CENTRE DE RECHERCHE EN CANCÉROLOGIE DE LYON

UMR INSERM-U1052/CNRS-5286/UCBL/CLB

Eradication of HBV?

a « one step at a time » calendar!

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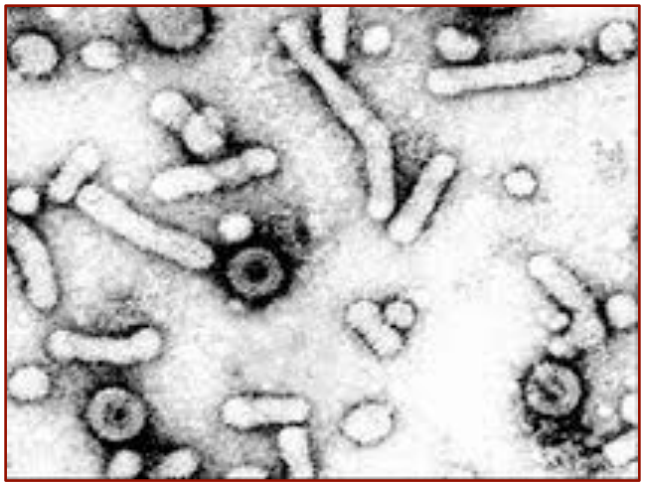
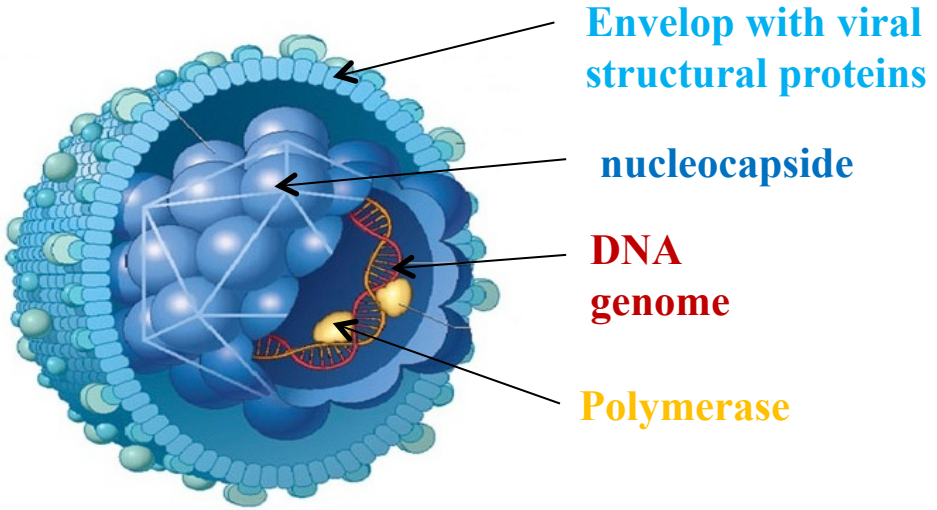
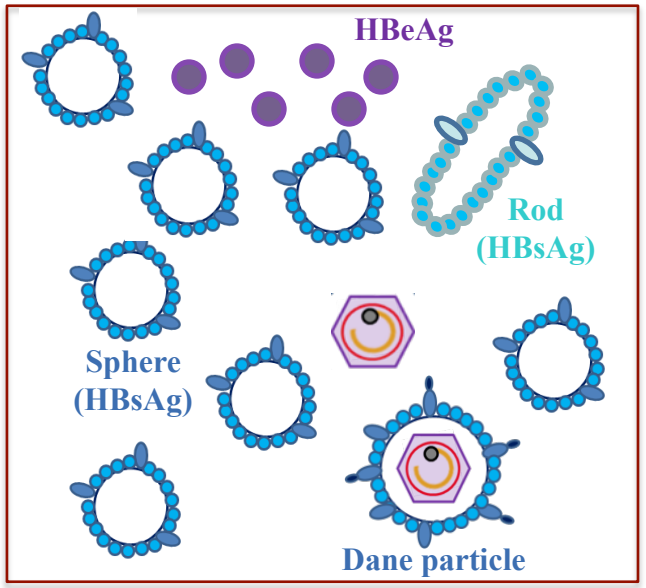
Centre Léon Bérard LYON ET RHÔNE-ALPES

Hôpitaux de Lyon

Circulating HBV particles, subviral particles, and antigens



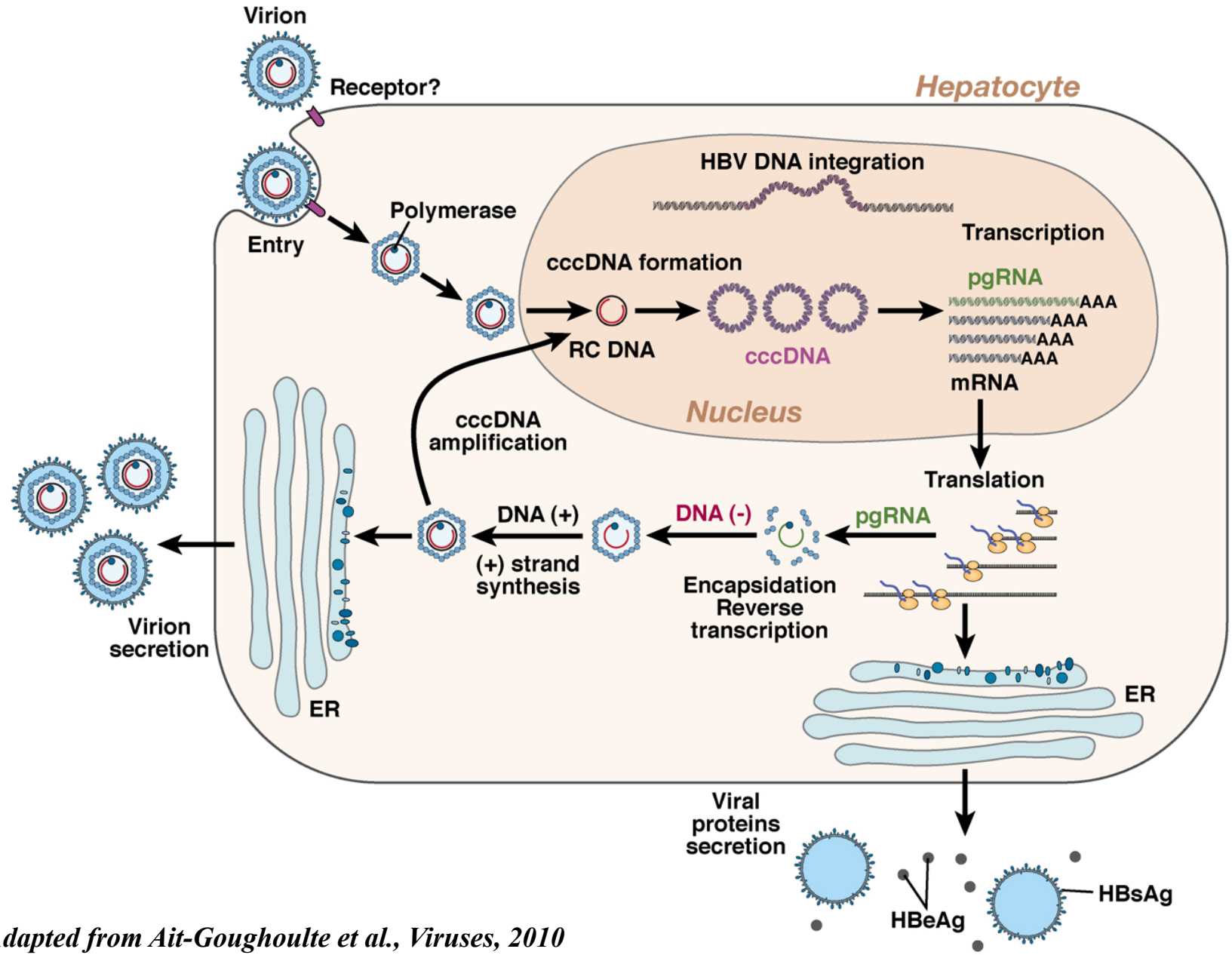




Adapted from Seeger et al. Field's in Virology , 2007

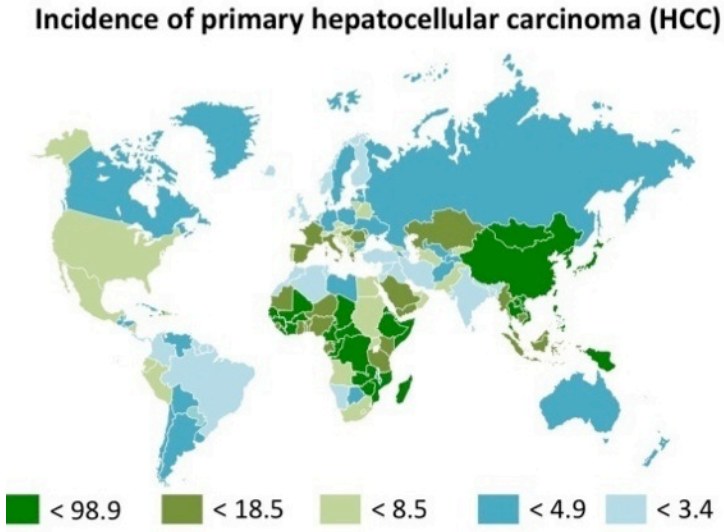
- ✓ Small envelopped virus of 42 nm = infectious particles or Dane particles
- ✓ 3 types of enveloppe protein: L, M, S
- ✓ Nucleocapsid = genome + 240 capsid proteins + polymerase (+ host factors)
- ✓ Subviral particles (sphere or rod-shaped) in large excess (1 Dane / 10,000 SVP)
- ✓ Viremia in patient: 10^{e9} vge/mL
- ✓ Antigenemia: 10^{e12}-10^{e13} SVP/mL

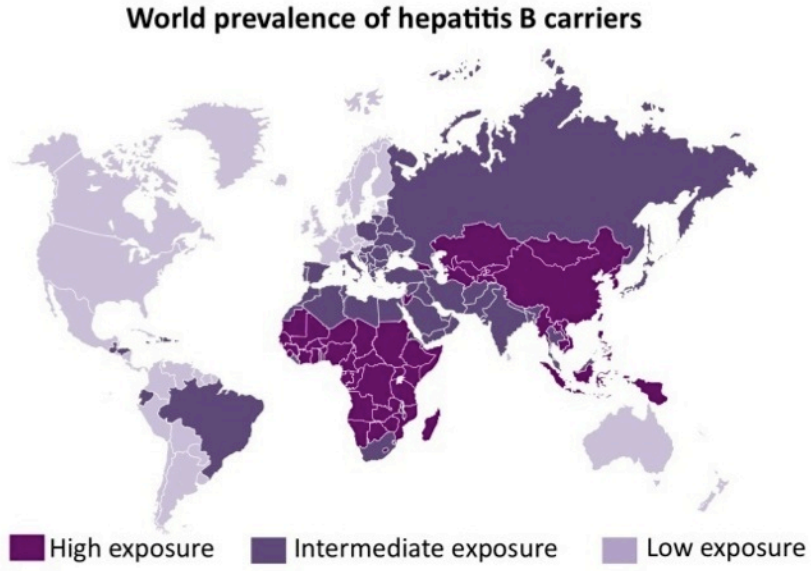
HBV life cycle



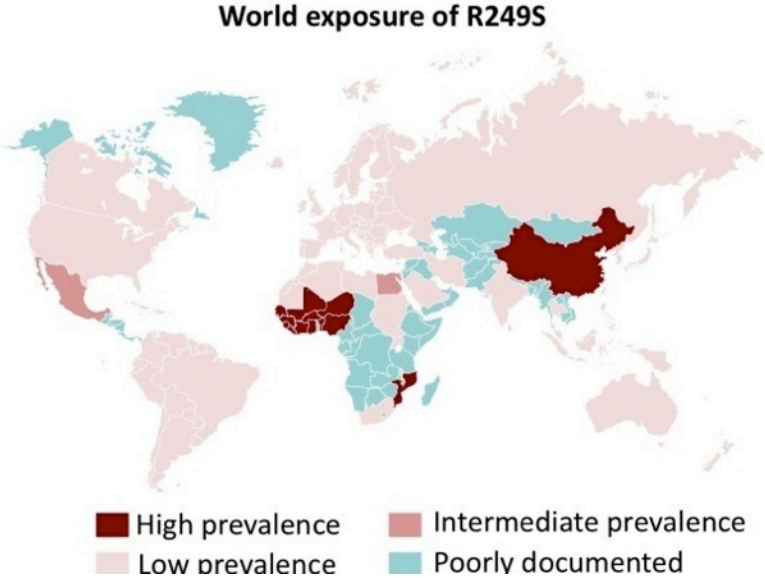
Adapted from Ait-Gougoulte et al., Viruses, 2010

