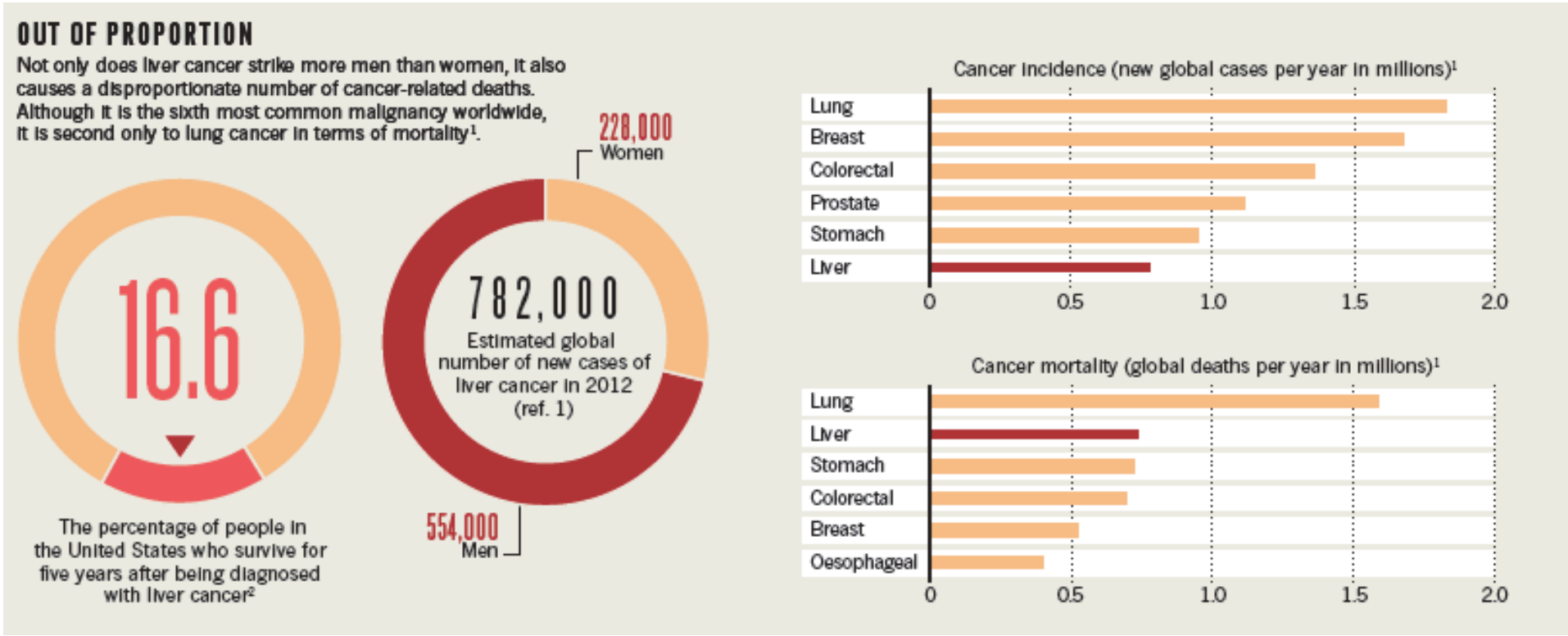




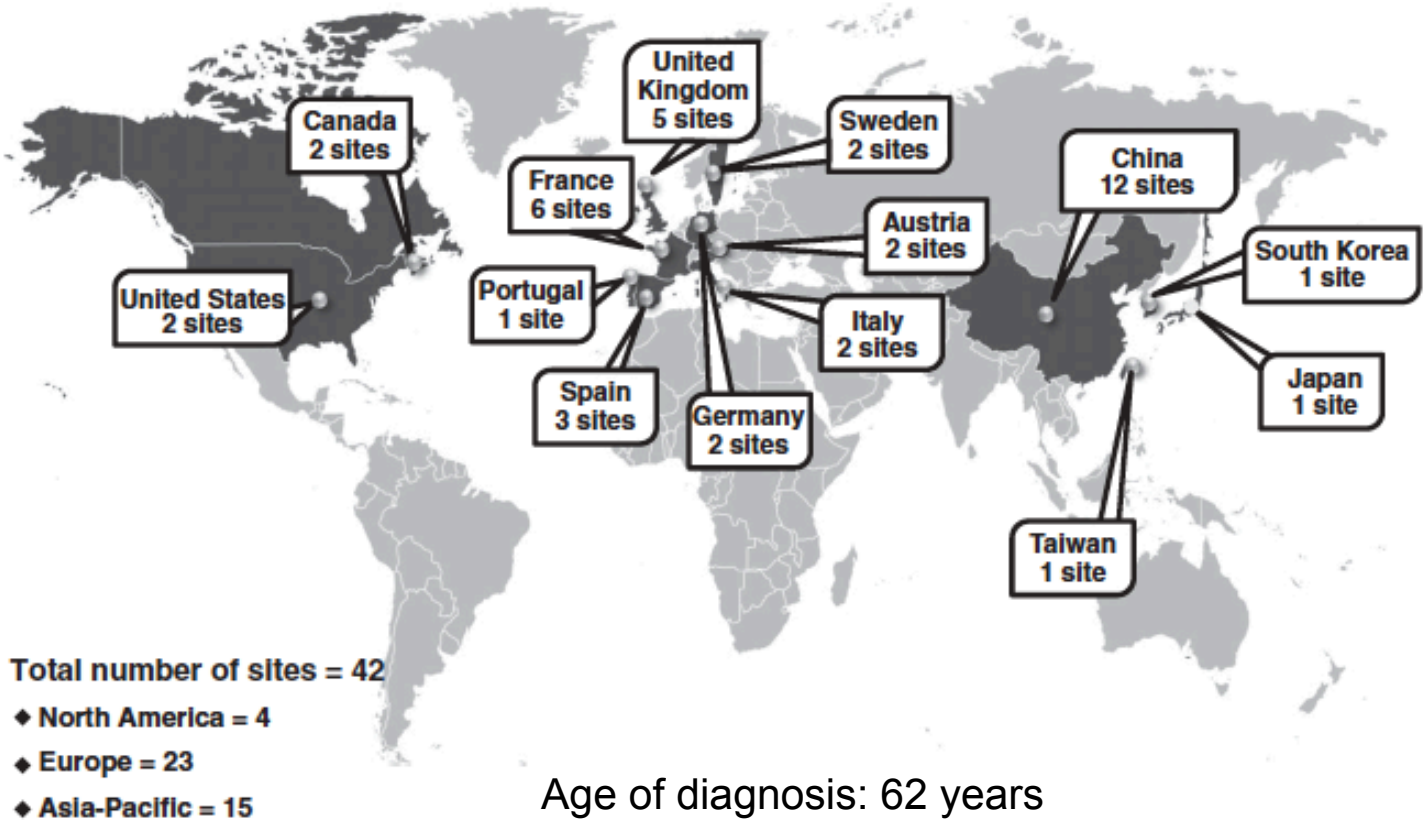
Hepatocellular carcinoma (HCC) is the most common of the three hepatobiliary malignancies



Globally, HCC is the second leading cause of cancer mortality



Epidemiology of HCC



Age of diagnosis: 62 years

Underlying cause:

Hepatitis C: North America, Europe, Japan

Hepatitis B: Rest of the world

Cirrhosis: 90%

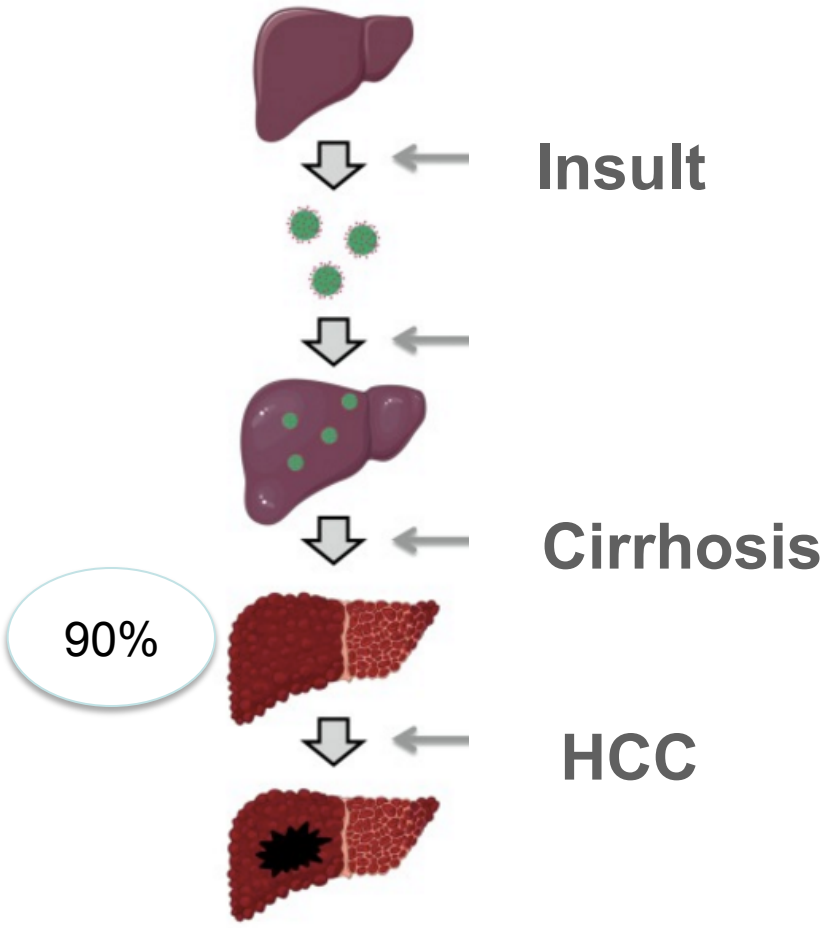
Park et al, Liver Int 2015
The Bridge study