HBV infection and HCC incidence



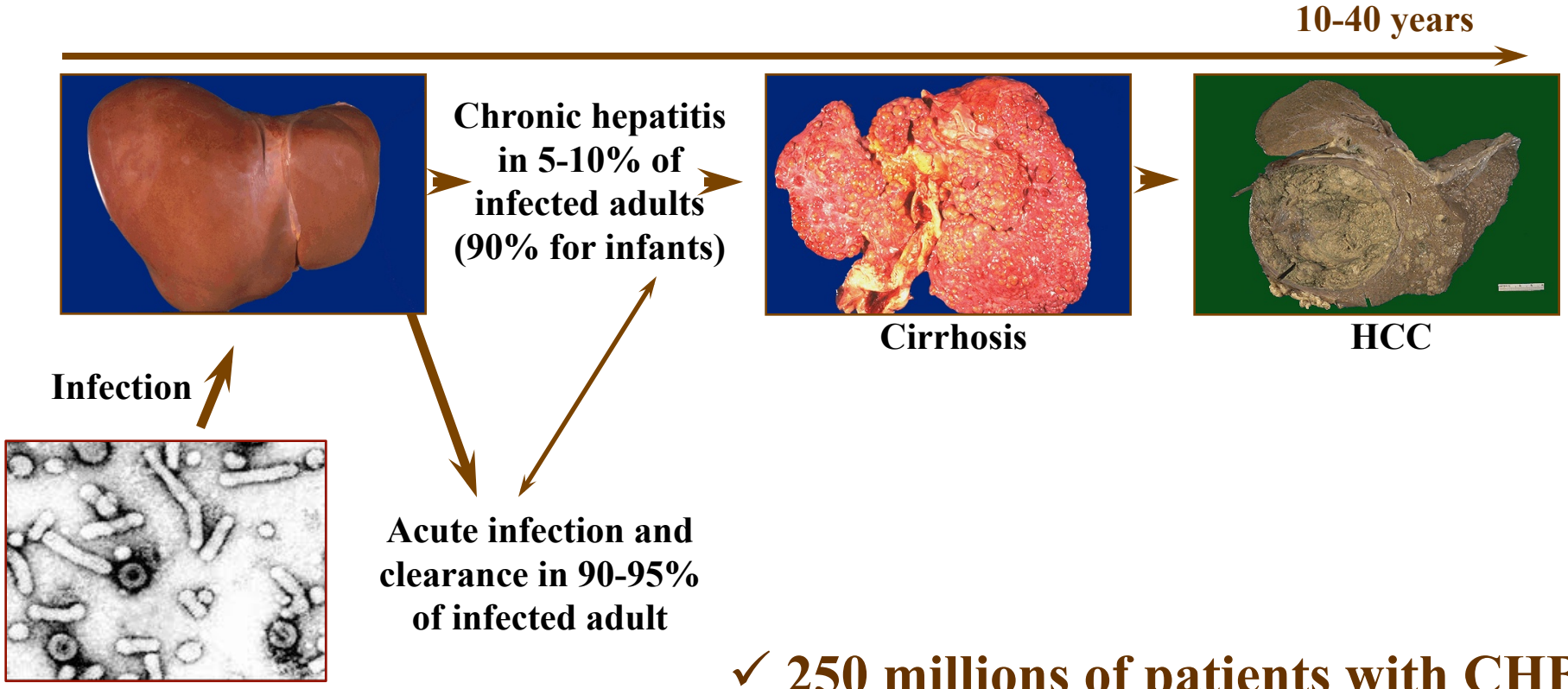


350 millions infected people



Gouas et al., Cancer lett., 2009

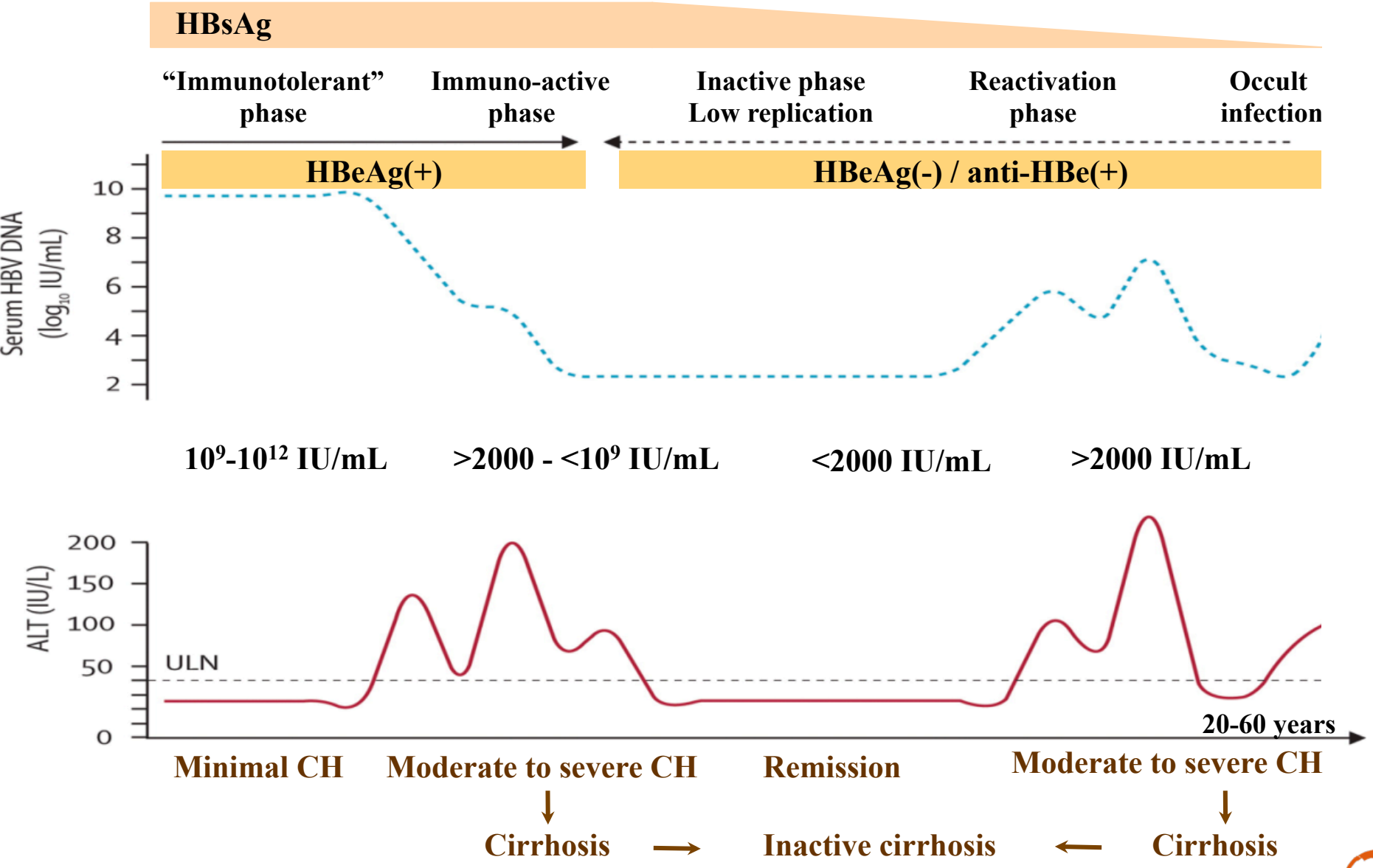
Natural history of HBV infections in humans



- ✓ 250 millions of patients with CHB
- ✓ Major cause of liver failure and HCC in endemic area
- ✓ HCC: 5th in term of prevalence, 3rd in term of death

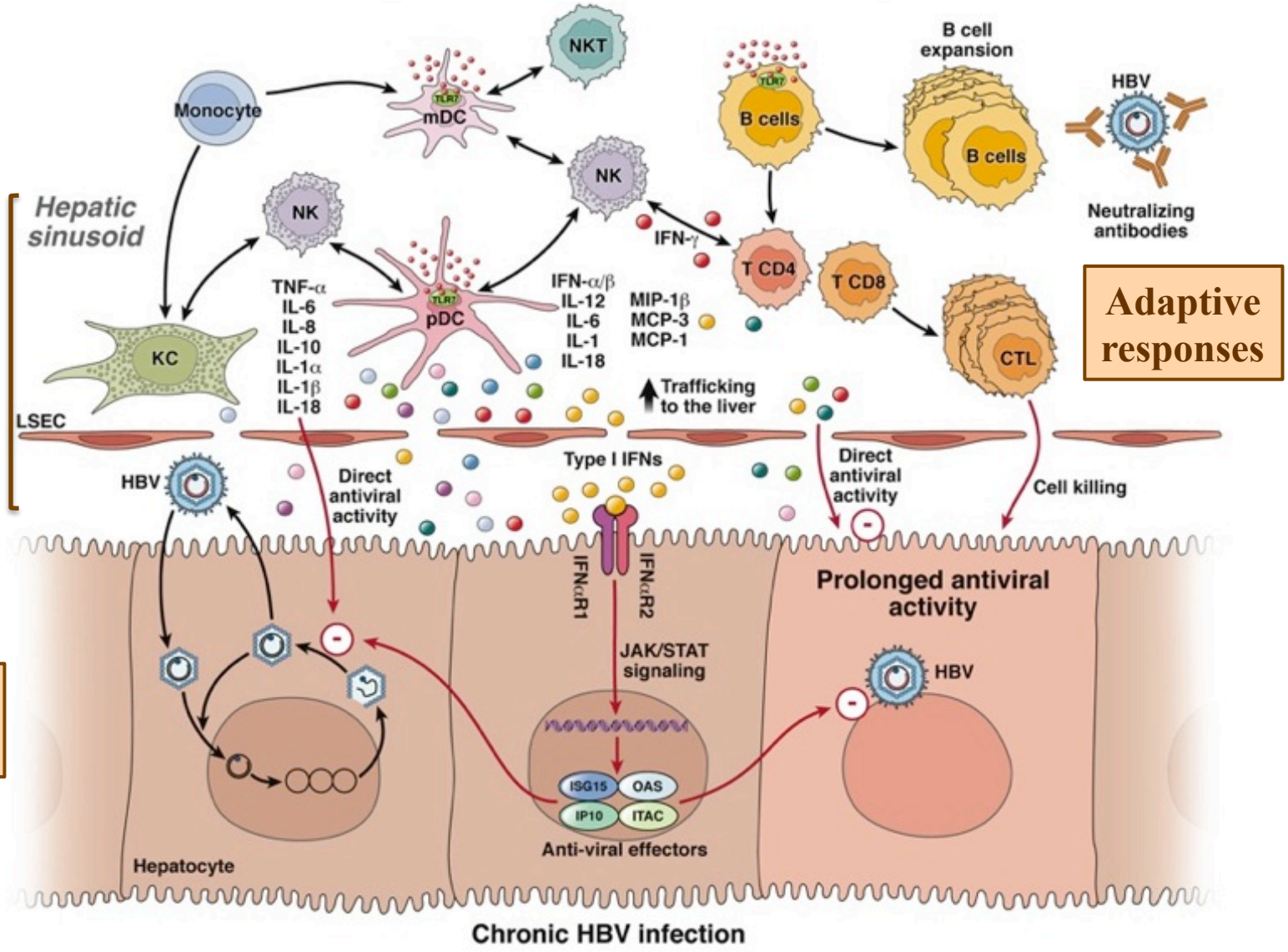
Ait-Goughoulte et al., Viruses, 2010

Natural history of HBV infections in humans



Adapted from Papatheodoridis, Lancet Inf Dis, 2008

Physiopathology of HBV infections



Bertoletti & Ferrari Gut 2012; Zoulim et al, Gastroenterology 2013

Liver Damage and HBV infection

- Liver damage results of immune killing of infected (only?) hepatocytes
- HCC not always seen on a background of cirrhosis
- Clonal expansion of hepatocytes not supporting HBV replication occurs even before cirrhosis
- Experimental models show that clonal hepatocyte repopulation is a major risk factor for HCC
- Current treatment slows/prevent cirrhosis & reduces HCC incidence

Zoulim & Mason, Gut 2012; 61 : 333-336

Antivirals approved for the treament of chronic hepatitis B

Drug Type	Approved	Phase 3	Phase 1-2
Nucleoside analogs	• Lamivudine • Entecavir • Telbivudine	• Emtricitabine* • Clevudine**	
Nucleotide analogs	• Adefovir dipivoxil • Tenofovir disoproxil fumarate		Tenofovir prodrug (TAF)
Cytokines	• Interferon alfa • Pegylated Interferon alfa-2a	•IFN lambda	Vaccine therapy IL7 TLR7 agonists
Others			Myrcludex (entry inhibitor) Capsid inhibitors

HIV
****development on hold***

Zoulim & Durantel, in press

Current treatment: sustained disease control achieved with NUCs/IFN in majority of patients

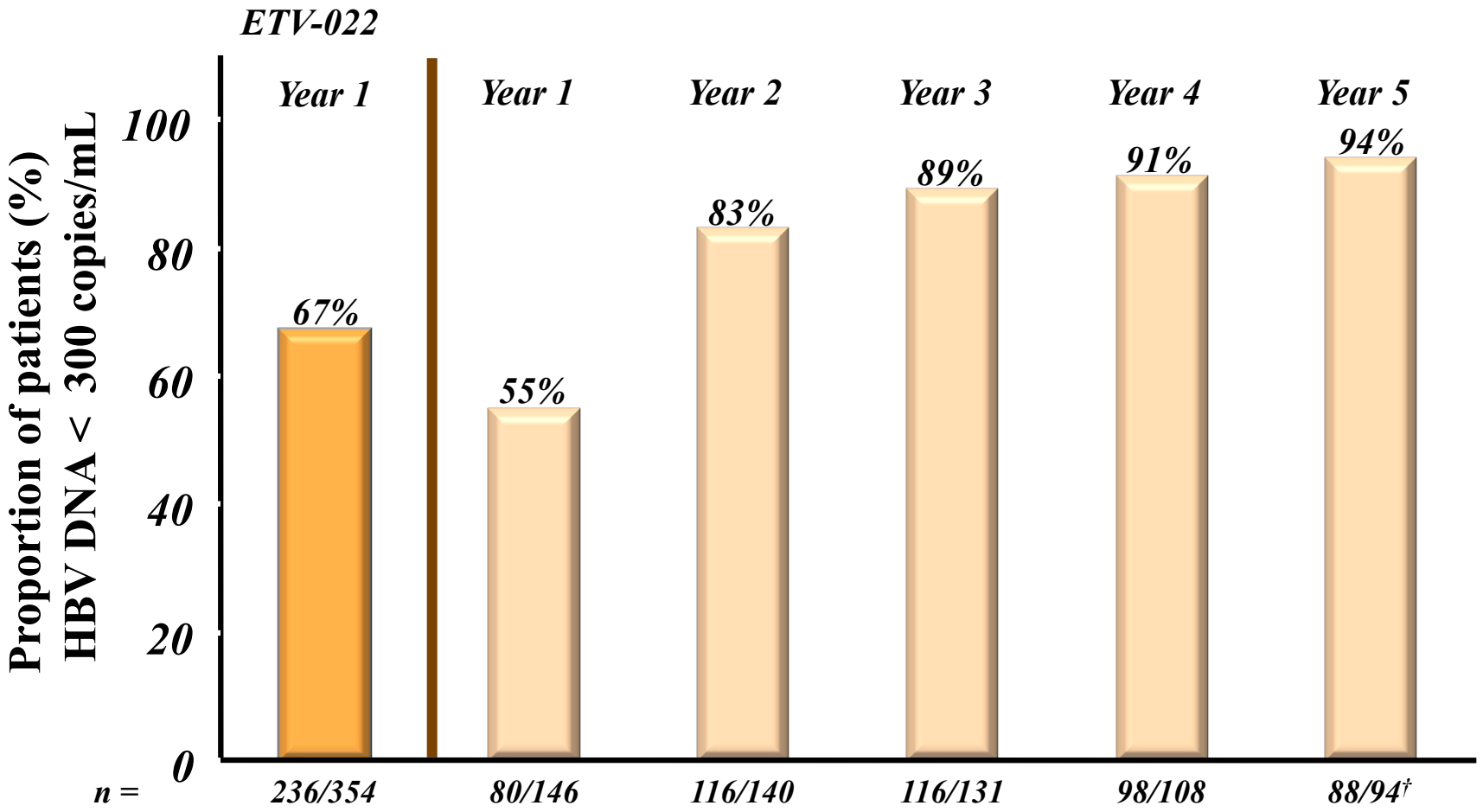
	Entecavir[1,2]	Tenofovir[3]	PEG-IFN α-2a[4,5]
HBeAg positive	n = 354	n = 176	n = 271
HBV DNA undetectable	67%	76%	25%[a]
HBeAg seroconversion	21%	21%	27%
ALT normalisation	68%	68%	39%
HBsAg loss	2%	3.2%	2.9%[b]
HBeAg negative	n = 325	n = 250	n = 177
HBV DNA undetectable	90%	93%	63%[a]
ALT normalisation	78%	76%	38%
HBsAg loss	0.3%	0%	0.6%[b]

Results at 48 weeks
[a]HBV DNA < 400 copies/mL; [b]At 72 weeks

ALT, alanine aminotransferase; IFN, interferon; NUCs, nucleos(t)ide analogues; PEG-INF, peginterferon α-2a;

1. Chang T-T, et al. N Engl J Med 2006;354:1001–10.
2. Lai C-L, et al. N Engl J Med 2006;354:1011–20.
3. Marcellin P, et al. N Engl J Med 2008;359:2442–55.
4. Lau GKK, et al. N Engl J Med 2005;352:2682–95.
5. Marcellin P, et al. N Engl J Med 2004;351:1206–17.

5-yr Entecavir for HBeAg-positive CHB
Virological response



** Different dosing regimen for naïve patients in the 901 study*

† Five patients who remained on treatment at the Year 5 visit had missing PCR values (NC=M).

Han S, et al. 59th AASLD, October 31-November 4 ,2008, San Francisco, USA. Poster 893.

6-yr tenofovir for HBeAg-pos. and neg. CHB
Virological response

Virologic Suppression at Year 6

Response	HBeAg- Patients (Study 102)		HBeAg+ Patients (Study 103)	
	Year 5	Year 6	Year 5	Year 6
HBV DNA < 400 copies/mL Intent-to-treat*, % (n/N)	**83** (291/350)	**81** (281/345)	**65** (160/248)	**63** (157/251)
HBV DNA < 400 copies/mL On treatment†, % (n/N)	**99** (292/295)	**99.6** (283/284)	**97** (170/175)	**99** (167/169)

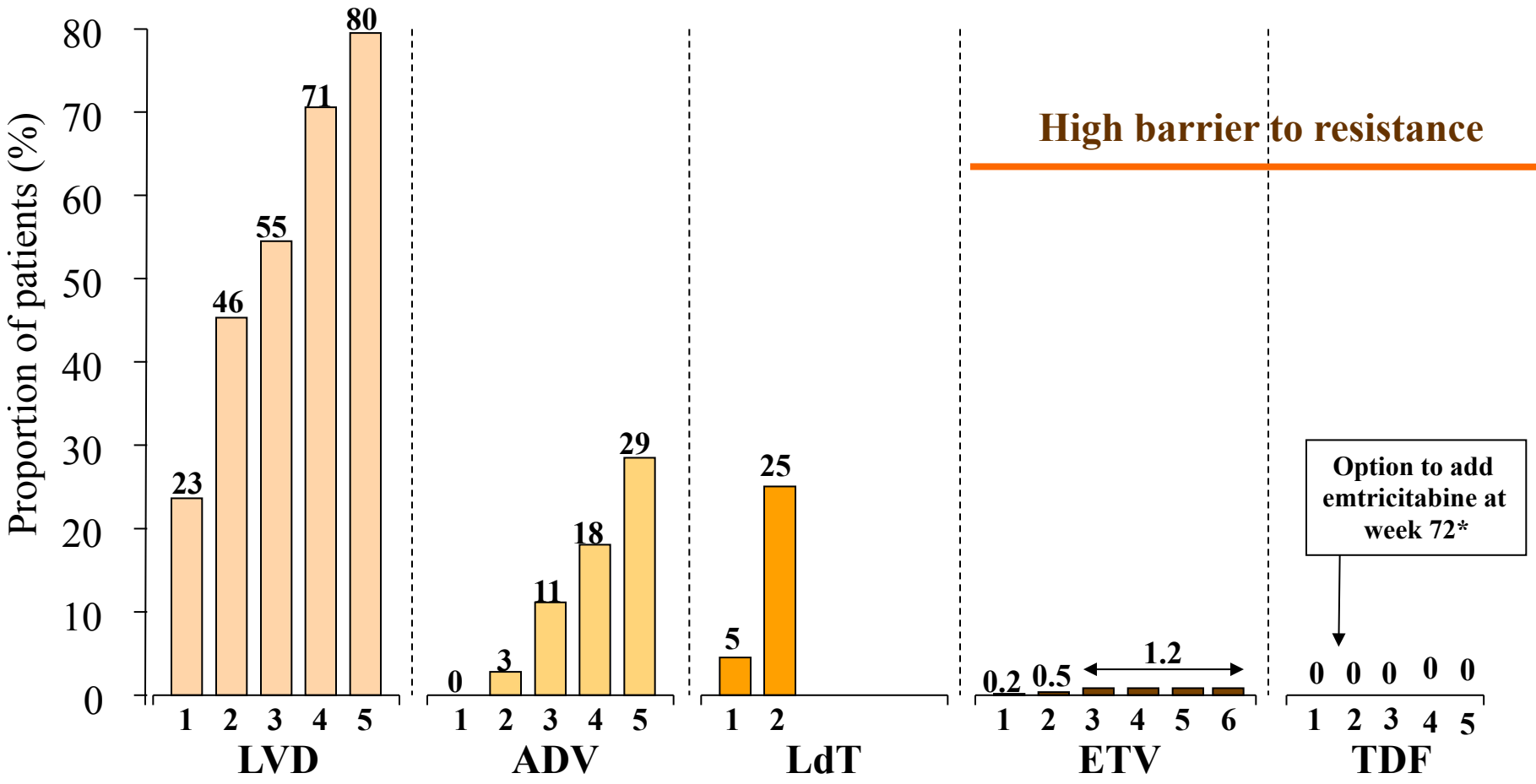
* **LTE-TDF (missing = failure/addition of FTC = failure)**
† **Observed (missing = excluded/addition of FTC = included)**

- 80% of 585 patients entering the open-label phase remained on study at Year 6; 73% of enrolled patients remained on study
- HBeAg loss/seroconversion rates of 50% and 37%, respectively, through 6 years
- 11% of HBeAg+ patients had confirmed HBsAg loss (8% with seroconversion)
- No resistance to TDF was detected through 6 years

Neither Truvada (TVD = TDF + FTC) or emtricitabine (FTC) are licensed for use to treat CHB

Marcellin et al., AASLD 2012

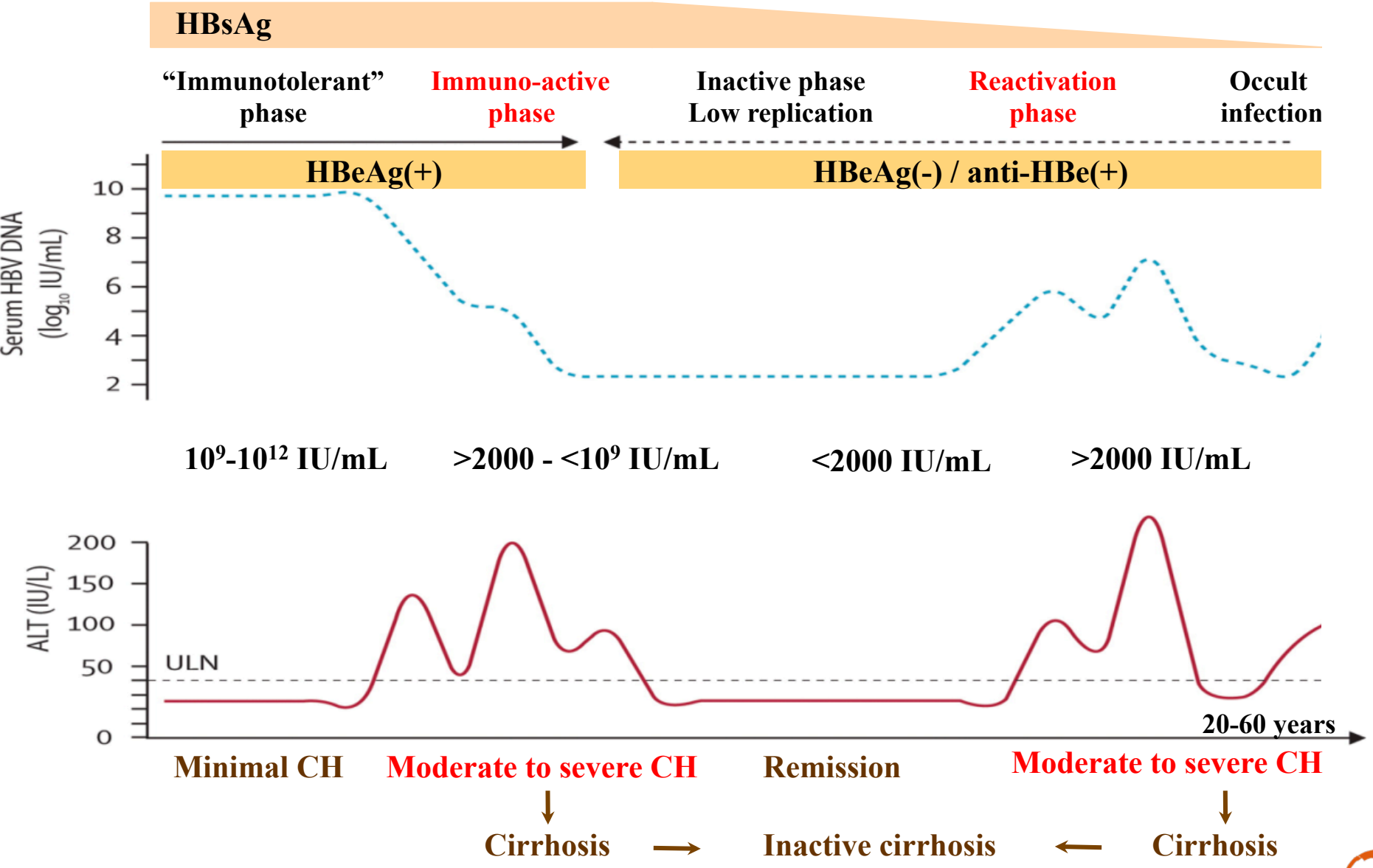
Rates of resistance with LVD, ADV, LdT, ETV and TDF among NA-naïve patients



*Patients confirmed to be viraemic at week 72 or beyond could add emtricitabine to TDF at the discretion of the investigator. Clinical data on the safety and efficacy of emtricitabine and TDF in CHB are pending

Gish, Jia, Locarnini & Zoulim, Lancet ID 2012

EASL recommendation to treat



Adapted from Papatheodoridis, Lancet Inf Dis, 2008

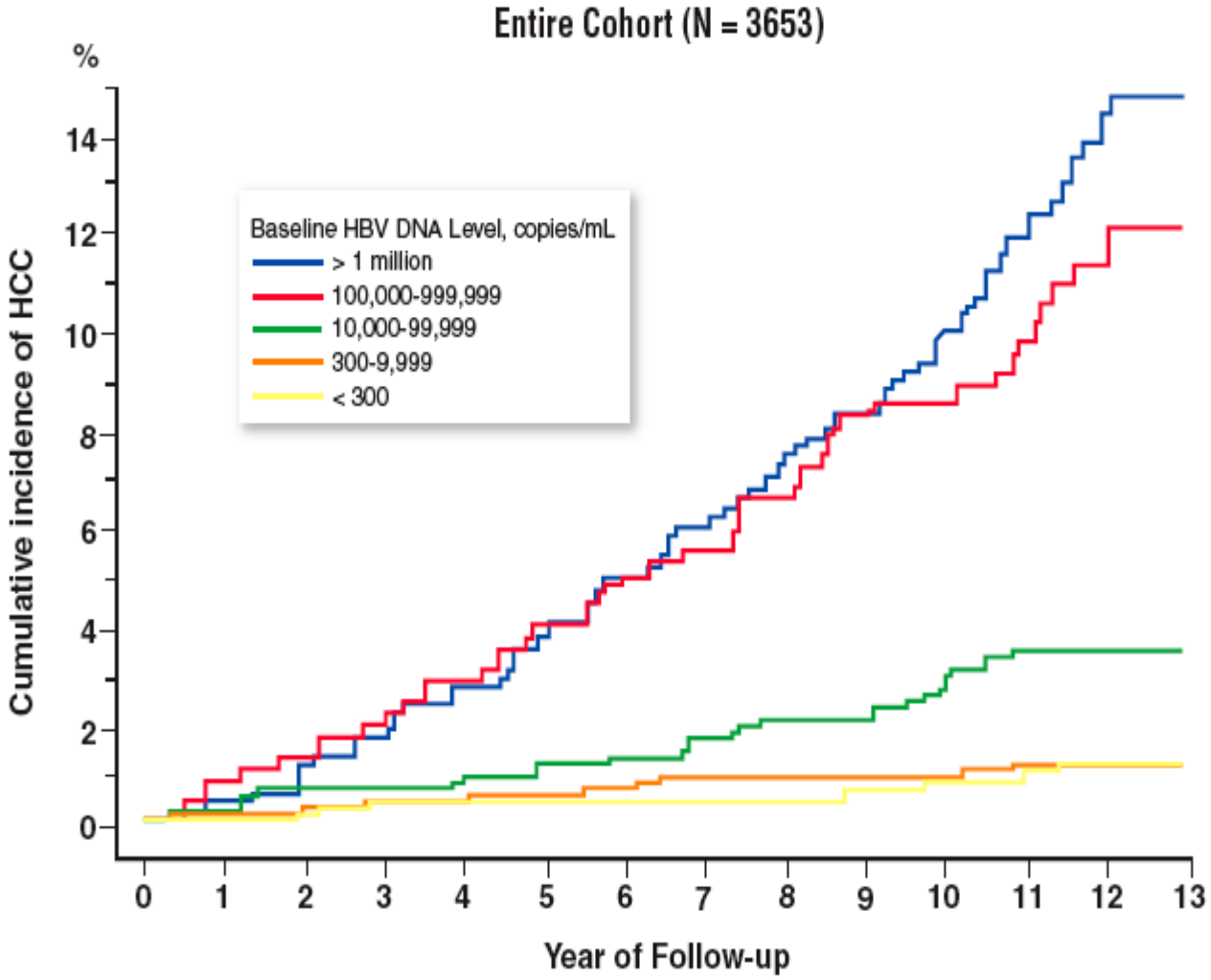
Should we treat immune tolerant patients ? (and patients with minimal hepatitis)

- **Absence of detectable liver disease (inflammation, fibrosis or cirrhosis)**
  - **> Treatment usually not indicated except for patients with family history of cirrhosis or HCC**

- **However, if HCC is the endpoint, there is no experimental support for treatment abstention**

EASL CPG 2012; Zoulim & Mason, Gut 2012; 61 : 333-336

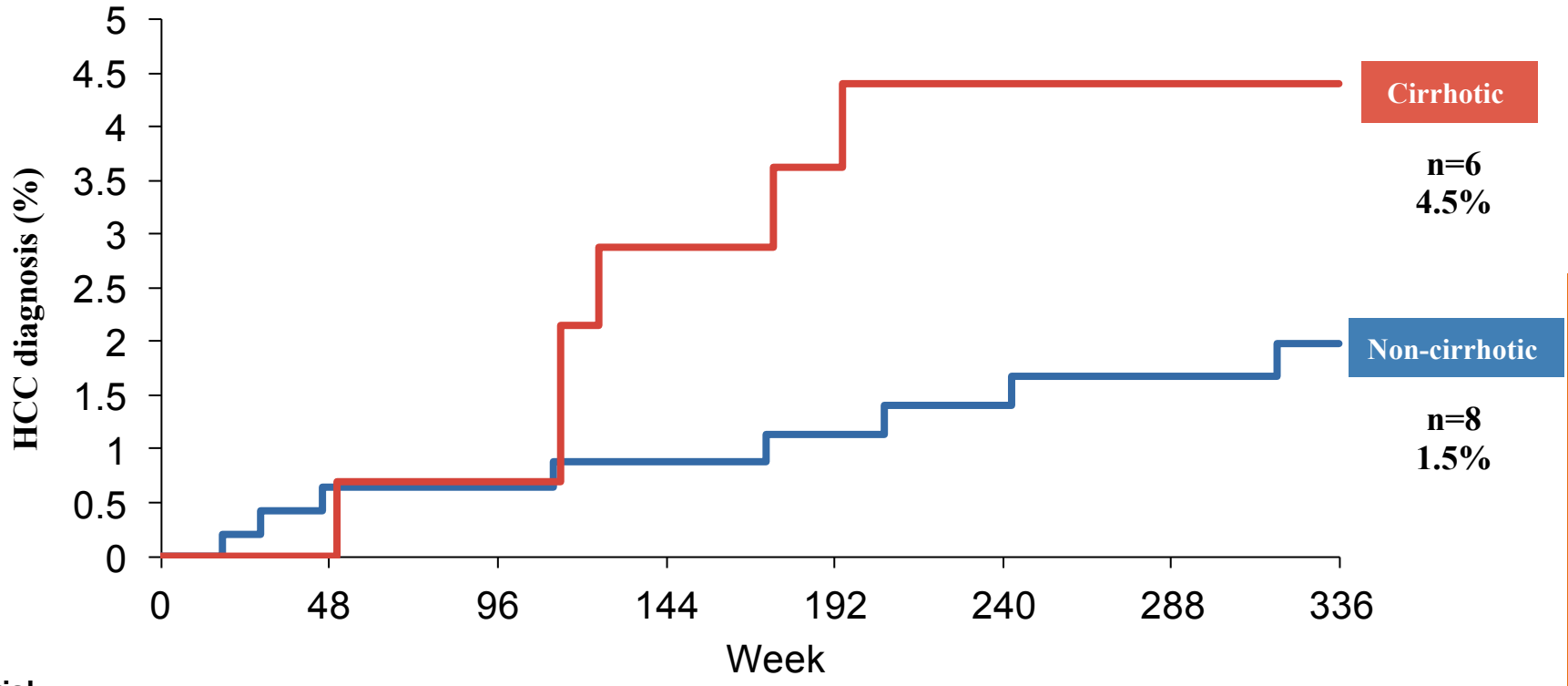
Viral load and incidence of HCC: the REVEAL study



Chen et al; JAMA 2006

Tenofovir Studies 102/103

HCC Incidence Based on Cirrhosis Status at Baseline



No. at risk

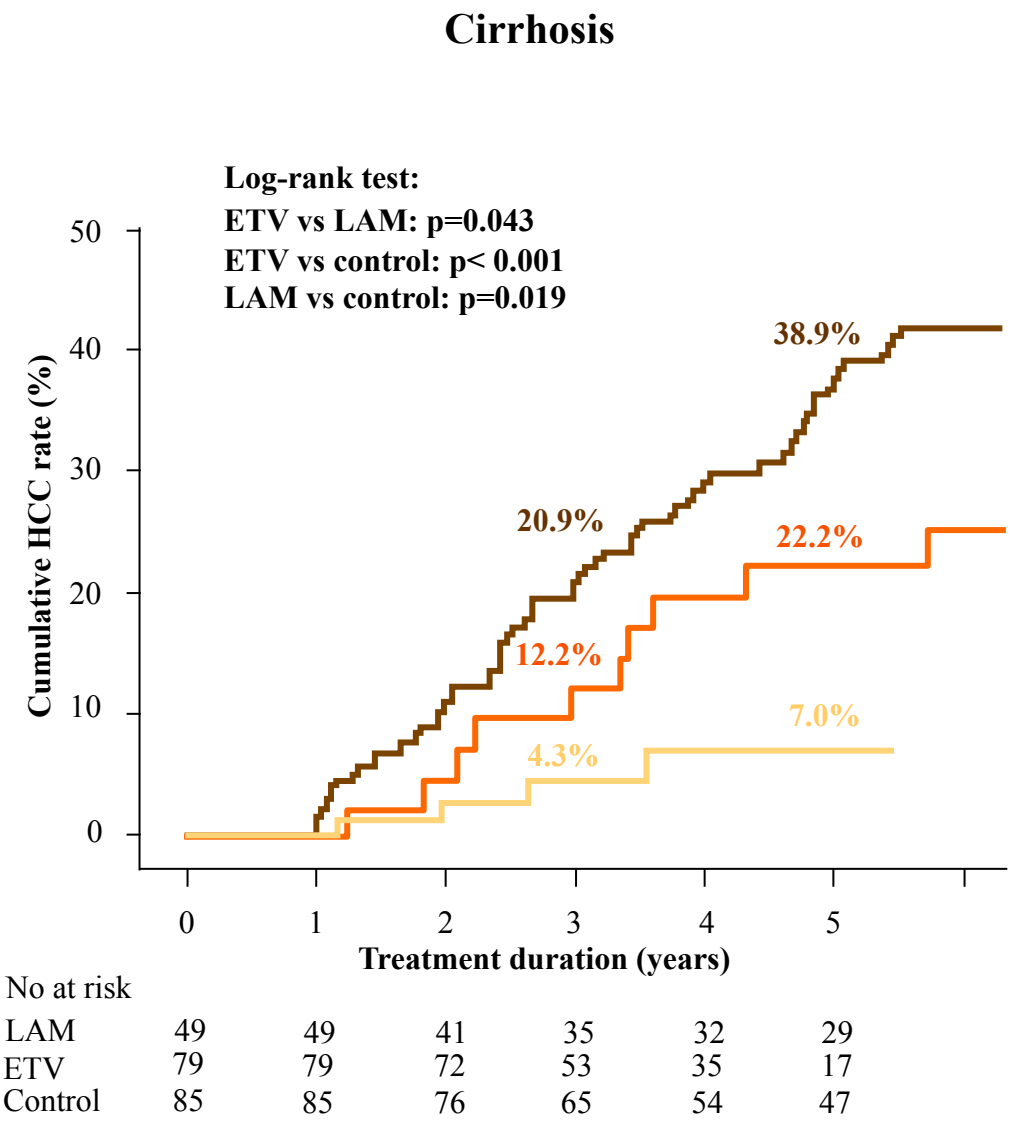
Non-cirrhotic	482	453	425	396	377	360	343	324*
Cirrhotic	152	146	137	132	126	120	115	109*