Risk factors of HCC

Hepatitis B
Hepatitis C
Alcohol

but also NAFLD
Hemochromatosis
PBC
Autoimmune hepatitis

Other host risk factors are:
Male
Older age
Asian or African ancestry
Family history of HCC
Aflatoxin expsoure
Alcohol or tobacco use
cirrhosis



Risk factors of HCC

HBV

Globally, 54% of HCC can be attributed to HBV infection

Factors that play a role are:

HBV DNA viral load
HBV genotype C

High serum HBsAg

Chen CJ, JAMA 2006; 295:65-73
Chan HL, Gut 2004; 53:1494-1498
Tseng TC, Gastro 2012; 142:1140-1149

Gender disparity

Table 1 | Age-standardized incidence rates for HCC*

Countries	Men	Women
Low resource		
Mongolia	116.6	74.8
Middle Africa	18.9	9.6
Eastern Africa	7.2	3.6
South-Eastern Asia	21.4	9.0
Melanesia	12.9	5.0
Western Africa	16.6	8.0
Polynesia	10.8	3.4
Intermediate resource		
China	37.4	13.7
Caribbean	6.3	4.4
South Africa	13.9	5.1
Central America	7.3	7.0
Western Asia	4.4	2.3
Northern Africa	7.5	2.5
South America	5.3	3.9
South Central Asia	3.4	1.6
High resource		
Korea	38.4	10.6
Southern Europe	9.8	3.2
Western Europe	7.2	2.1
Eastern Europe	4.6	1.9
Northern America	6.8	2.2
Australia/New Zealand	5.0	2.0
Northern Europe	3.8	1.6

*All values expressed per 100,000 of the population in 2008 (data from GLOBOCAN 2008[1]). Abbreviation: HCC, hepatocellular carcinoma.

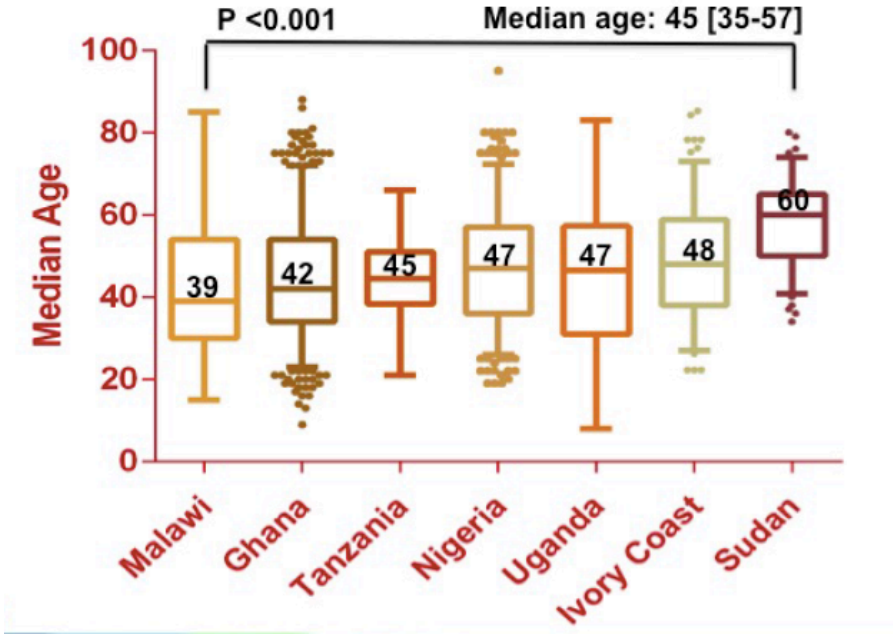
Epidemiology of HCC in Africa

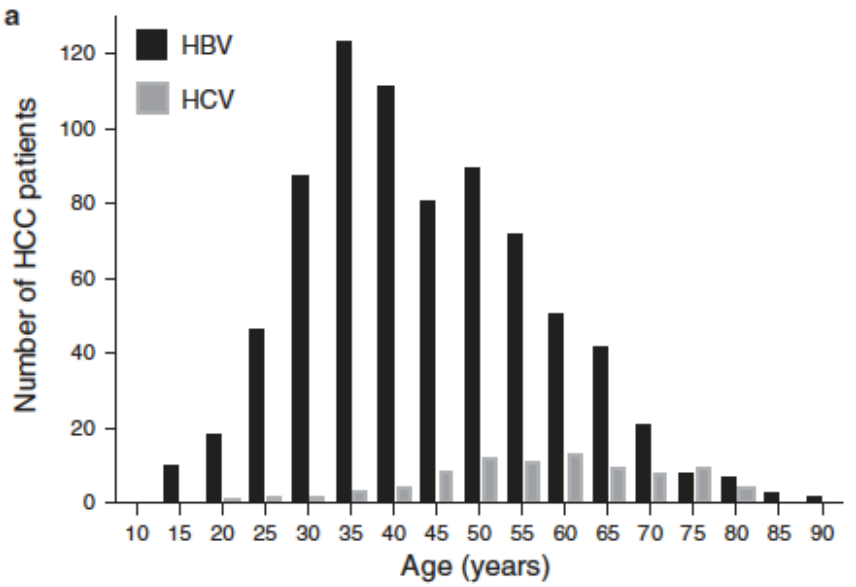
2000 patients with HCC

Cameroon
1 site (n=59), March, 2013, to December, 2015, 24 million
Egypt
2 sites (n=1251), April, 2009, to March, 2016, 93 million
Ethiopia
1 site (n=33), February, 2015, to January, 2016, 102 million
Ghana
4 sites (n=491), August, 2006, to April, 2016, 28 million
Côte d'Ivoire
1 site (n=277), August, 2009, to December, 2015, 23 million
Nigeria
9 sites (n=363), December, 2009, to April, 2016, 187 million
Sudan
1 site (n=51), January, 2015, to April, 2016, 41 million
Tanzania
1 site (n=21), January, 2008, to January, 2016, 55 million
Uganda
1 site (n=20), March, 2015, to May, 2015, 40 million

55% Hepatitis B

HCC in Africa occurs at a relatively young age (mean age 42 (range 34-55)



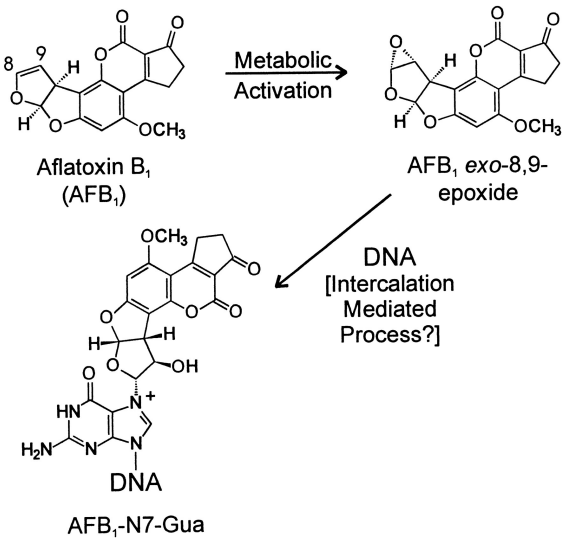


Yang et al, Lancet Gastroenterol 2017

In 14 non-African countries the mean age of HCC diagnosis ranged from 52-69

Aflatoxins

a fungal toxin that contaminates maize, nuts etc
Metabolized in the liver, and forms adducts with DNA
is a known carcinogen
exposure leave a p53 signature (249ser)



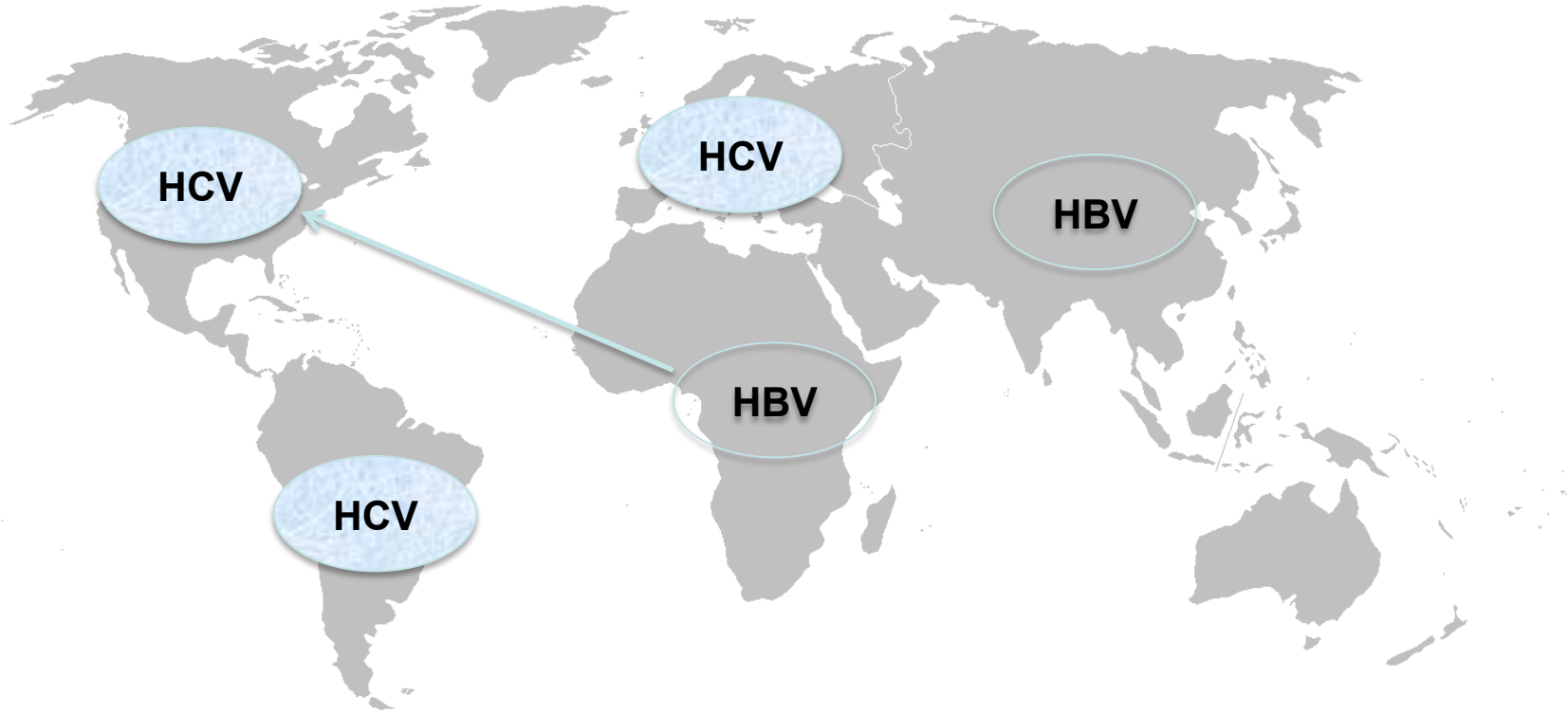
HCC risk:
4x higher in persons with high aflatoxin levels
7x higher in persons with chronic HBV
60x higher in person with high aflatoxin+ chronic HBV