***Patients completing 336 weeks in study as defined by protocol**
Kim WR, et al. J Hepatol 2013 Supp 1;58(43):S19 - Oral#43

REACH-B is a risk calculator developed in non-cirrhotic pts so It may underestimate the risk

Long-term ETV treatment reduces HCC incidence in patients with HBV infection

HCC incidence lower with ETV than with LAM



- **LAM cohort:**
  - PS matched LAM cohort (n=182)
  - Treated with LAM (1995–2007)
  - Received no rescue therapy if LAM failure

Group analysis showed a greater HCC suppression effect with ETV than with LAM, as compared with control group

Hosaka T, et al. Hepatology 2013

Molecular and clinical studies advocate for early antiviral therapy to prevent HCC

- Association between viral replication and integration
- Persistent viremia associated with higher risk of HCC development
- Liver damage occurs even if clinically silent (initially)
- Viral suppression associated with decreased liver damage and delayed risk of HCC

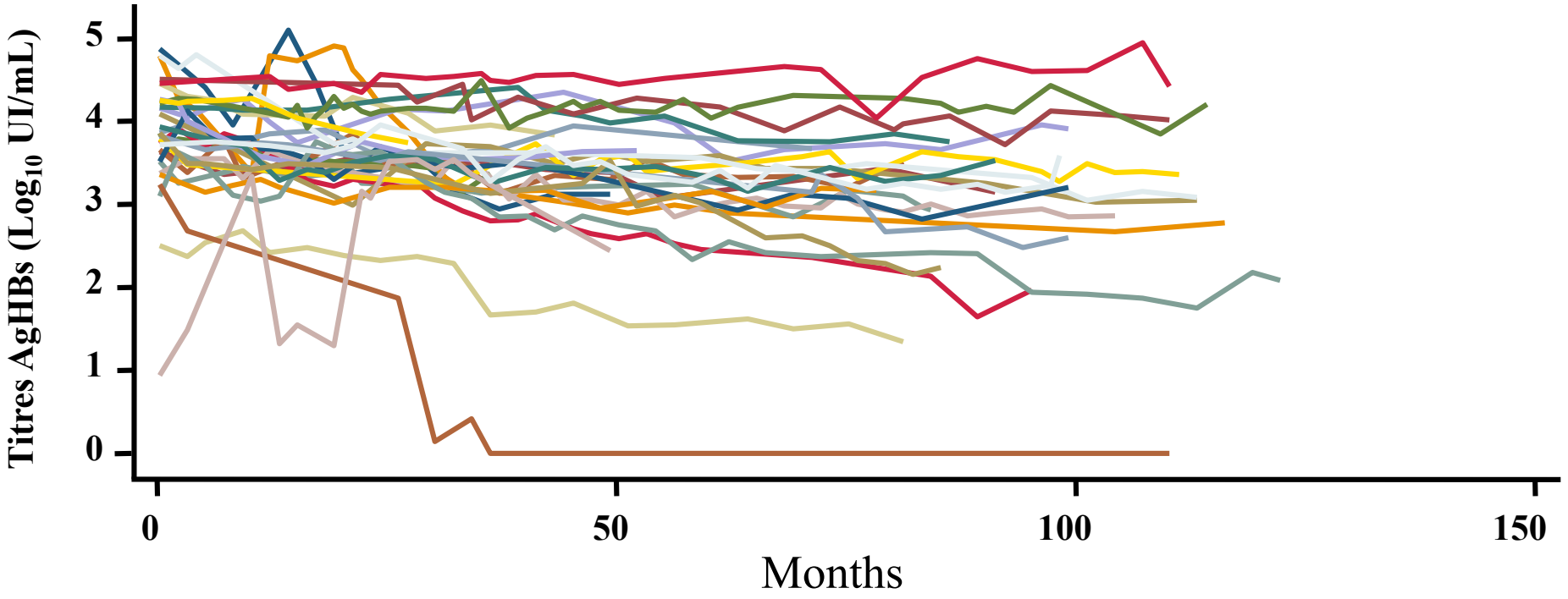
Zoulim & Mason, Gut 2012; 61 : 333-336

Hepatitis B - clinical needs

- **Better understanding of disease pathogenesis**
  - **biomarkers / prediction of disease progression**
- **Prevention of HCC**
- **« Early » treatment intervention**
- **Prevention of resistance in resource limited countries**
- **Improve treatment / HBV cure ?**
  - **Reduce antigenemia**
  - **Reduce cccDNA**
  - **Restoration of immune response → immune control**
  - **Treatment with « finite » duration; end point HBs loss**

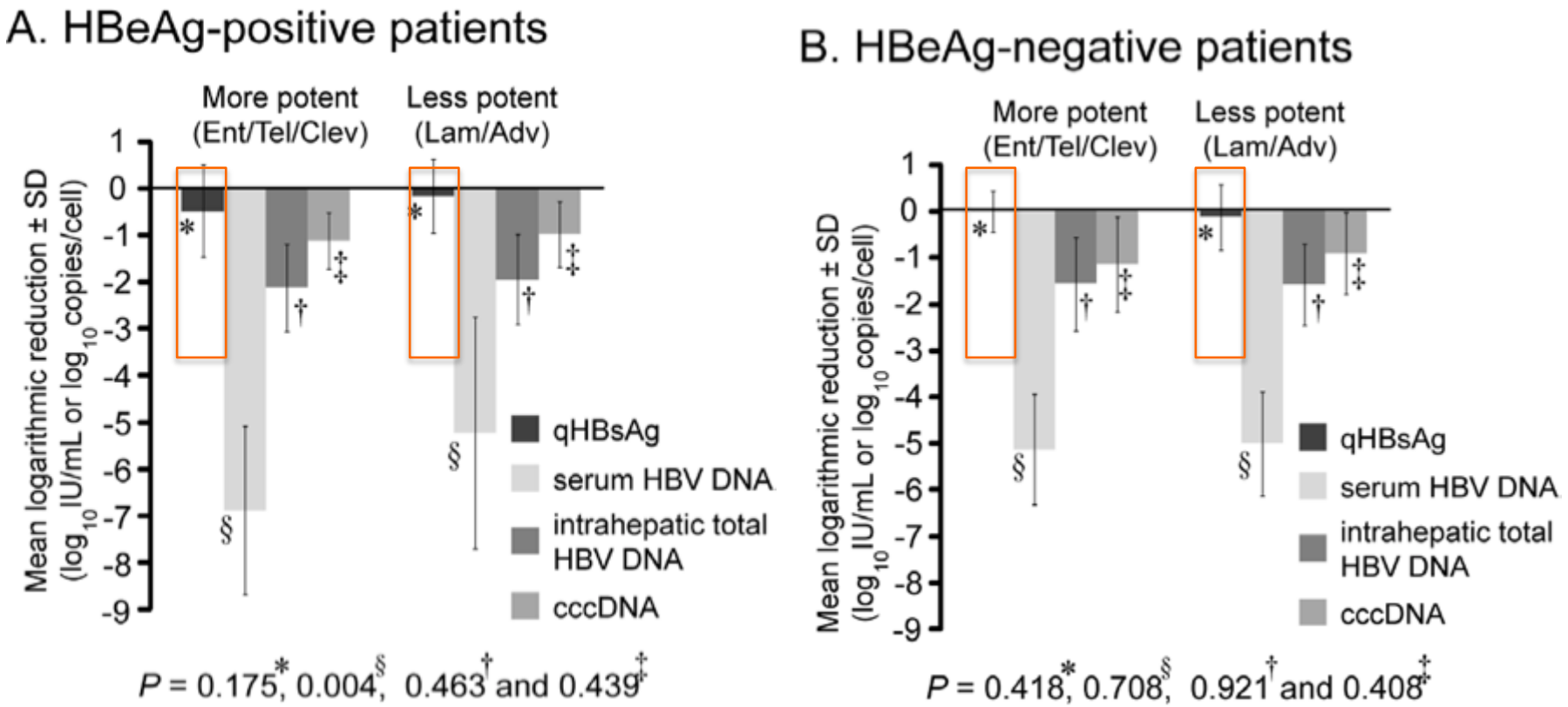
Long-term kinetics of HBsAg during NUC administraiton

30 patients, median FU: 102 months (IQR: 88-119 months)



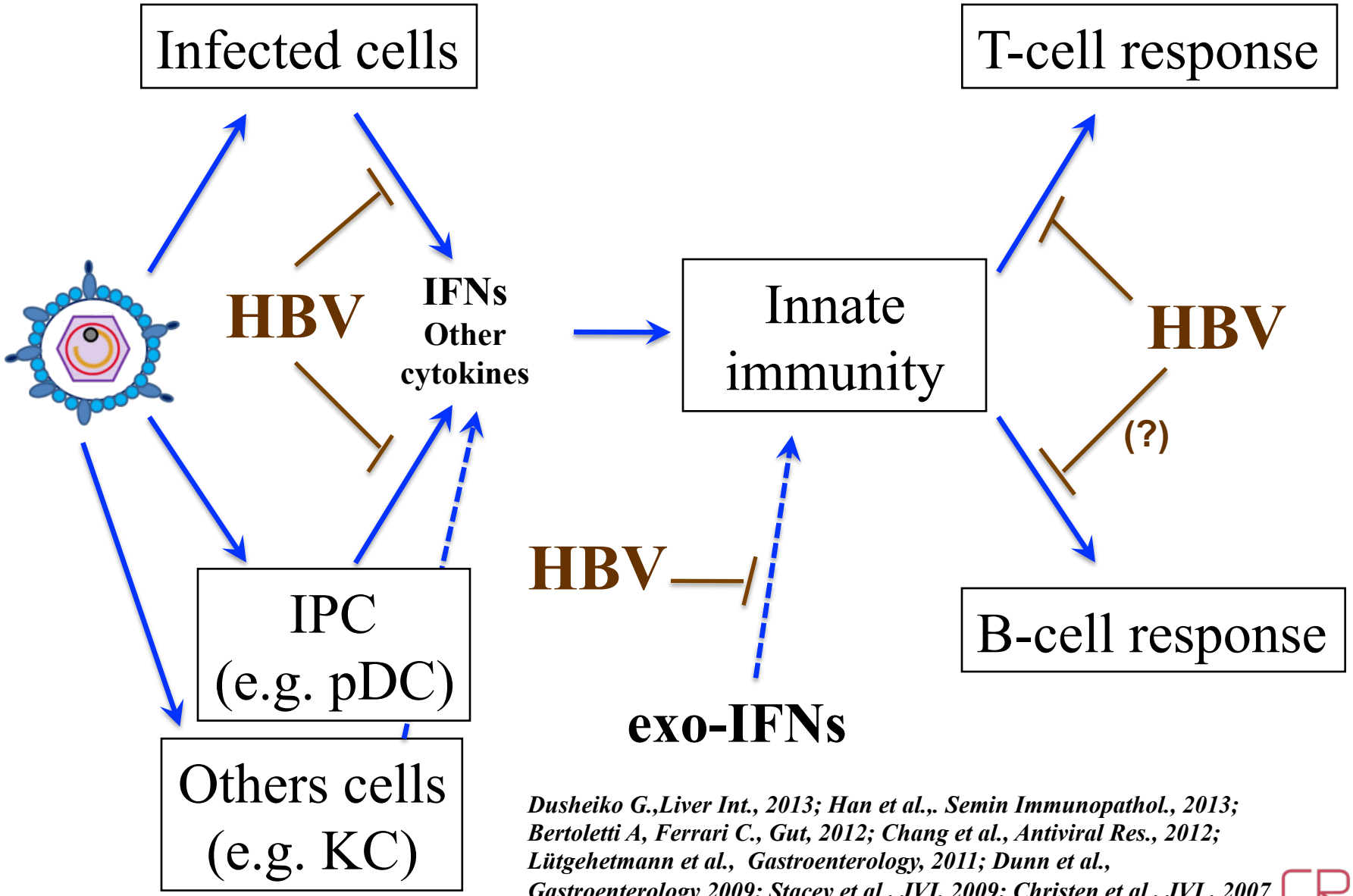
Chevaliez S., et al., J Hepatol 2013

NUCs are good for virosupression, but little impact on other viral parameters



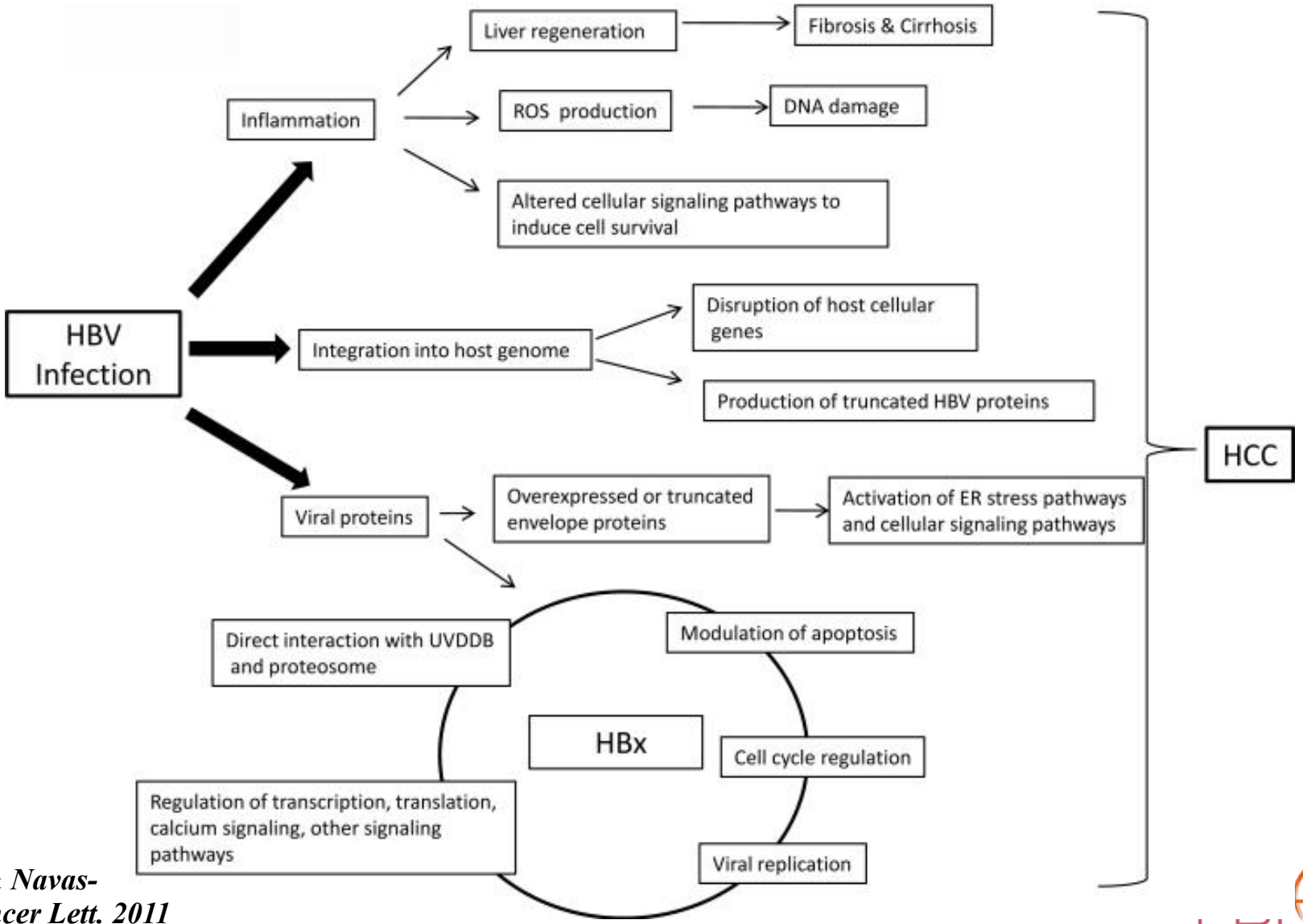
Wong et al, Clin Gastroenterol Hepatol 2013

HBV inhibit immune responses
Secreted antigens (HBs, HBe, HBc) responsible?



Dusheiko G.,Liver Int., 2013; Han et al.,. Semin Immunopathol., 2013; Bertoletti A, Ferrari C., Gut, 2012; Chang et al., Antiviral Res., 2012; Lütgehetmann et al., Gastroenterology, 2011; Dunn et al., Gastroenterology 2009; Stacey et al., JVI, 2009; Christen et al., JVI., 2007

Role of HBx and HBV genome integration in carcinogenesis



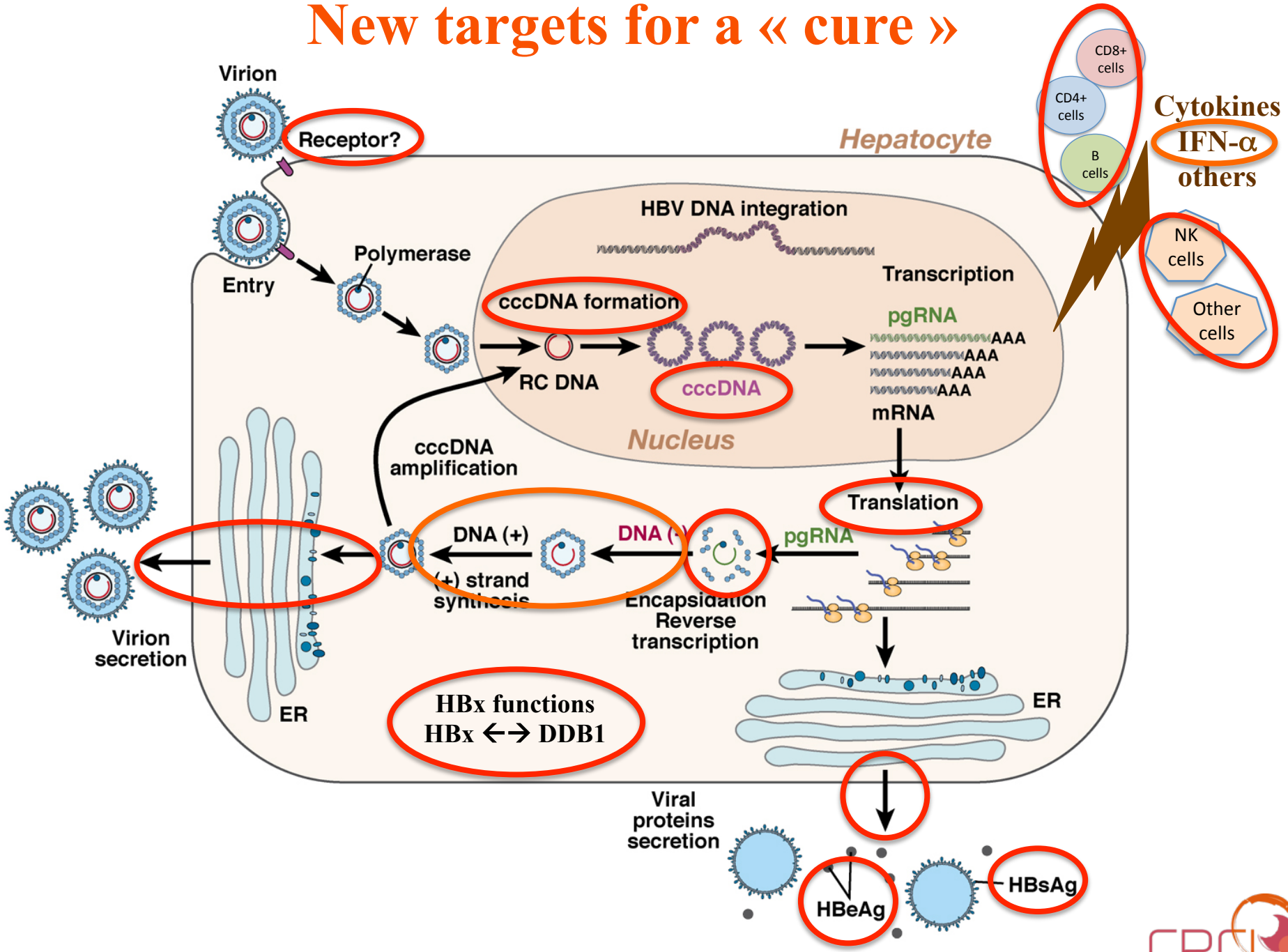
Bouchard & Navas-Martin, Cancer Lett. 2011

Eradication of HBV?

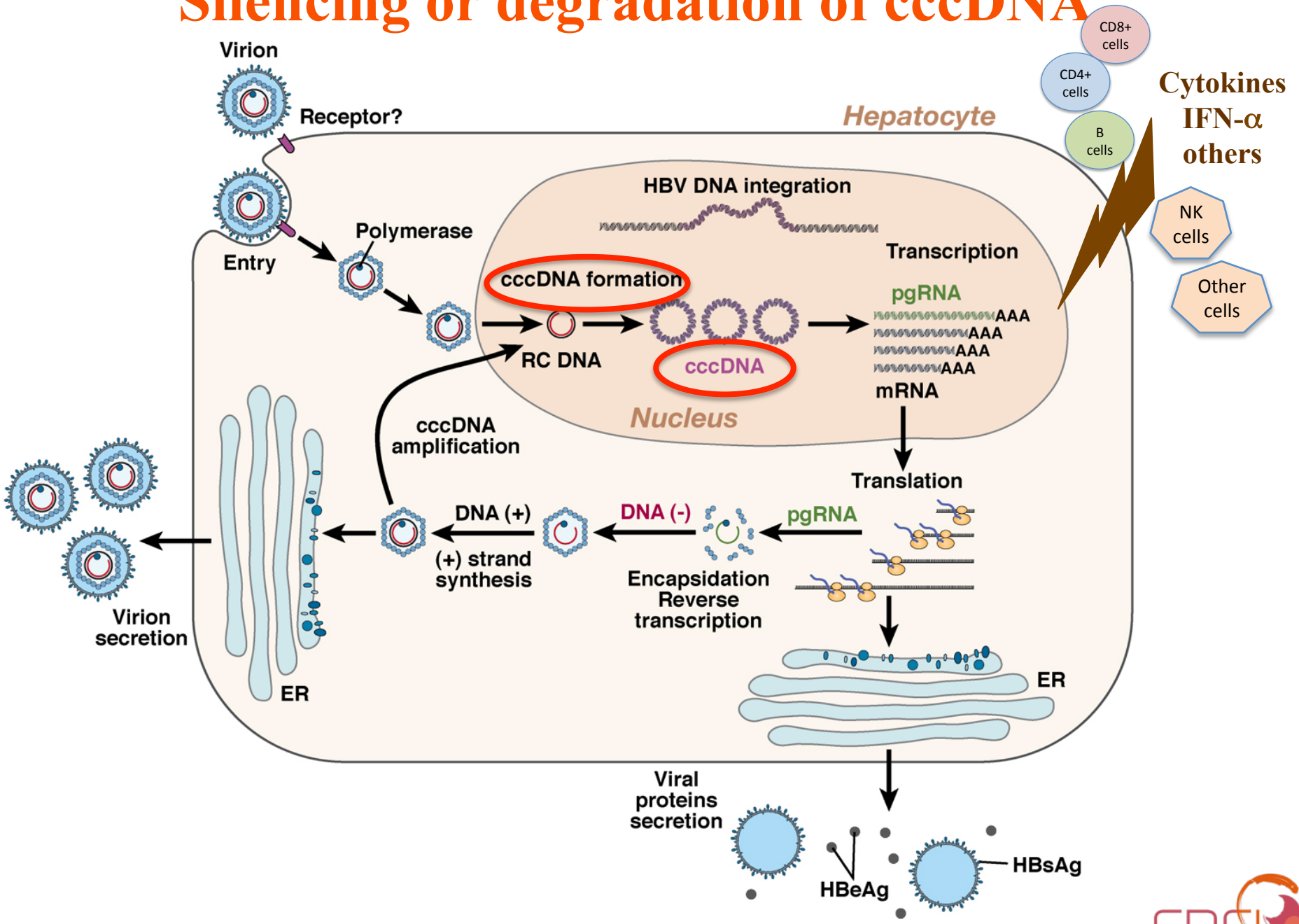
Definitions around the concept of HBV cure

- « Absolute cure » vs « functional cure » vs « virological cure »
- Virological definition
  - Eradication of cccDNA
  - Clearance of HBsAg
- Clinical definition
  - HBsAg seroconversion even if cccDNA persistence (similar to spontaneous resolution of infection)
- Control of infection (by immunity)
  - Persistence of low amount of transcriptionally inactive cccDNA, i.e. « HBsAg occult infection »
  - Inactive carrier state, but residual risk of HCC *(Cho et al., Gut, 2014)*

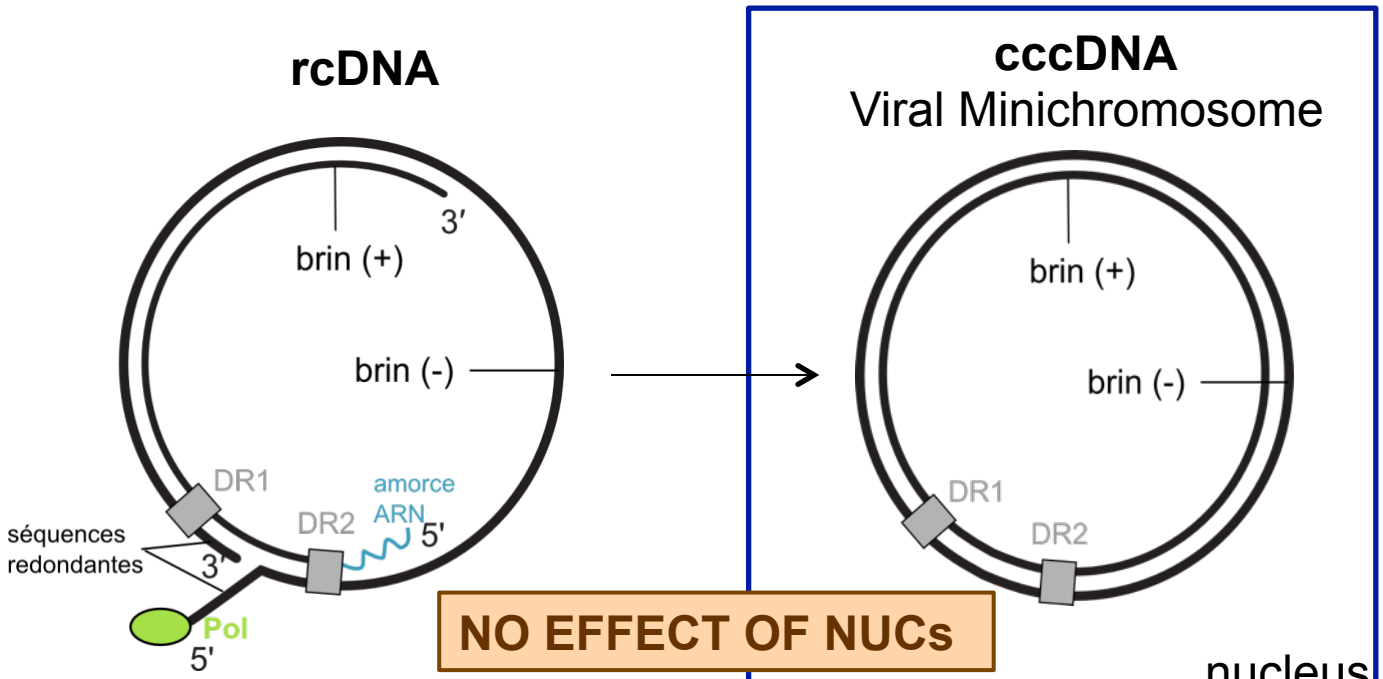
New targets for a « cure »



Silencing or degradation of cccDNA



Formation of cccDNA

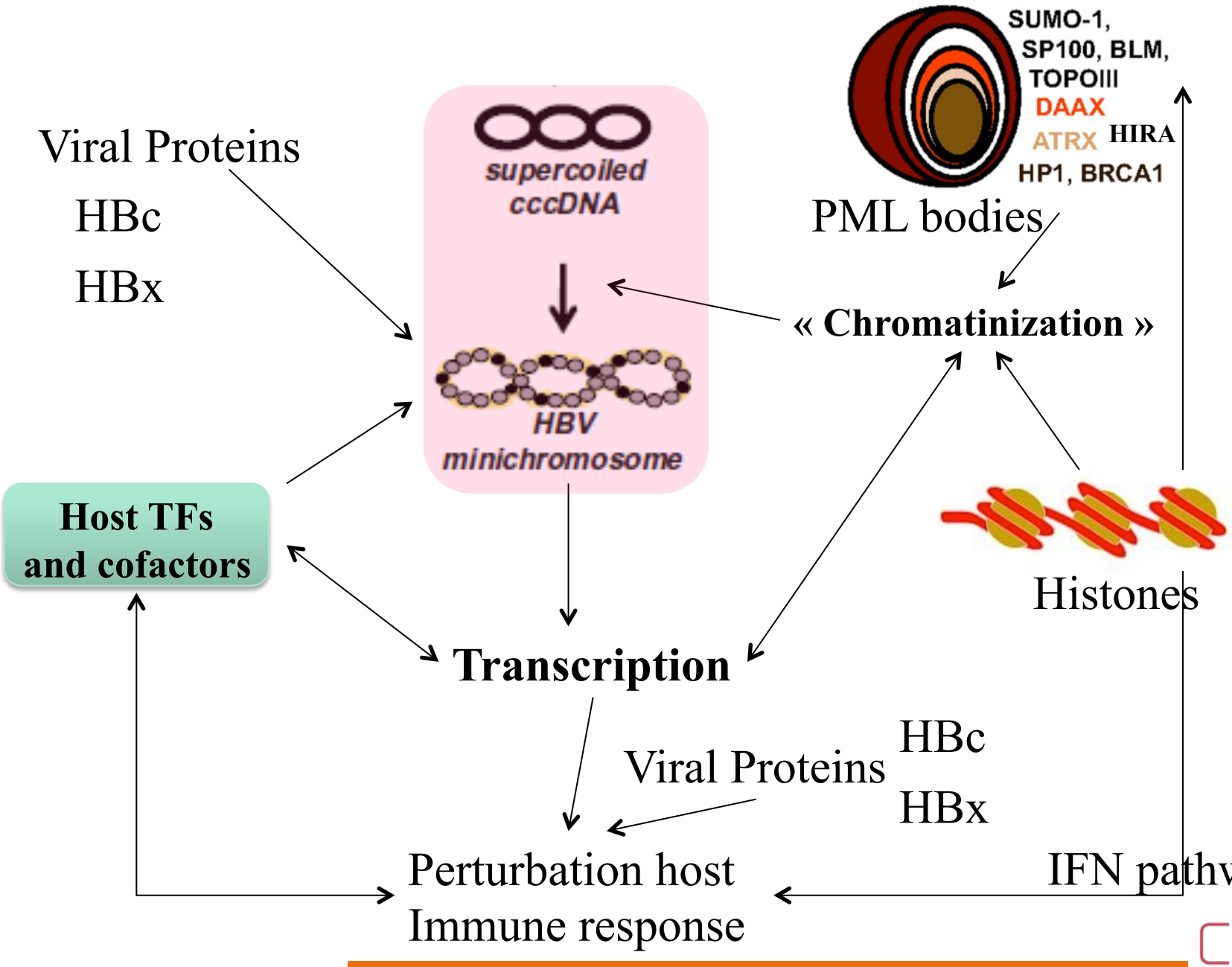


NO EFFECT OF NUCs

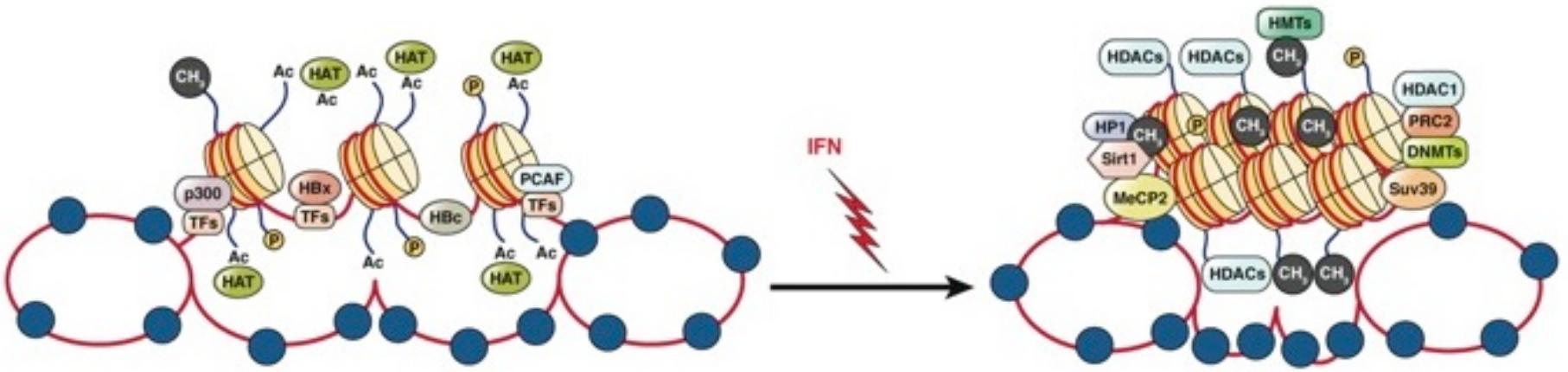
Viral polymerase
DNA repair enzymes
(TDP2 and others)
Exonucleases
Ligase
Other cellular enzymes?