Likely, also synergy with HCV, but aflatoxin is more prevalent in areas with high HBV

Coffee and aspirin

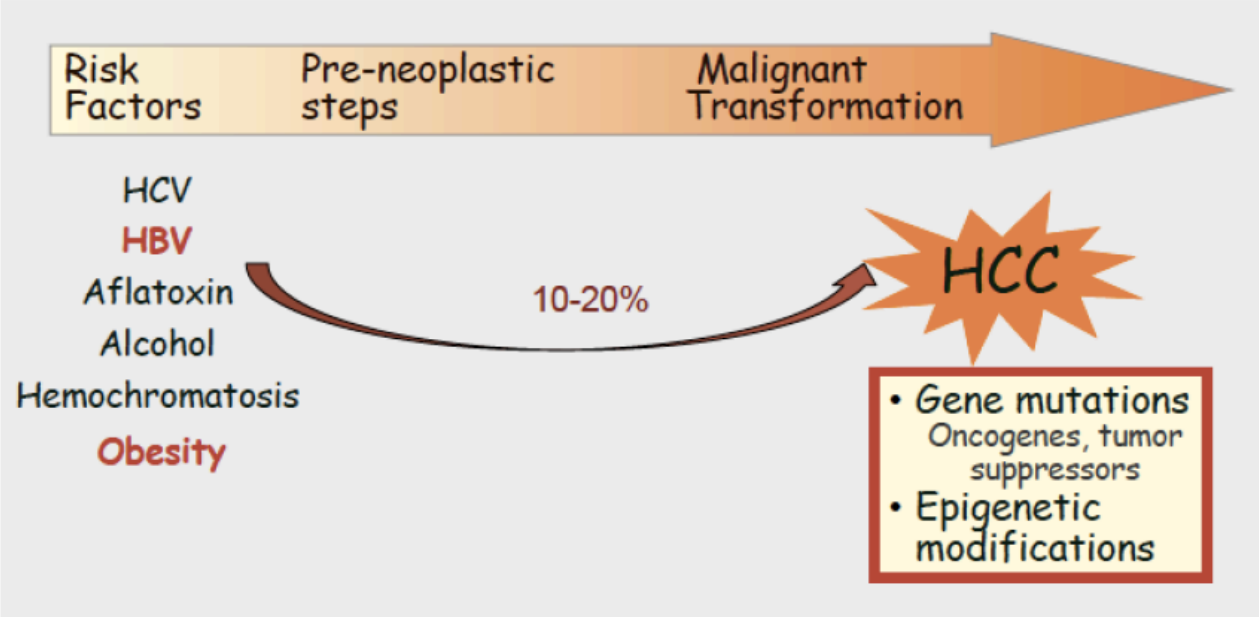
Reduction of HCC has been described for coffee (40%), the anti-diabetic drug metformin and aspirin (49%).

Global differences in HCC risk factors



In high rate countries: HBV and aflatoxin are major risk factors

In low rate countries: HCV, alcohol and diabetes/obesity are major factors



So, what's going on?

Why are individuals with HBV more likely to get HCC?

The development of HCC is accelerated by HBV, since host genes are modified near the HBV integration site, which may cause host cell genome instability and carcinogenic proteins

Integration of HBV DNA into the host hepatocyte genome is key to HCC

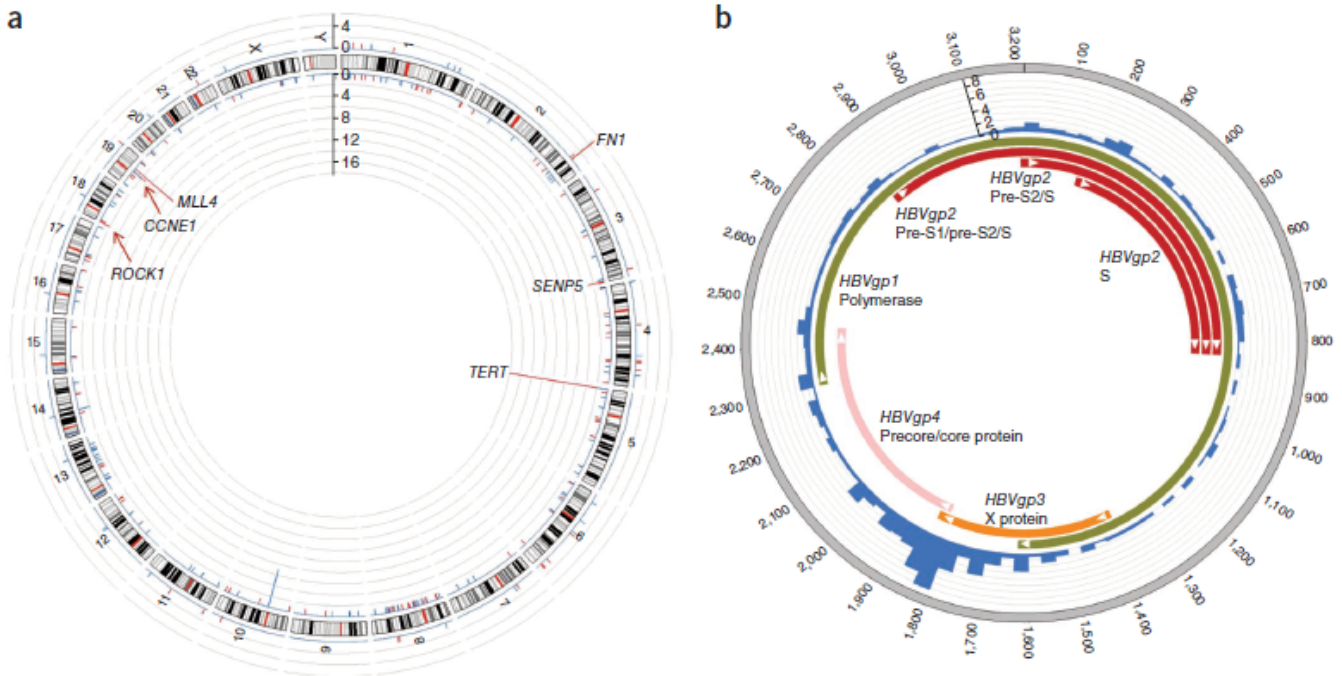
Integration of HBV DNA is found in 80-90% of cancer cells, and in 30% of liver tissue adjacent to the tumor Sung WK, Nature Genetics 2012; 44:765-769

Integration can affect the function of host genes as a cis-acting elelment, and activate proto-oncogenes and silence tumor-suppressor genes

HBV DNA integration can also cause host genome instability, and induce mutations, deletions, and rearrangements

HBV expresses diverse active proteins, such as HBx, which can alter apoptosis, intracellular signaling and epigenetics. HBx is highly expressed in the tumor