Tuttleman et al Cell 1986
Delmas et al AAC 2002
Cortes Ledesma et al Nature 2009
Sohn et al, Plos One 2009
Boeck et al Plos Pathogen 2010

Host/viral factor involved in cccDNA chromatinization?



Epigenetic control of cccDNA
Use of epidrugs ?



✓ IFN-α treatment
✓ Spontaneous during immunosuppression

HIGH-REPLICATIVE STATE ⇄ **LOW-REPLICATIVE STATE**

✓ HBx
✓ HBc ?

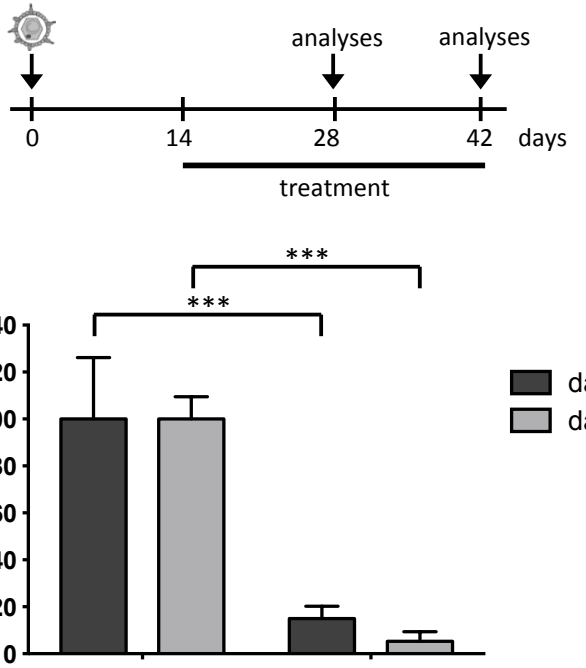
➢Epigenetic regulation:

- Histones acetylases, deacetylases, methyltransferases
- Transcription factors
- Binding of viral proteins: HBc & HBx

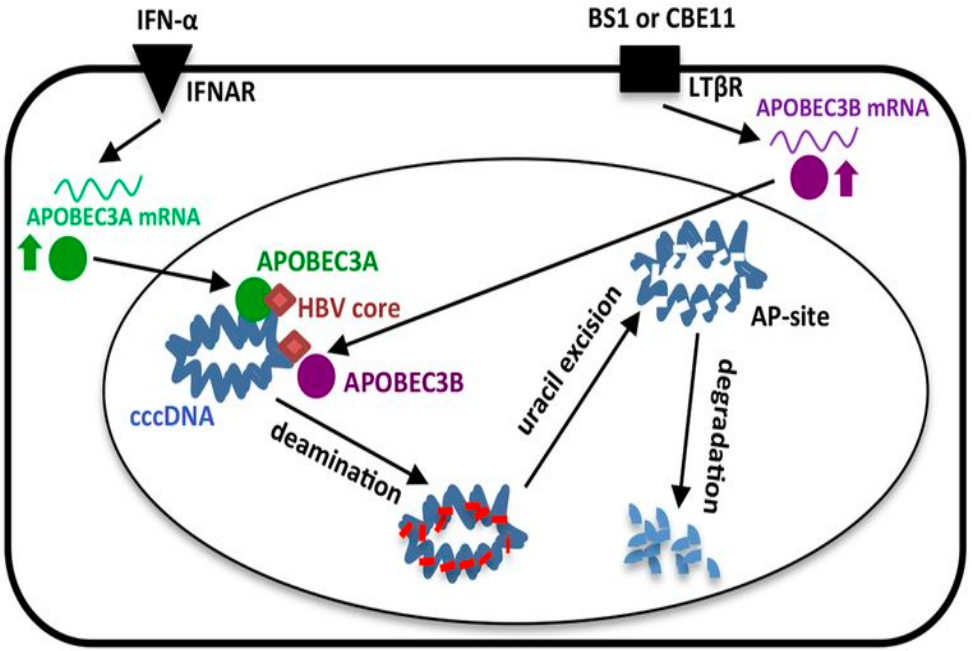
Belloni et al., JCI 2012
Pollicino et al. Gastroenteroplogy 2006
Levrero et al. J Hepatol, 2009
Lucifora et al, J Hepatol 2011

cccDNA degradation via induction of « intracellular » effectors

- IFN-α /Lymphotoxin-β can induce **APOBEC3A/B dependent degradation** of HBV cccDNA



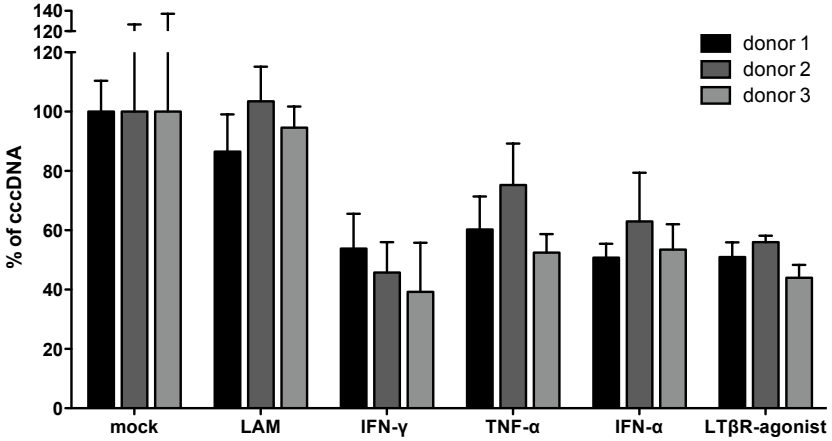
95% degradation of cccDNA
in HBV-infected HepaRG cells



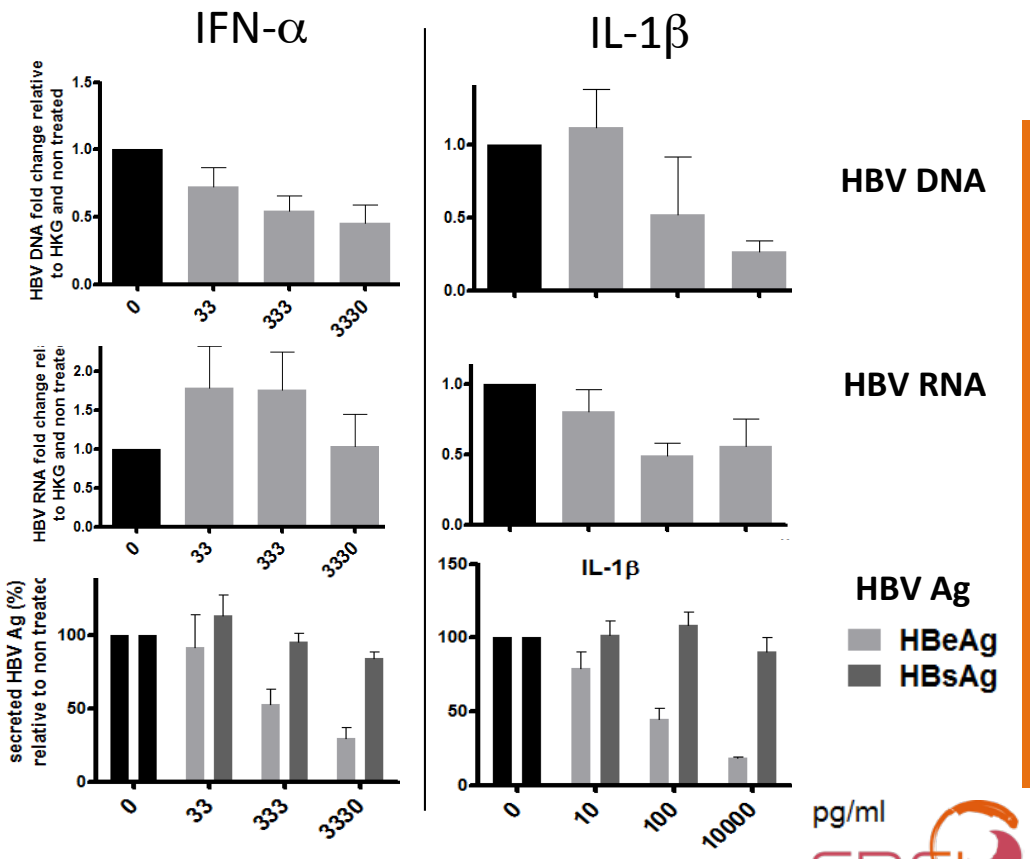
Lucifora et al, Science 2014

cccDNA degradation via induction of « intra-cellular » effectors: an ubiquitous mechanisms ?

Effect of various cytokines on cccDNA in HBV infected PHH cells

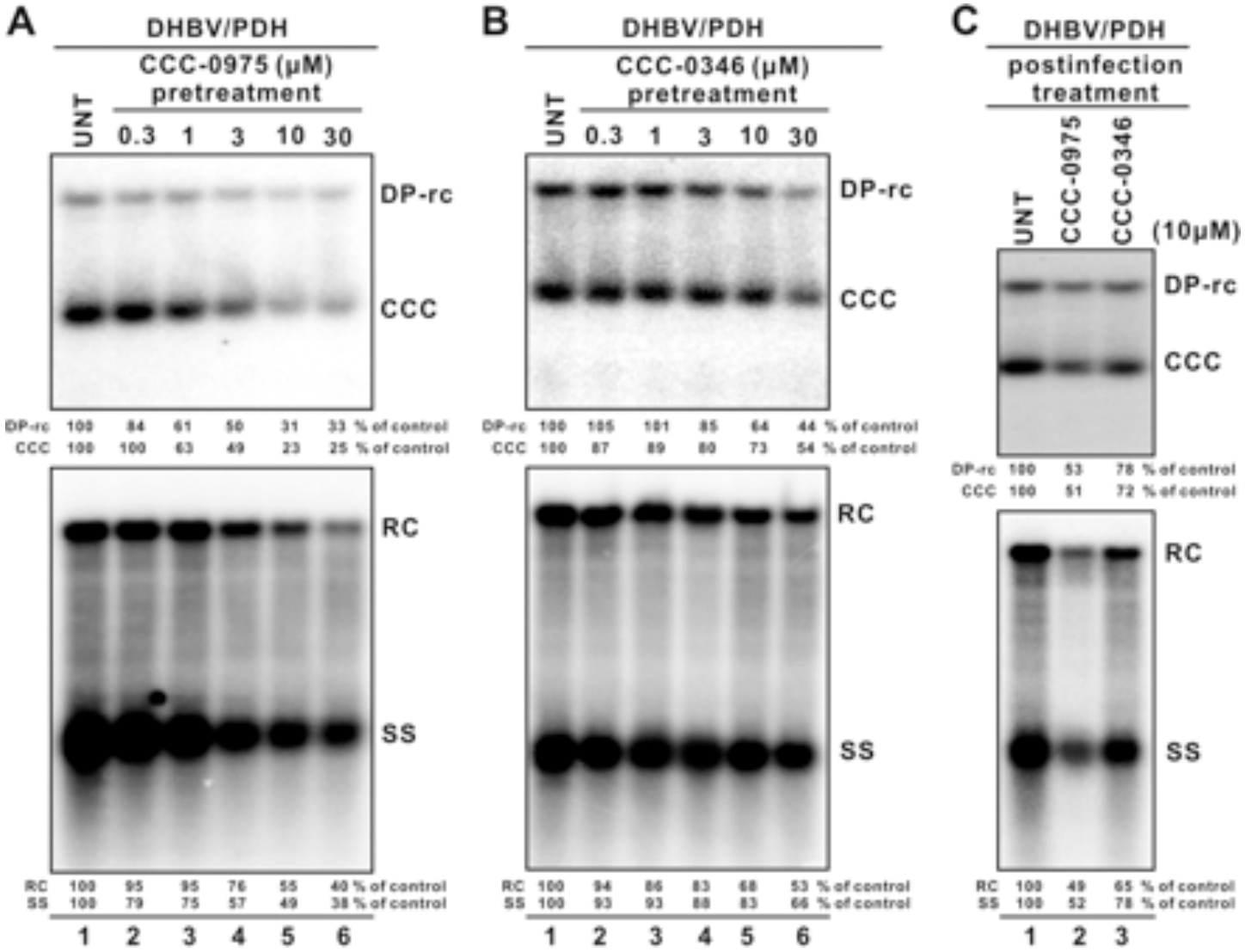


Antiviral properties of IL1-b and IFN-a in the model of HBV infected HepaRG cells

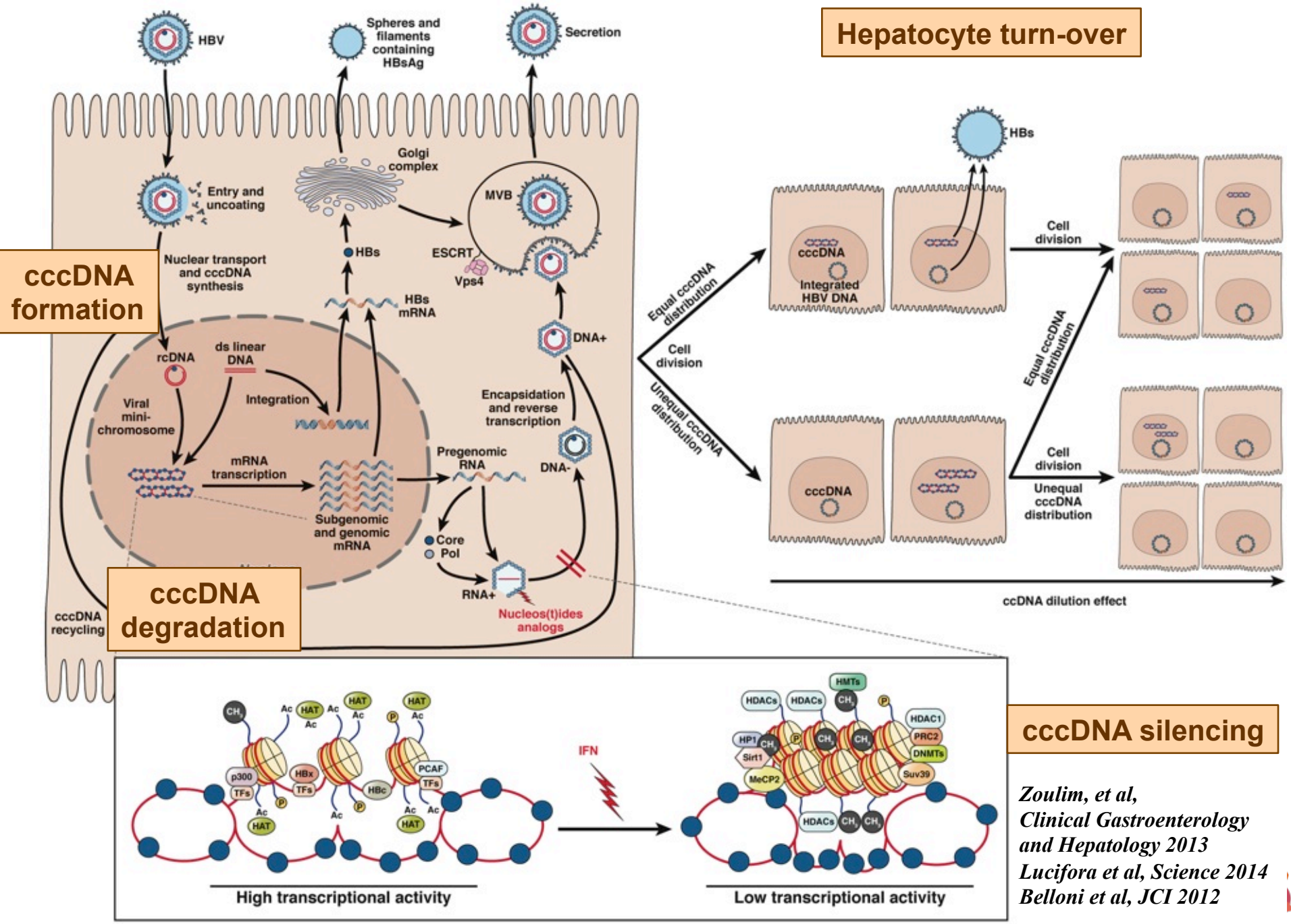


Xia et al., submitted (Protzer's lab)
Isorce et al., in prep.