HBV integration is not a random event



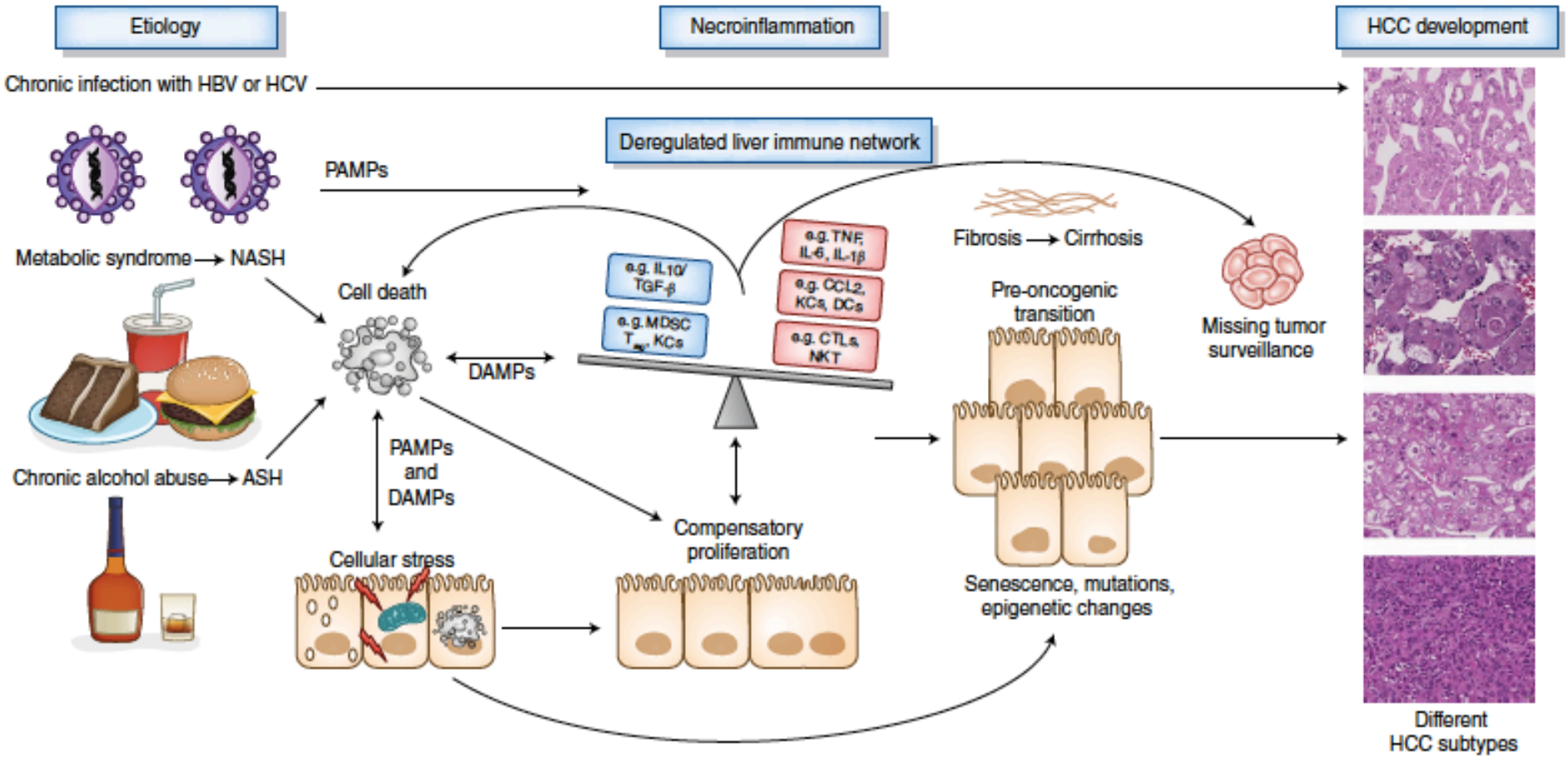
41% of all inserted genes are the TERT, MLL4 and CCNE1 genes

- TERT: Teolomerase reverse transcriptase
- MLL4: mixed-lineage leukemia 4
- CCNE1: cyclin E1

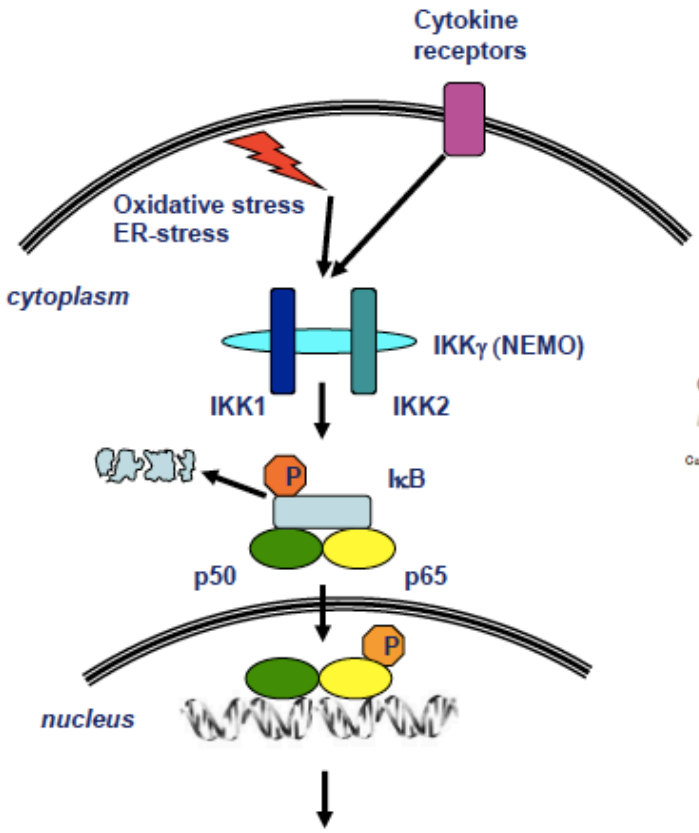
40% of breakpoints are near the 1800th nt of the HBV genome (area that contains an enhancer, X gene and core promoters)

Sung, Nat Gen 2012; 44: 765-769
Murakami, Gut 2005; 54: 1162-1168
Paterlini, Oncogene 2003; 22: 3911-3916

Role of necroinflammation in development of HCC



The development of HCC is accelerated by prolonged HBV-induced hepatic inflammation



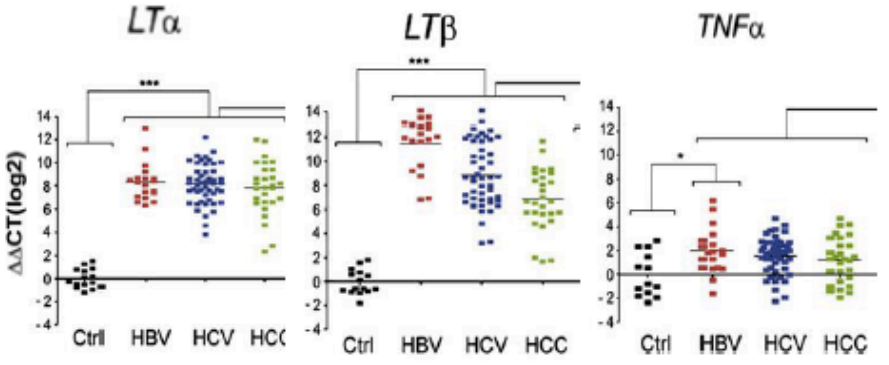
modified from F Marra, Gut 2008;57:570

HBx (HBV) and NS5a (HCV) activate NFkB signaling by multiple mechanisms

Lymphotoxin (LT) α and β and their receptor (LTβR) are upregulated in HBV- or HCV-induced hepatitis and HCC.

Cancer Cell Article Cell

A Lymphotoxin-Driven Pathway to Hepatocellular Carcinoma

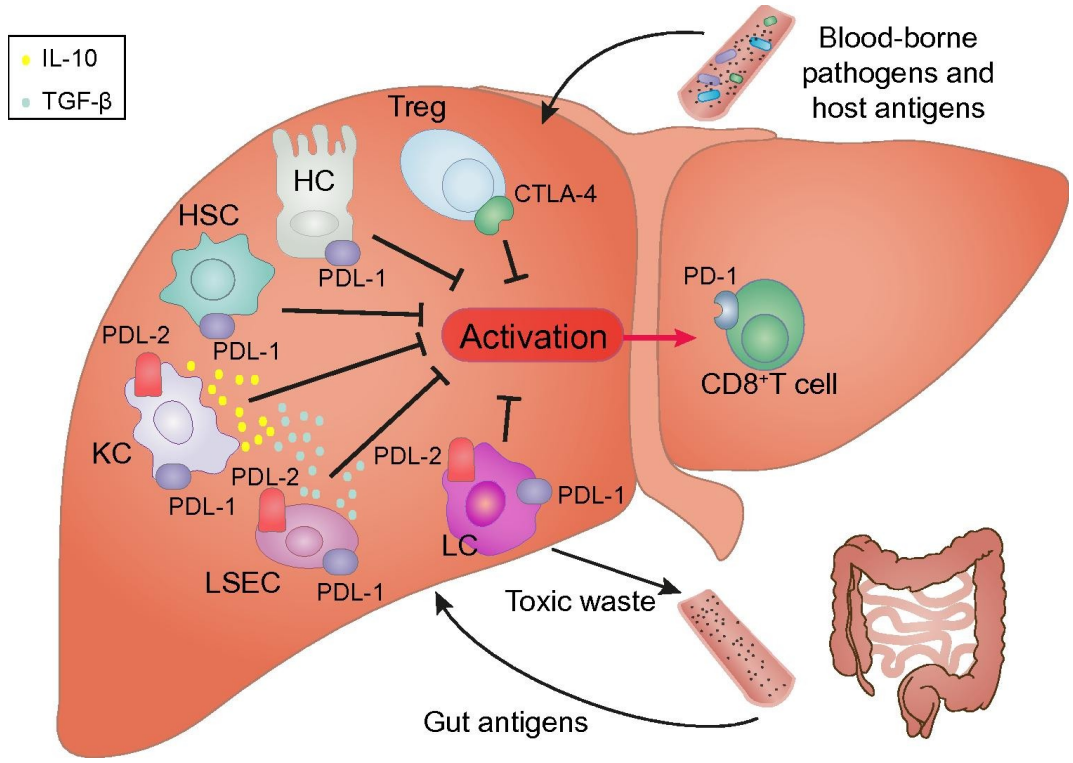


Haybaeck et al, Cancer Cell 16, 296, 2009

Also induction of IL-6/STAT3 and TGF-β has been reported

Tolerance inducing mechanisms in the liver

Immune suppression ⟷ *Immune activation*



Immune surveillance

Immune response and HCC

Tumor-associated **macrophages** express exhaustion/inhibitory receptors

Natural killer cells are less frequent, show reduced cytokine production, and have a reduced lytic activity