Inhibition of cccDNA formation with sulfonamide derivatives

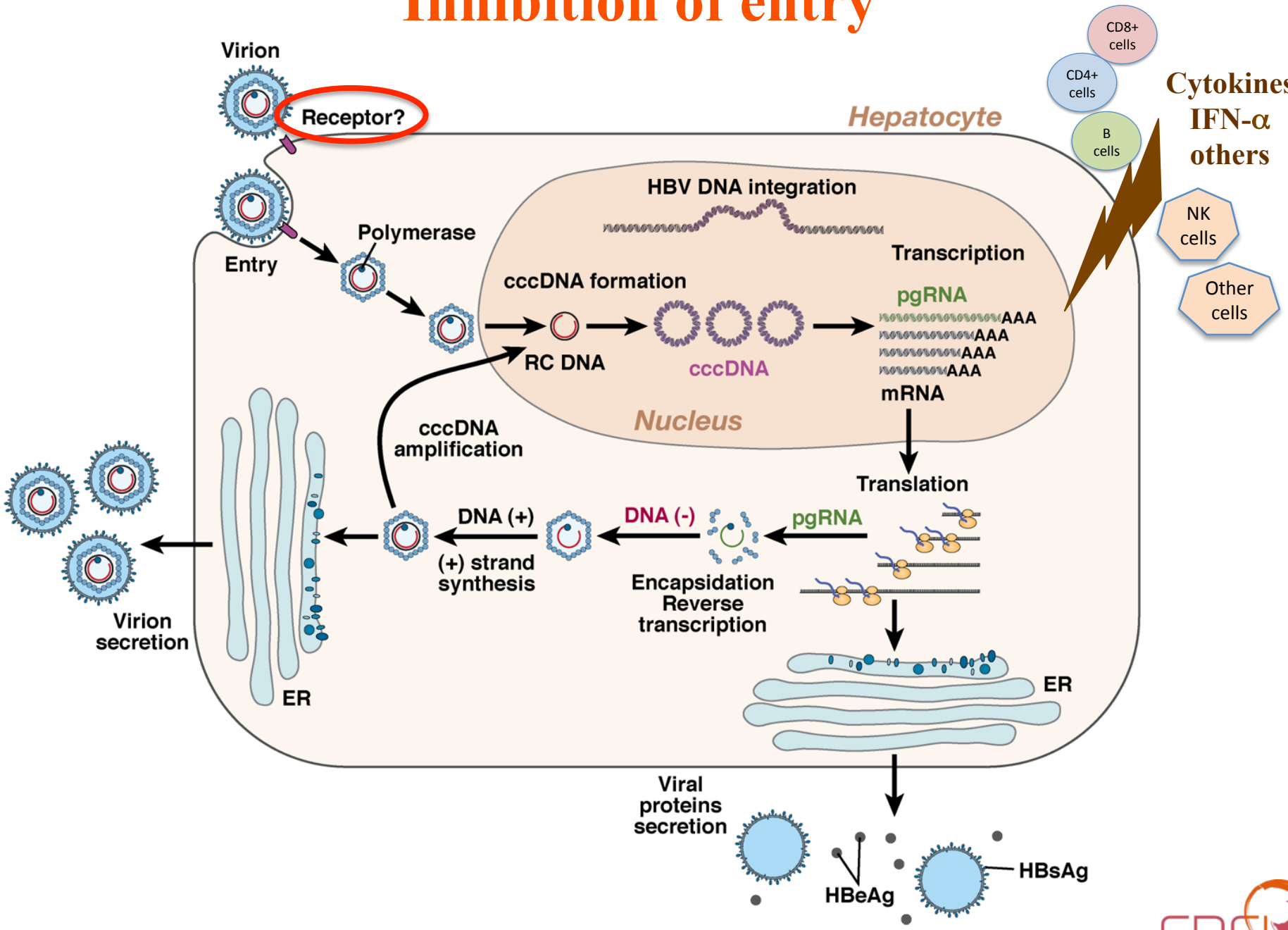


Cai et al, Antimicrob Agents Chemother. 2012



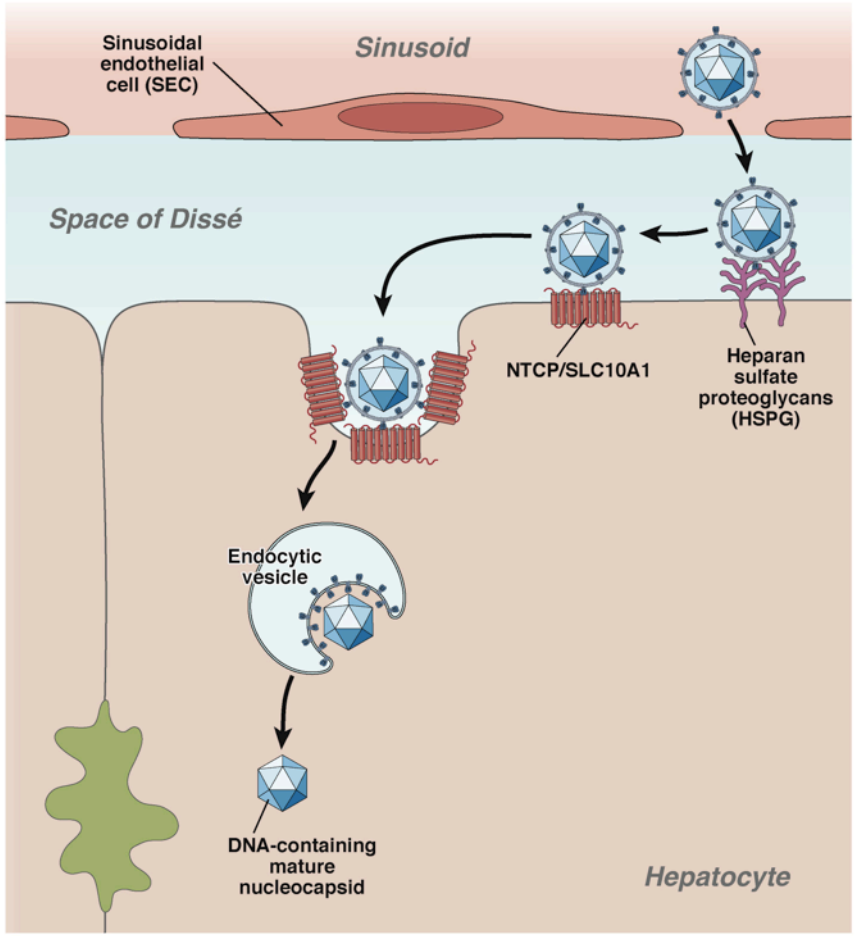
Zoulim, et al, Clinical Gastroenterology and Hepatology 2013
Lucifora et al, Science 2014
Belloni et al, JCI 2012

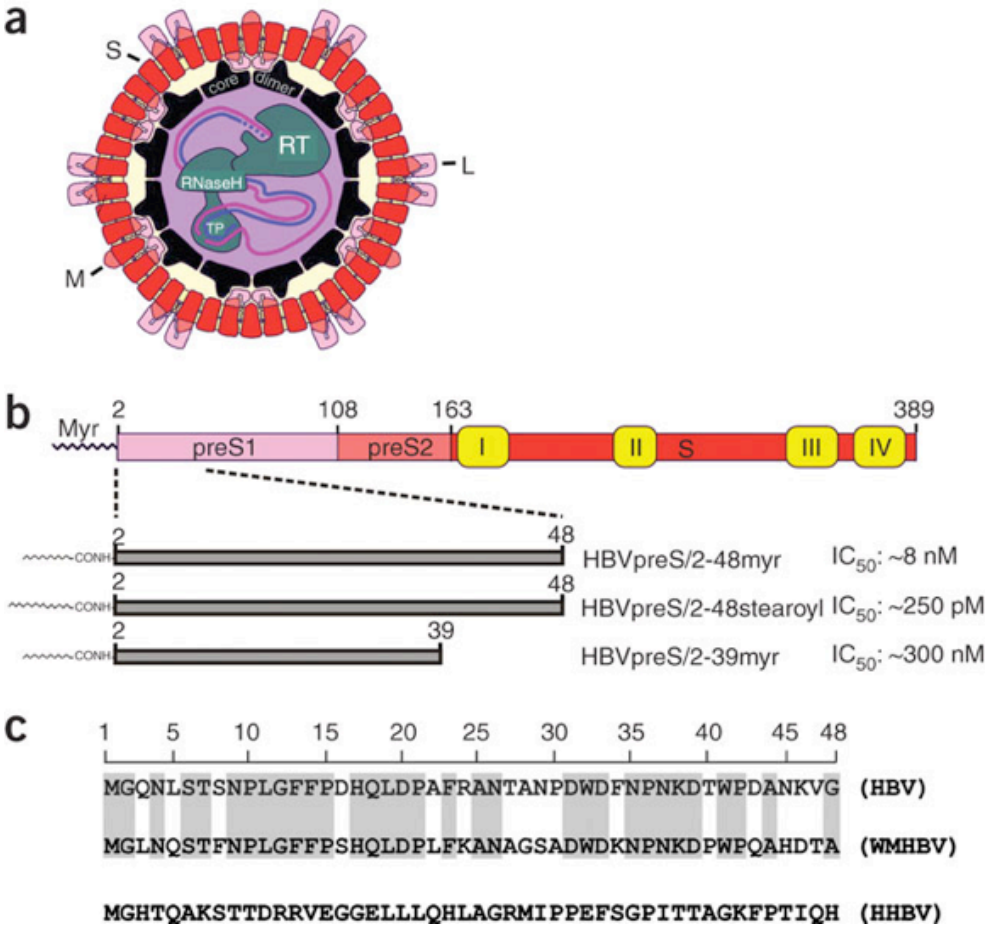
Inhibition of entry



The current model of HBV entry in hepatocyte

HBV/NTCP interaction as a target





Petersen et al, Nature Biotechnol 2008
Urban et al, Gastroenterology 2014

Myrcludex and co…

Table 1. Overview of HBV entry inhibitors including their latest clinical status

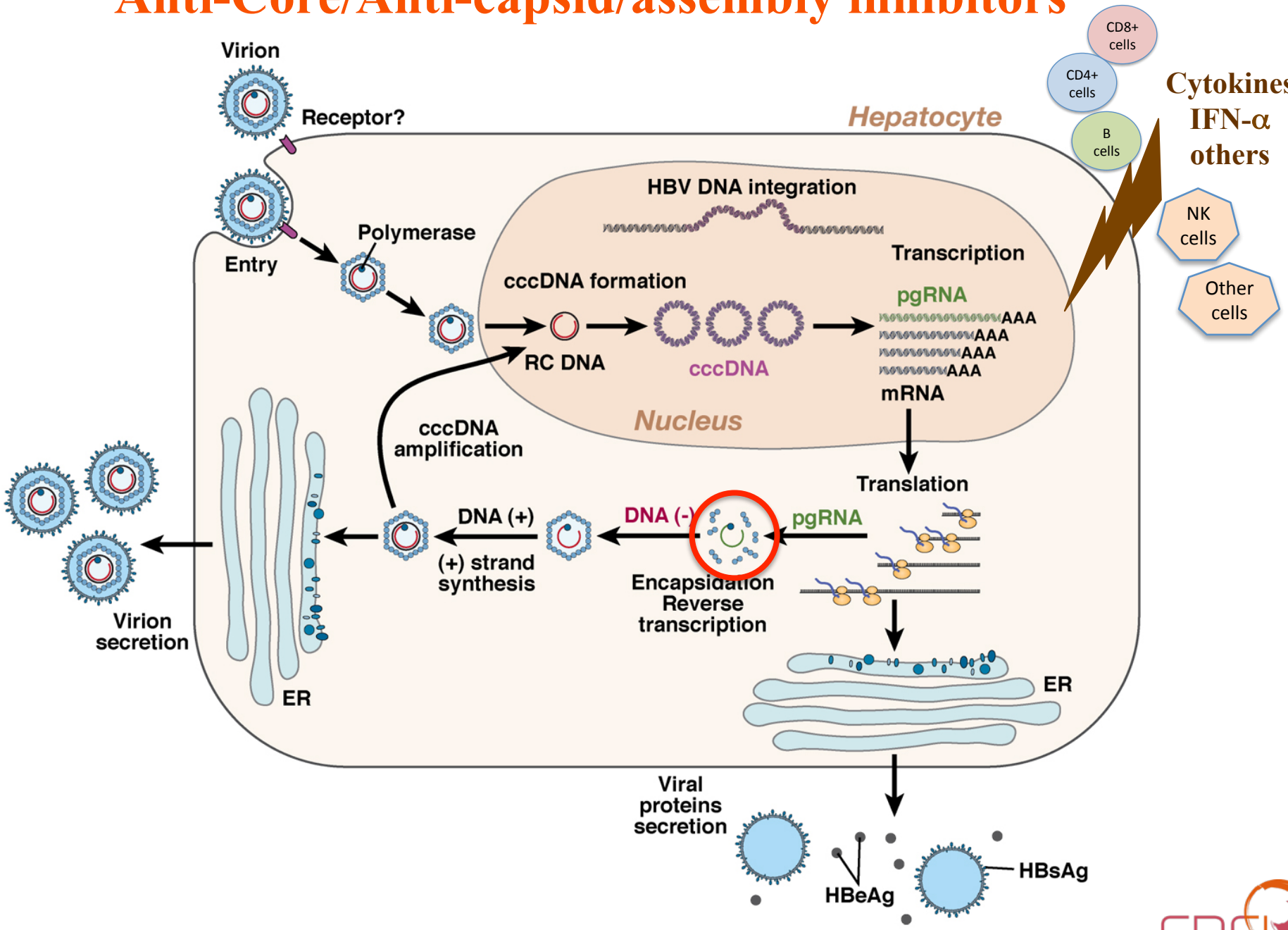
Class	Substance	Molecule	Target	Clinical status	IC50 HBV inhibition‡	Patients plasma concentration†	Reference
Antibodies	HBIG	Polyclonal Ab	S	Approved			Samuel, 1993 [7]
	Ma18/7	Monoclonal Ab	preS1	Preclinical			Heermann, 1984 [16]
	KR127	Monoclonal Ab	preS1	Preclinical			Hong, 2004 [18]
	17.1.41/19.79.5	Monoclonal Abs	S	Phase I			Galun, 2002 [15]
Attachment inhibitors	Heparin	Glycosaminoglycan	S, M, L	Approved*	9.4 μg/ml	~5 μg/ml	Schulze, 2007 [3]
	Suramin	Small molecule	S, M, L	Approved*	25 μM	50 μM	Petcu, 1988 [21]
	SALP	Peptide	HSPG	Preclinical	0.37 μM	n/a	Krepstakies, 2012 [26]
NTCP inhibitors	Myrcludex B	Peptide	NTCP	Phase II	80 pM	n/a	Schulze, 2010 [33]
	Cyclosporin A	Cyclic peptide	NTCP	Approved*	1 μM	0.7 μM	Nkongolo, 2013 [41]
	SCYX1454139	CsA-derivate	NTCP	Preclinical	0.17 μM	n/a	Watashi, 2013 [43]
	Ezetimibe	Small molecule	NTCP/NPC1L1	Approved*	18 μM	0.2 μM	Lucifora, 2013 [46]
Bile salts	e.g. Taurocholic acid	Small molecule	NTCP	–	20–30 μM	2–4 μM	Ni, 2013 [5]

* Approved for indications other than HBV infection. ‡ As determined in vitro using HepaRG cells. † During therapy in the standard indication.