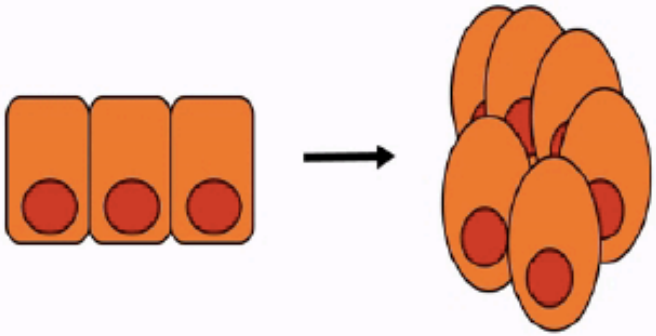
Dendritic cells produce less IL-12 and more IL-10, and have a poor maturation

T cells have an exhausted phenotype and are functionally impaired

Regulatory T cells are present at higher frequencies and express high levels of inhibitory molecules

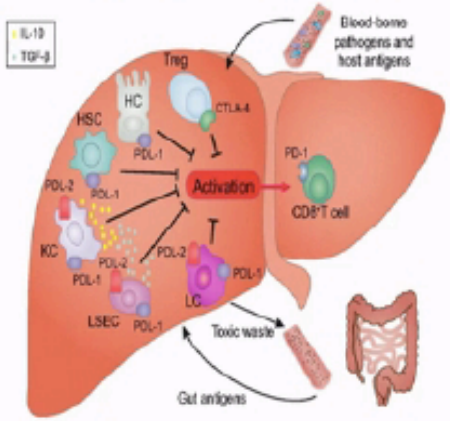
T cells do not efficiently eliminate tumor cells

Tumor-cell intrinsic factors



- Low antigenicity of HCC-associated Ags
- ↓ MHC molecules
- ↑ PD-L1

Microenvironment-related suppressive factors



- Immunosuppressive innate immunity: defective **DC, ↑MDSC** and **TAMs**
- Abundance of **suppressive cytokines** (IL-10, TGF-β...)
- **↑ inhibitory checkpoints**: PD-L1/PD-1 → T cell exhaustion

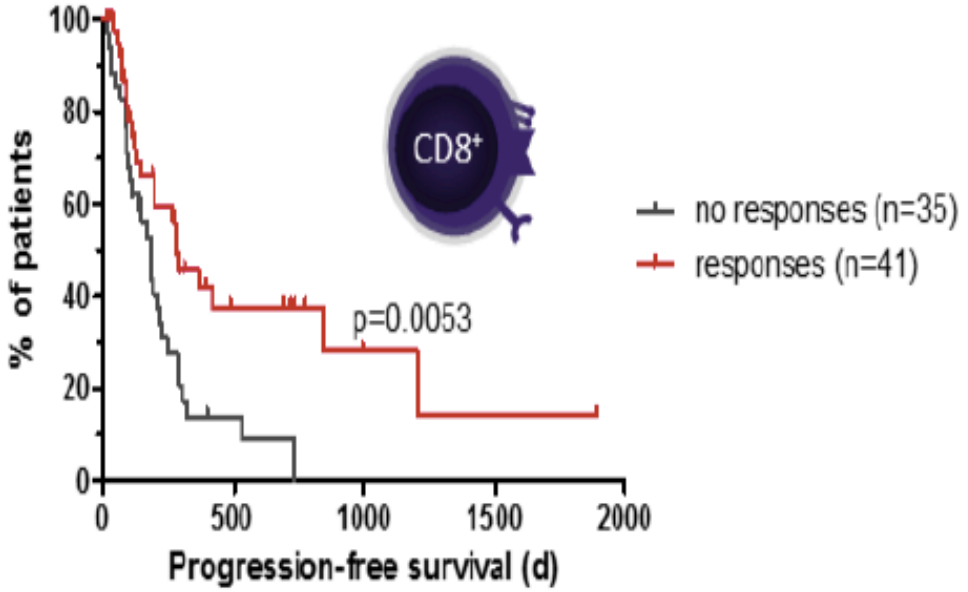
Kurohkochi K et al Hepatology 1996; Calderaro J et al Hepatology 2016; Kuang DM et al J Exp Med 2009; Han Y et al Hepatology 2014; Makarova-Rusher OV et al J Hepatol 2015; Yeung OW et al J Hepatol 2015

Tumorantigen-specific T cell responses and patient survival

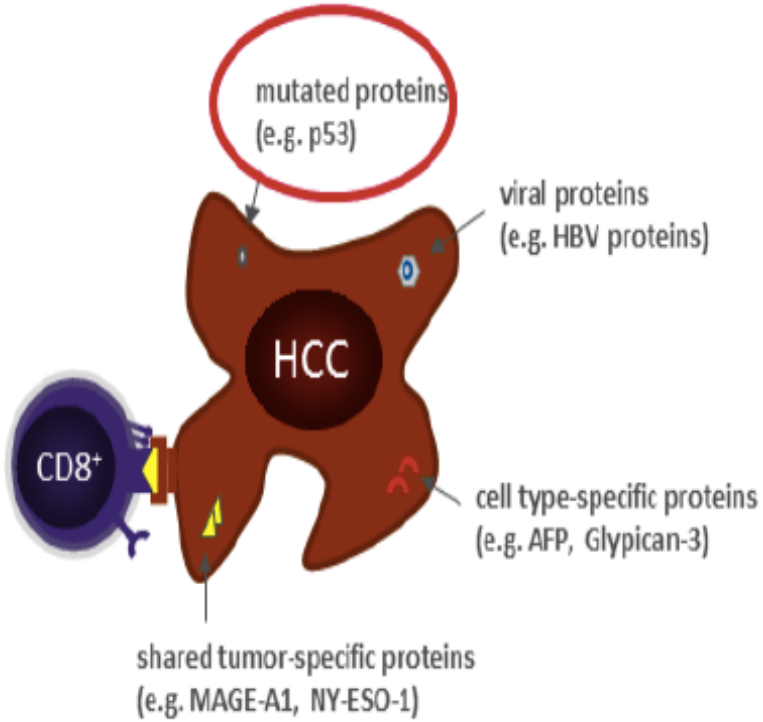
Immunodominance and Functional Alterations of Tumor-Associated Antigen-Specific CD8$^+$ T-Cell Responses in Hepatocellular Carcinoma

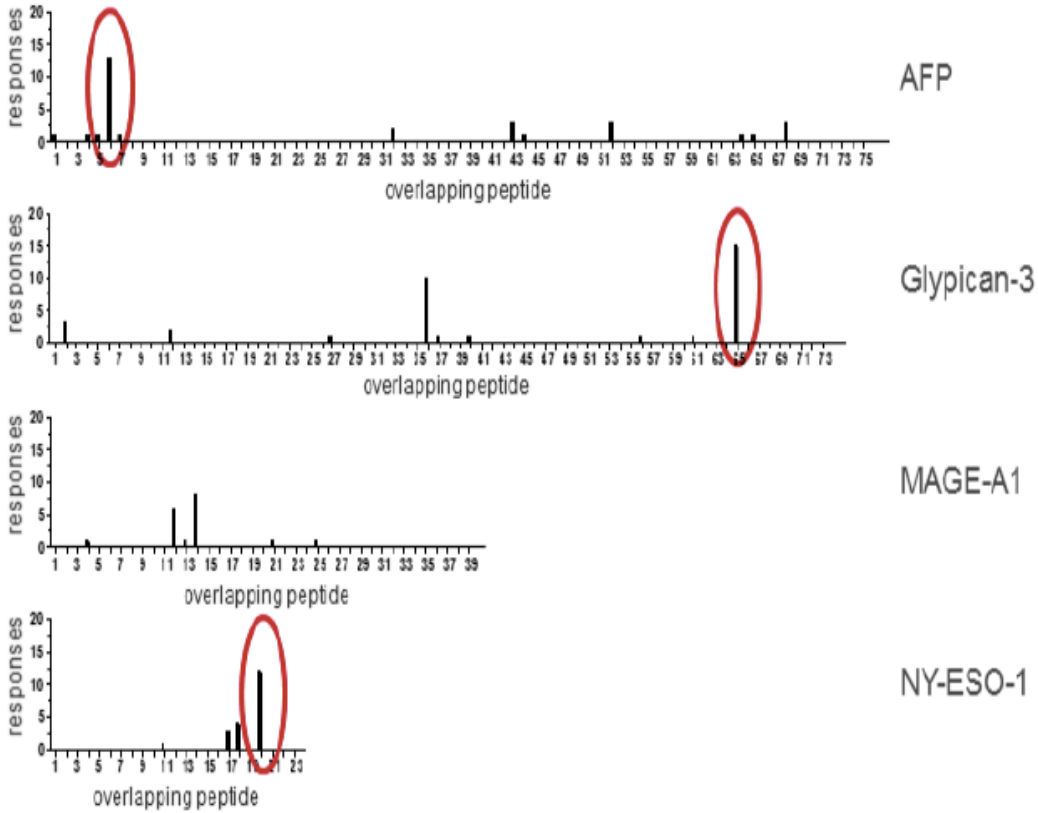
Tobias Hecken,[1,2,3*] Sathalie Schmidt,[1*] Sandra Hild,[1] Emma Gostick,[4] Oliver Drognitz,[5] Robert Zeiser,[6] Peter Schemmer,[7] Helge Bruns,[7] Thomas Giermann,[8] David A. Price,[4] Hubert E. Blum,[1] Christoph Neumann-Haefelin,[1] and Robert Thimme[1]

Hepatology 2014



Tumor antigens in HCC



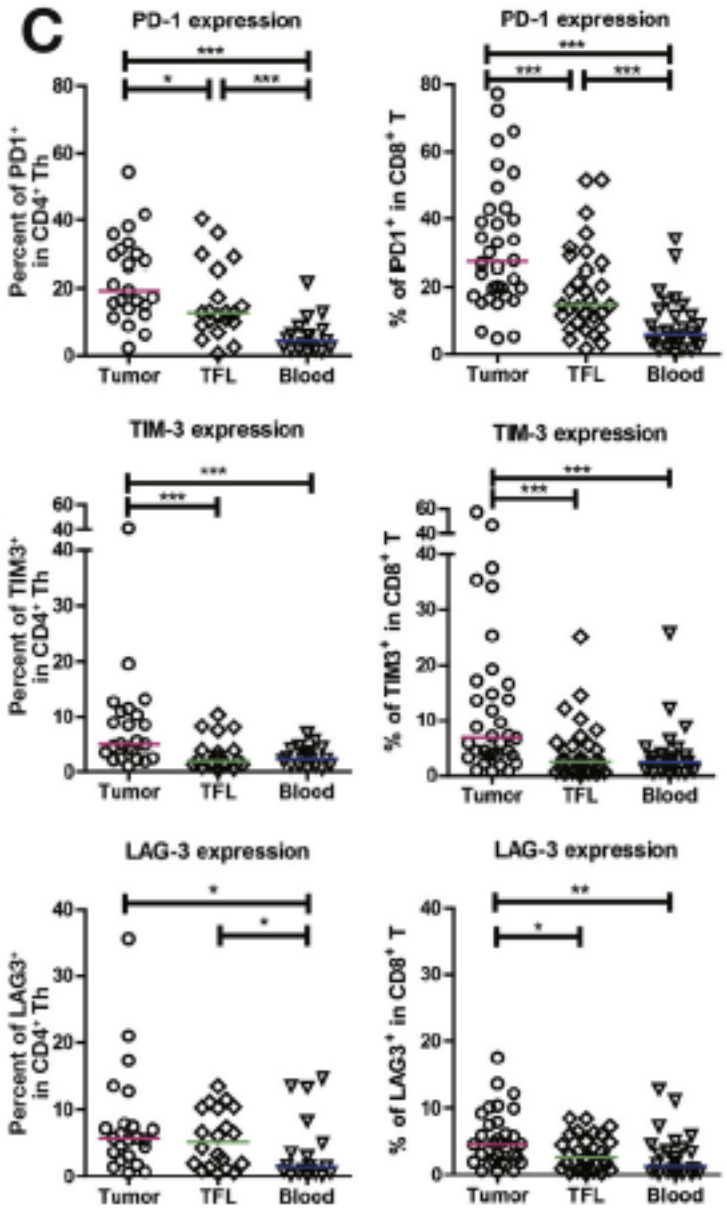


Effector T cells are exhausted and functionally impaired

PD-1

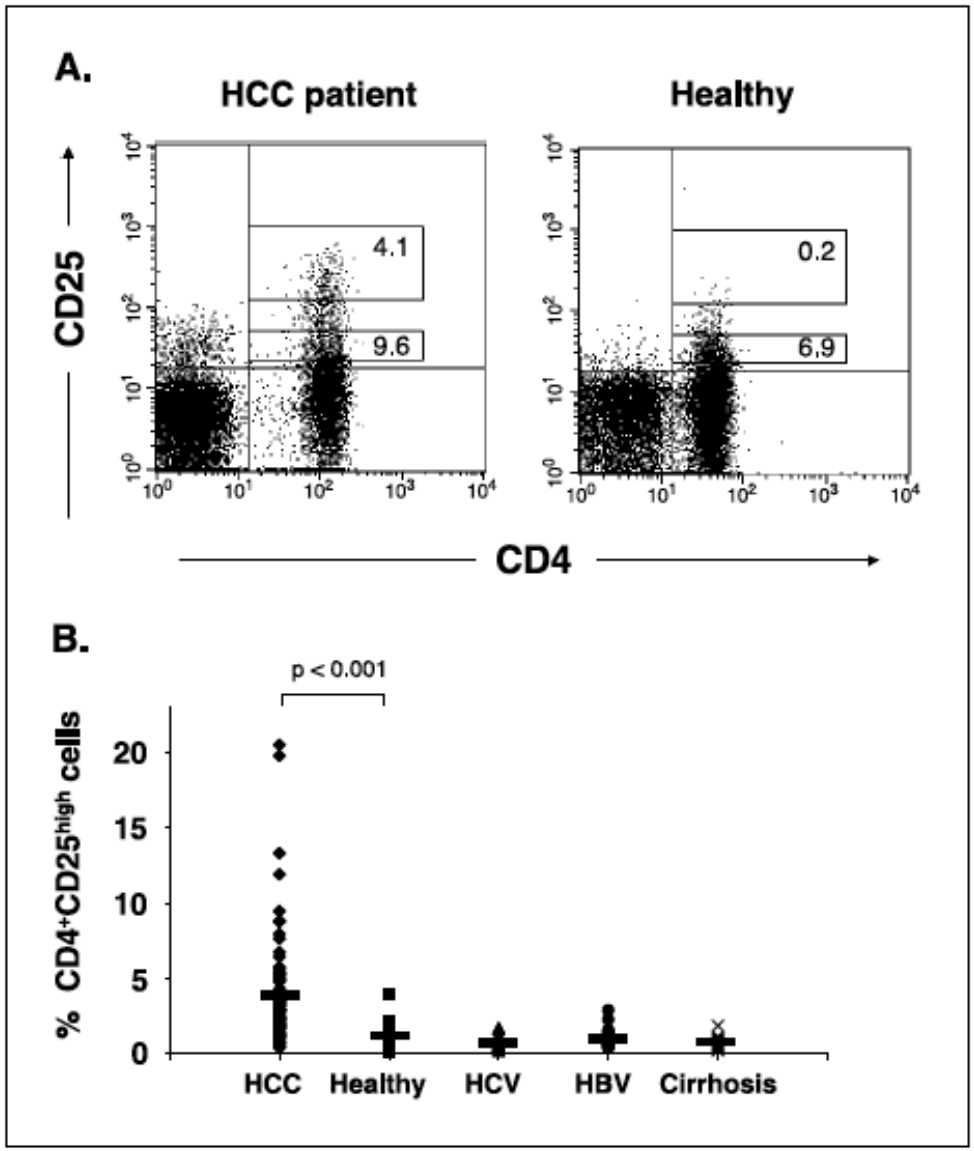
Tim3

LAG3



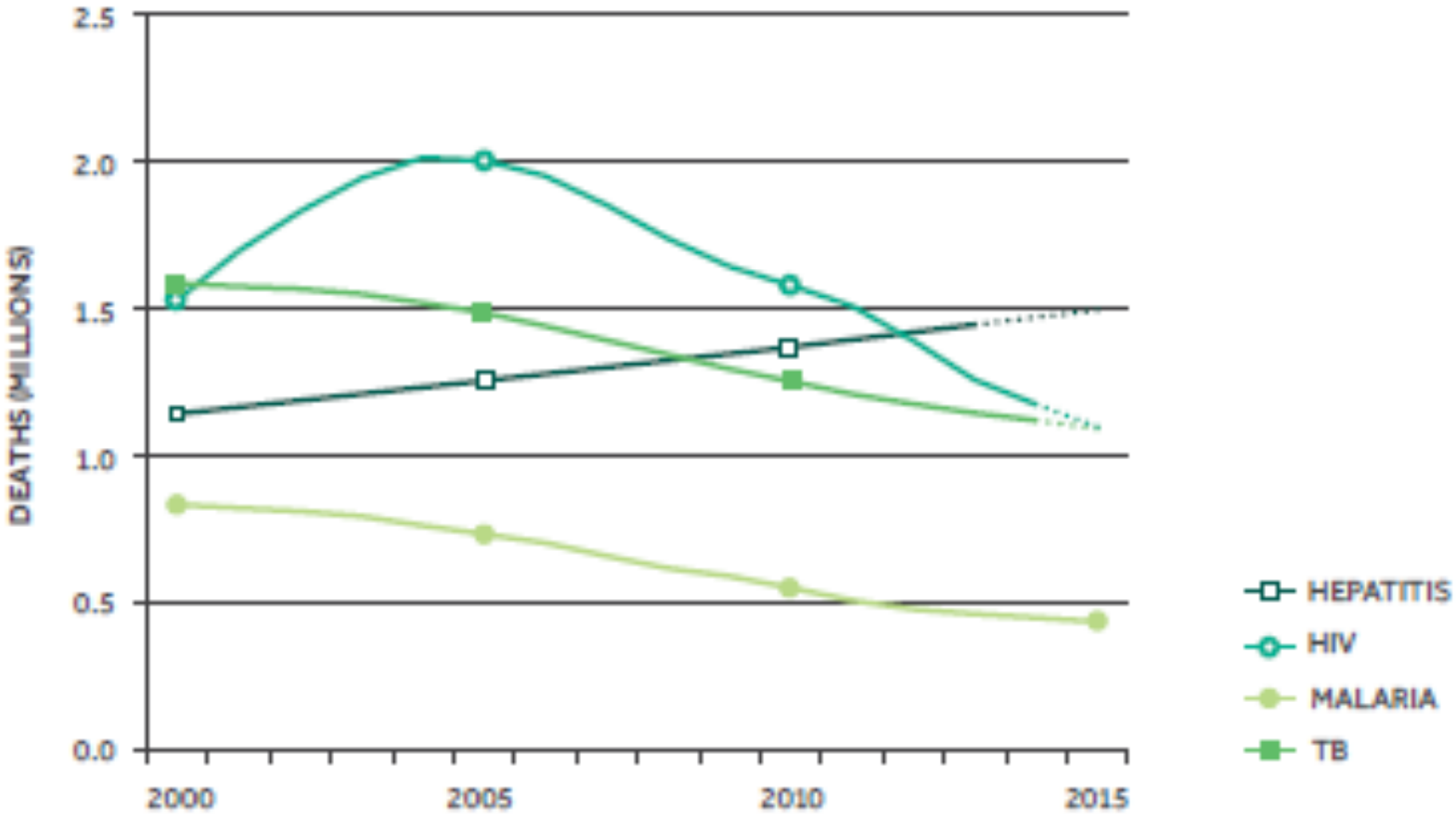
TFL =
tumor free liver

Enrichment of regulatory T cells in HCC



Ormandy, 2005

Globally, the estimated numbers of HCC-related deaths are still increasing, 2000-2015



WHO report, June 2016

HCC is a preventable cancer

Main risk factor worldwide is hepatitis B
- Vaccine preventable disease

Main risk factor in the “West” is hepatitis C
- Fully curable disease

Second risk factor is alcohol
- Fully preventable

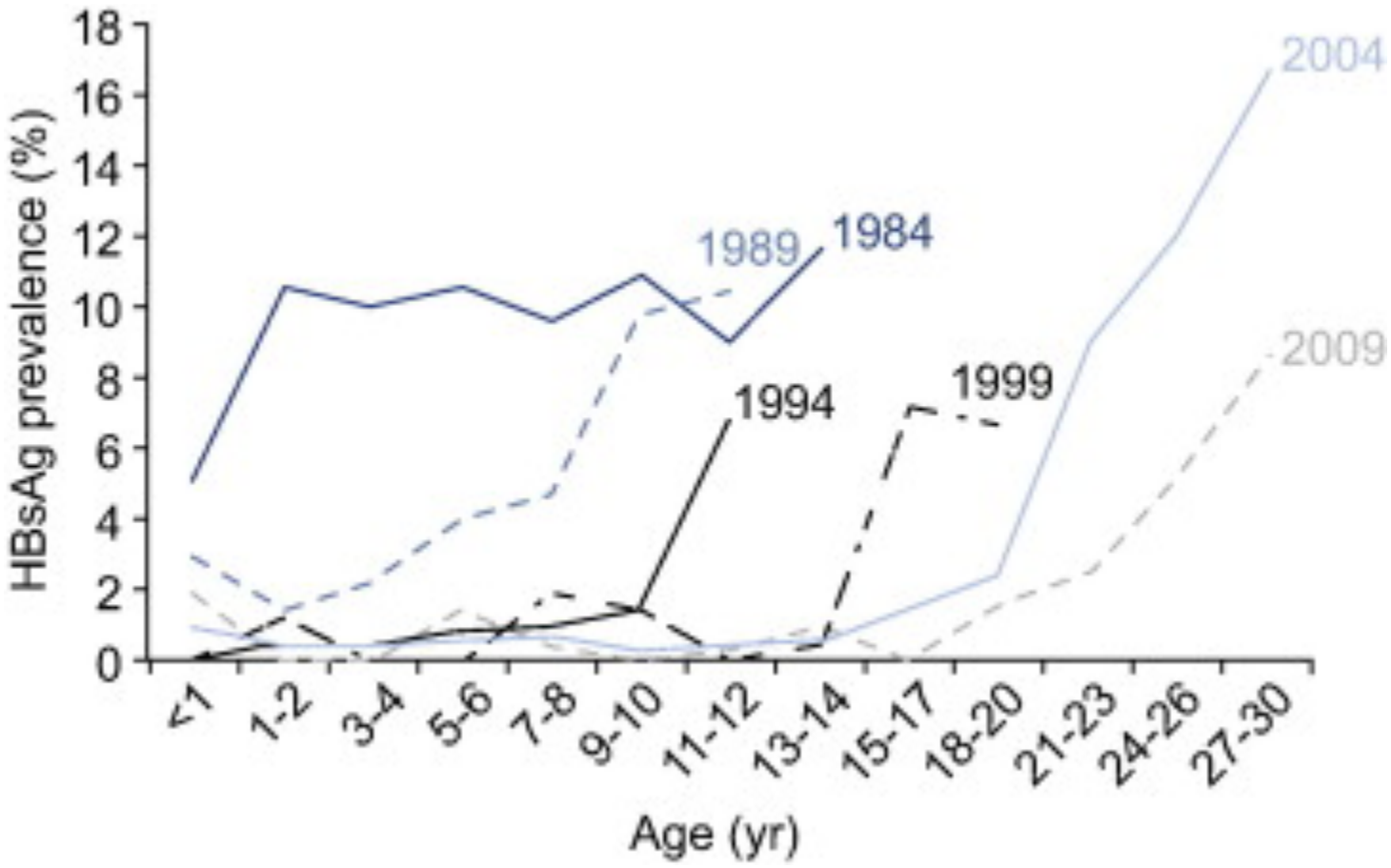
Prevention of HCC development

HBV vaccination

Early surveillance of HCC – most HCC cases are diagnosed (too) late

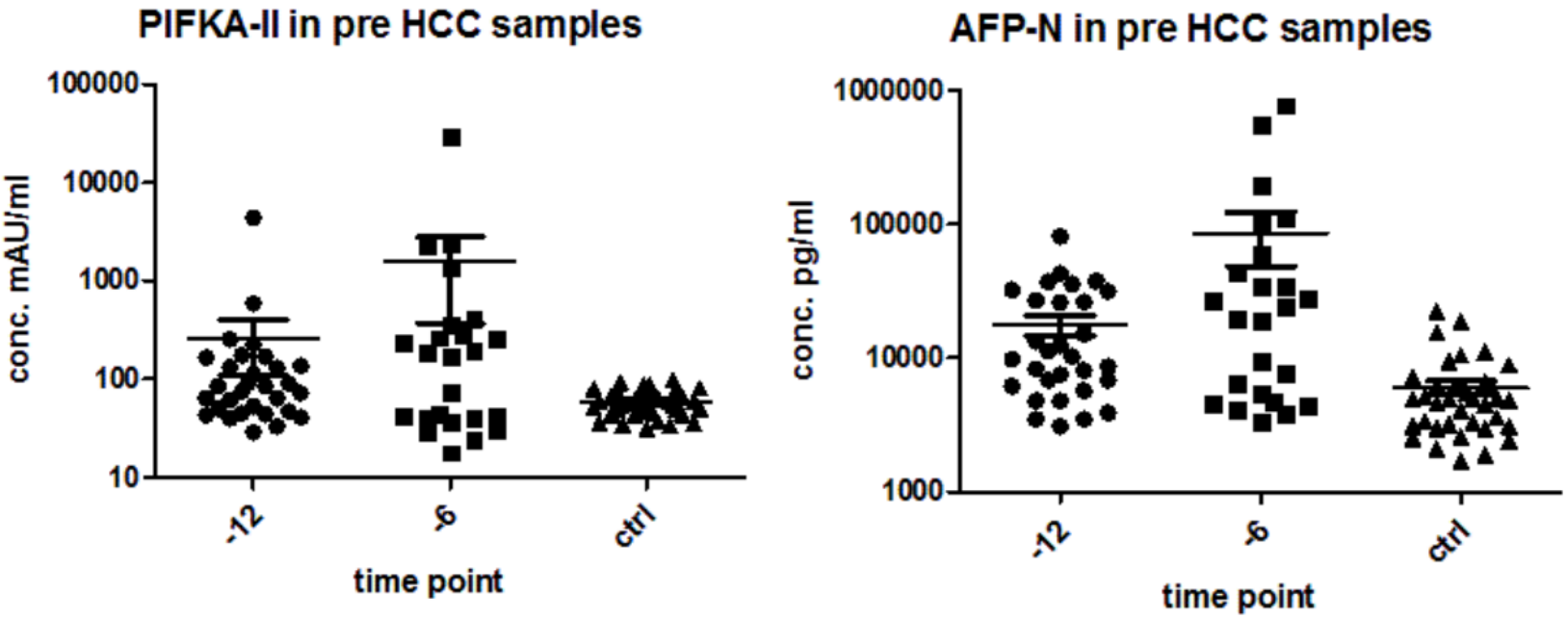
Antiviral treatment

Age-specific HBsAg seropositive rates in Taiwan



In 1984, none of the subjects were under universal vaccination coverage. In 1989, only children <5 years of age were covered.

Prediction or diagnostic markers for HCC



Diagnostic blood markers include PIVKA, CA19, AFP, AFP-L3 (not CEA)

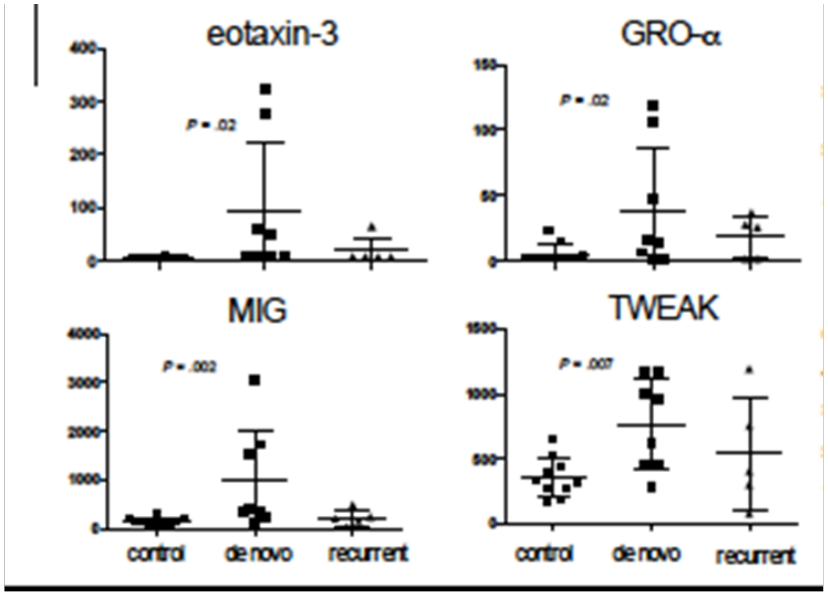
Gastroenterology 2018;154:515–517

BRIEF REPORT

Levels of Cytokines in Serum Associate With Development of Hepatocellular Carcinoma in Patients With HCV Infection Treated With Direct-Acting Antivirals

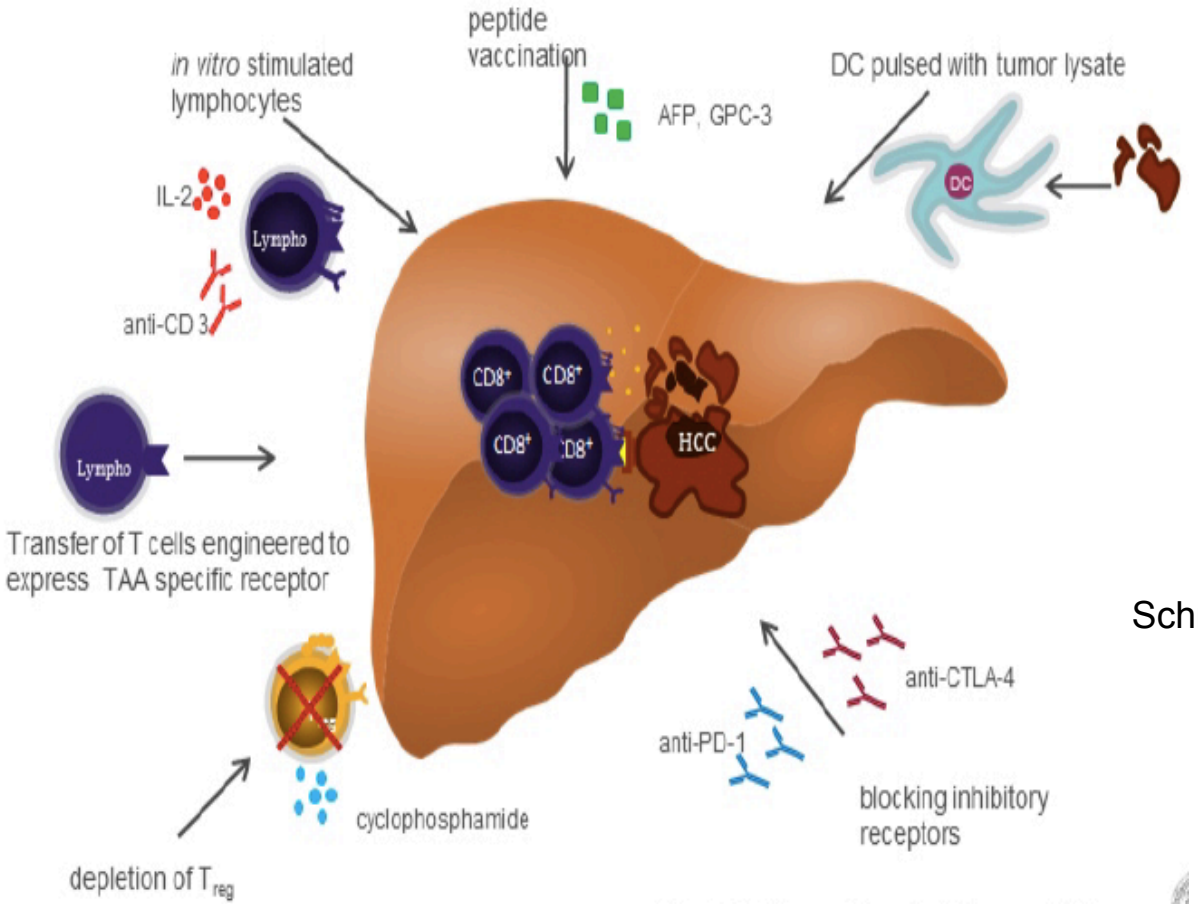
Jose D. Debes,[1,2,*] Marjolein van Tilborg,[2,*] Zwier M. A. Groothuismink,[2] Bettina E. Hansen,[2,3,4] Julian Schulze zur Wiesch,[5] Johann von Felden,[5] Robert J. de Knegt,[2] and Andre Boonstra[2]

12 cytokine signature in patients with HCV treated with direct acting antivirals (sofosbuvir/ledipasvir)



Control = HCV patients treated with DAA, no HCC
De novo = HCV patients treated with DAA, who develop de novo HCC within 2 years
Recurrent= HCV patients treated with DAA, who develop recurrent HCC within 2 years

Immunotherapy for HCC

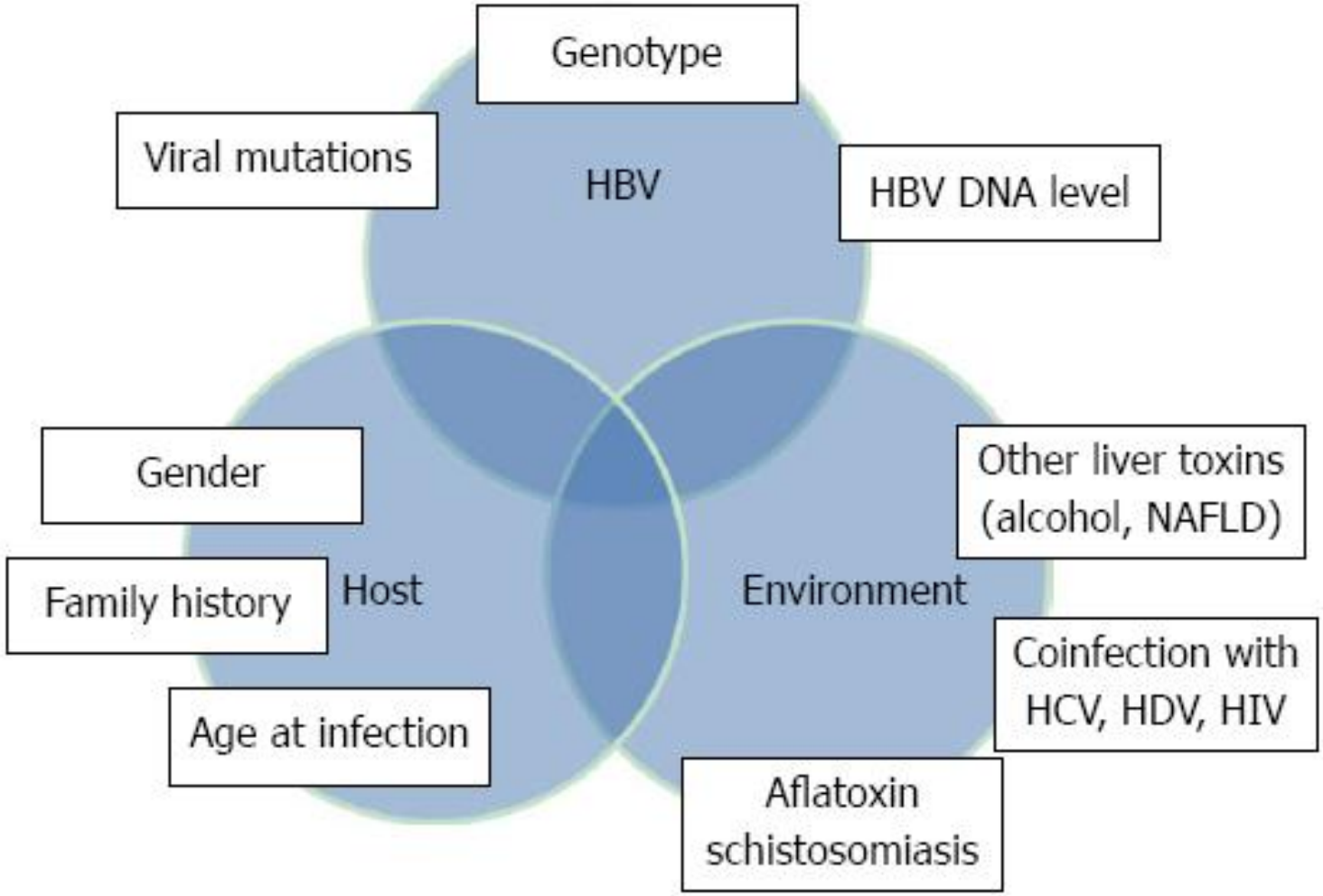


Schmidt/ Thimme, 2016

Clinical trials with antibodies against CTLA-4 and PD1/PD-L1

Good safety profile (pruritis, diarrhea, rash)

15-20% of patients had tumor responses, which lasted for a median of 17 months
45% of patients had stable disease, lasting more than 6 months in most cases


Genotype
Viral mutations
HBV
HBV DNA level
Gender
Other liver toxins
(alcohol, NAFLD)
Family history
Host
Environment
Coinfection with
HCV, HDV, HIV
Age at infection
Aflatoxin
schistosomiasis

Hepatocellular carcinoma, epidemiology and risk factors

Healthy Liver

Fibrosis/
Cirrhosis

Hepatocellular carcinoma