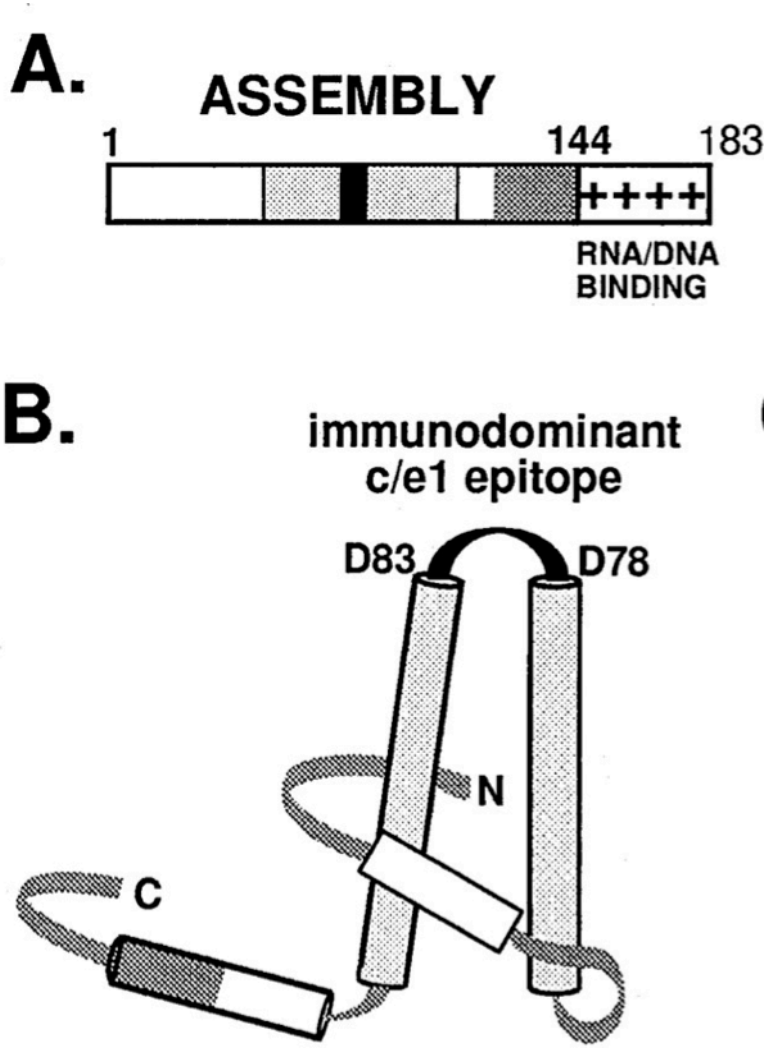
- ✓ **Entry inhibitors useful to prevent re-infection of graft**
- ✓ **Usefulnesss in the context of chronic infection to be proven…**
  - **Involvement of hepatocytes turn over**

Lempp & Urban, Intervirology 2014

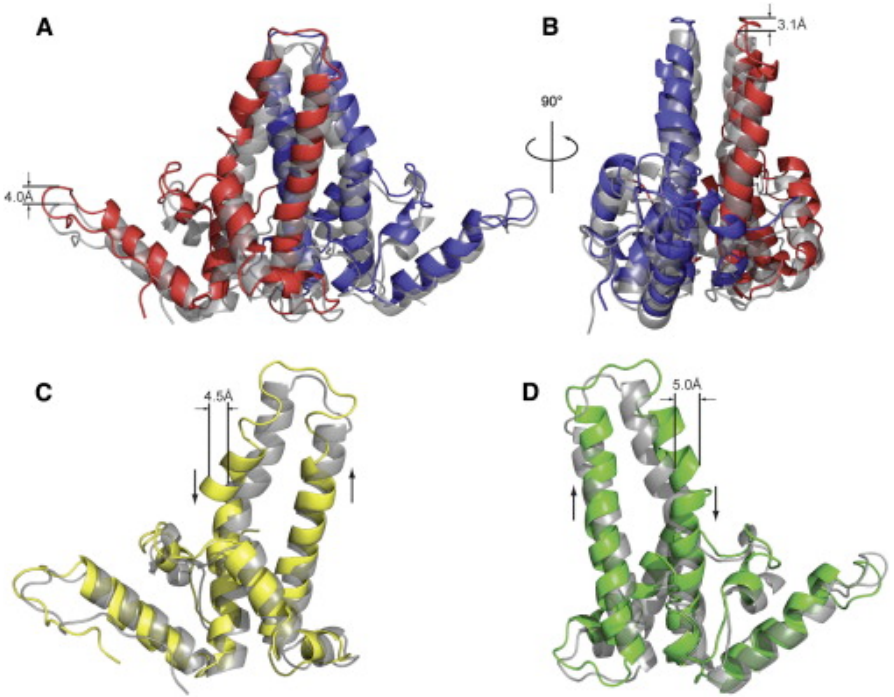
Anti-Core/Anti-capsid/assembly inhibitors



How HBc looks like



HBc1–149 dimer structure

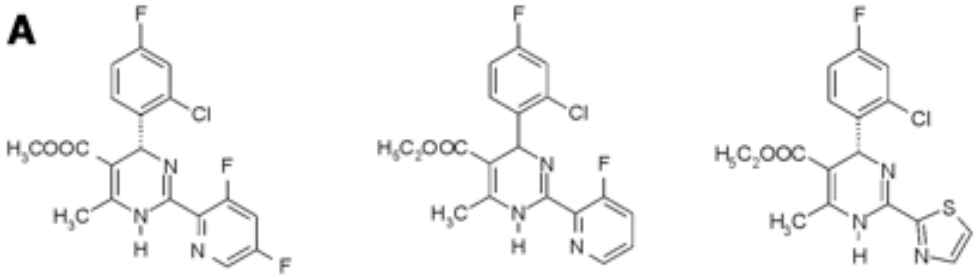


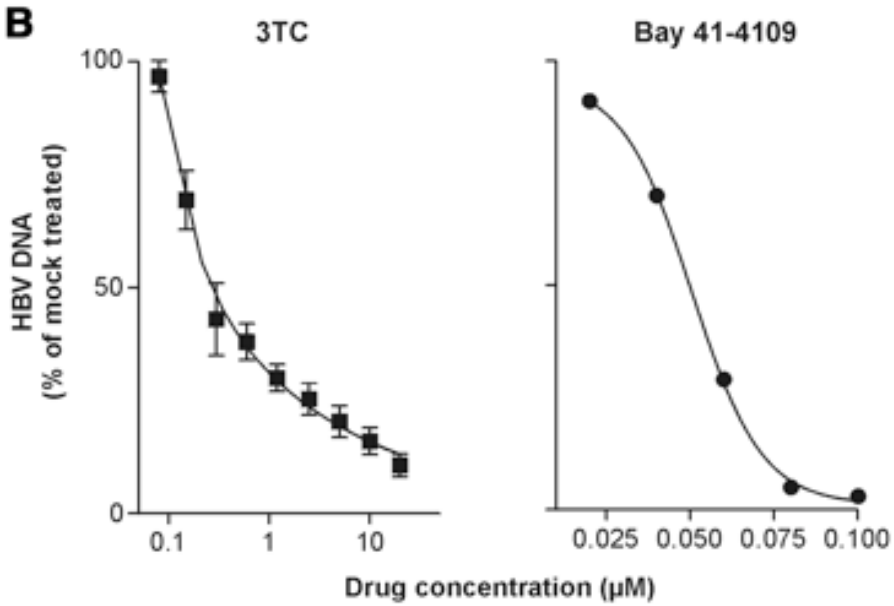
Katen et al., Structure 2013
Alexander et al, PNAS 2013
Kratz et al., PNAS 1999

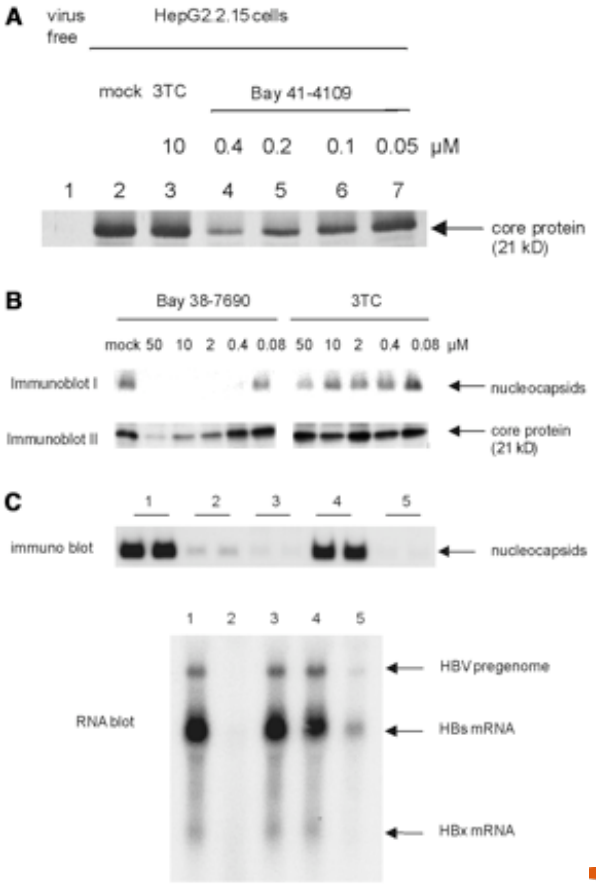
HAP inhibitors

Heteroaryl-dihydropyrimidines

Destabilization of nucleocapsids

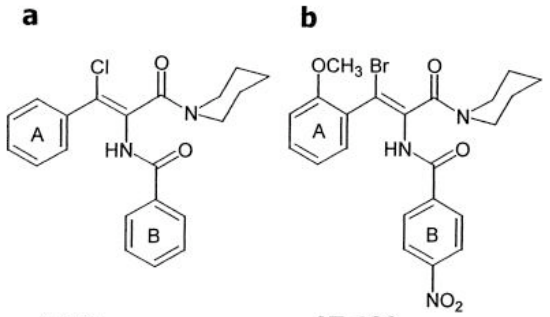






Deres et al, Science 2003

Phenylpropenamide derivatives

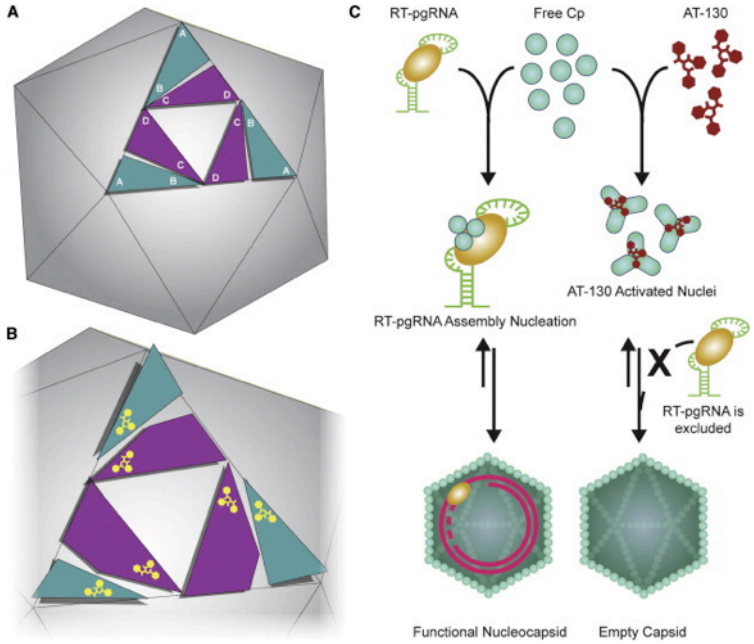


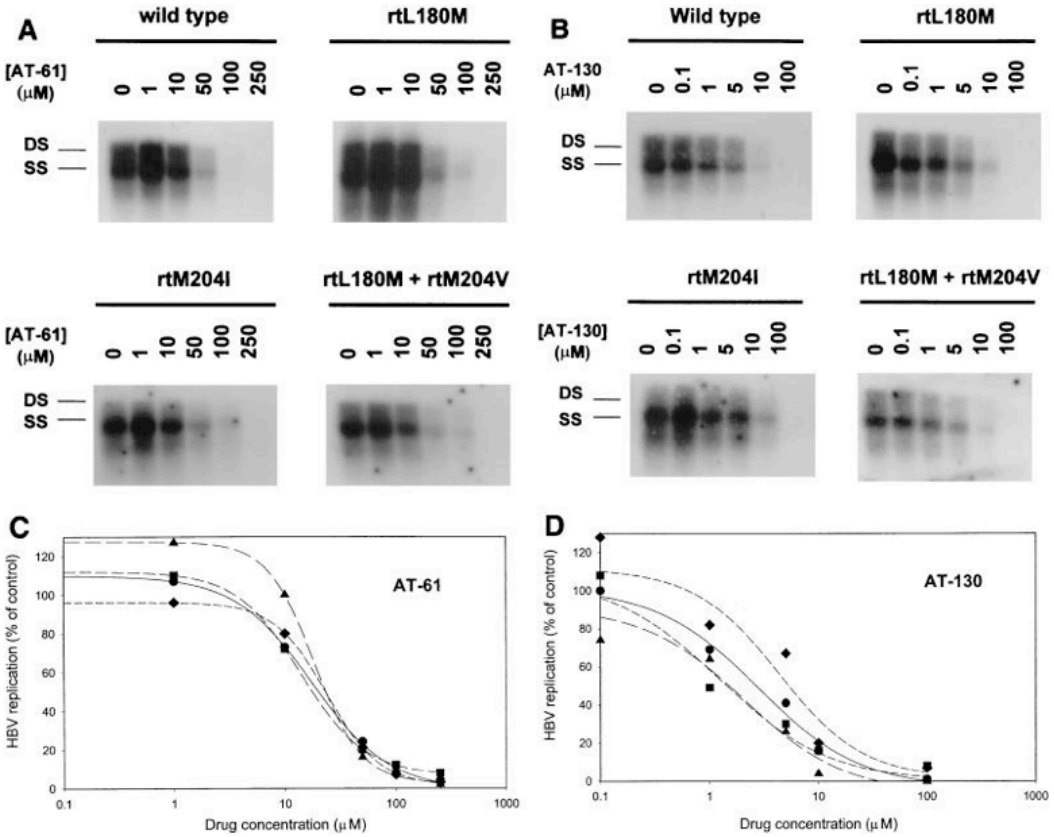
AT-61
$C_{21}H_{21}ClN_2O_2$
Mol. Wt.: 368.86

AT-130
$C_{22}H_{22}BrN_3O_5$
Mol. Wt.: 488.33

AT-like compounds

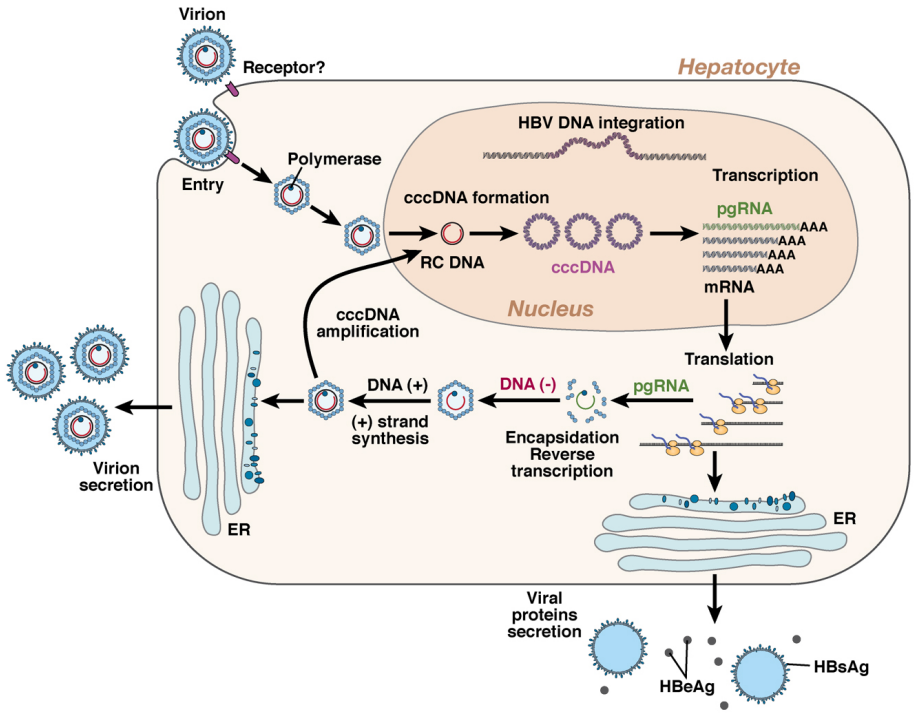
Prevent pgRNA encapsidation





Antimicrob Agents Chemother. 2002 Sep;46(9):3057-60
Katen et al., Structure 2013

Anti-capsids: more than assembly inhibitors?



Nucleocapsid assembly pgRNA packaging → Viral DNA synthesis

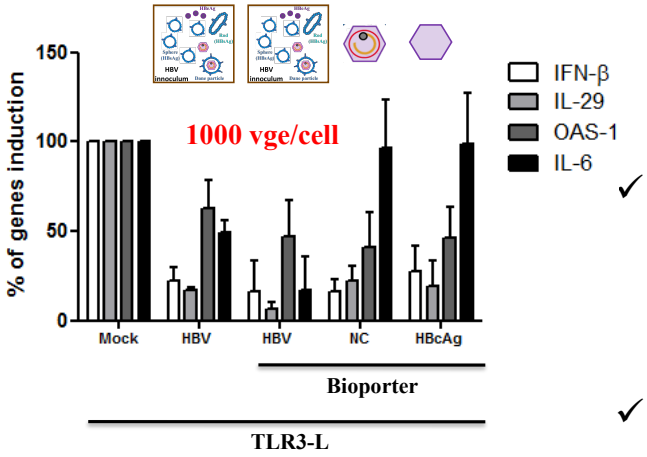
Cytoplamic HBc

HBV core

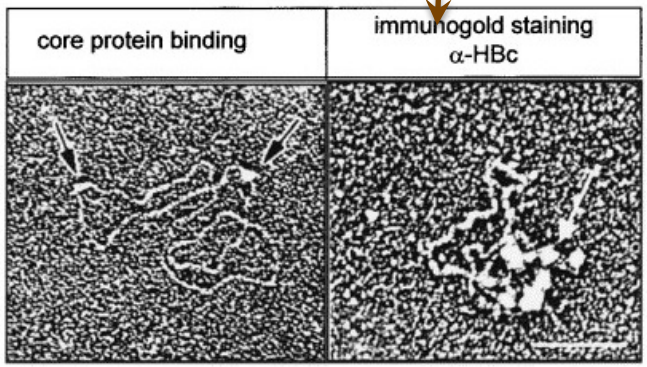
Nuclear HBc

Activation of cccDNA transcription ←→ Stability?

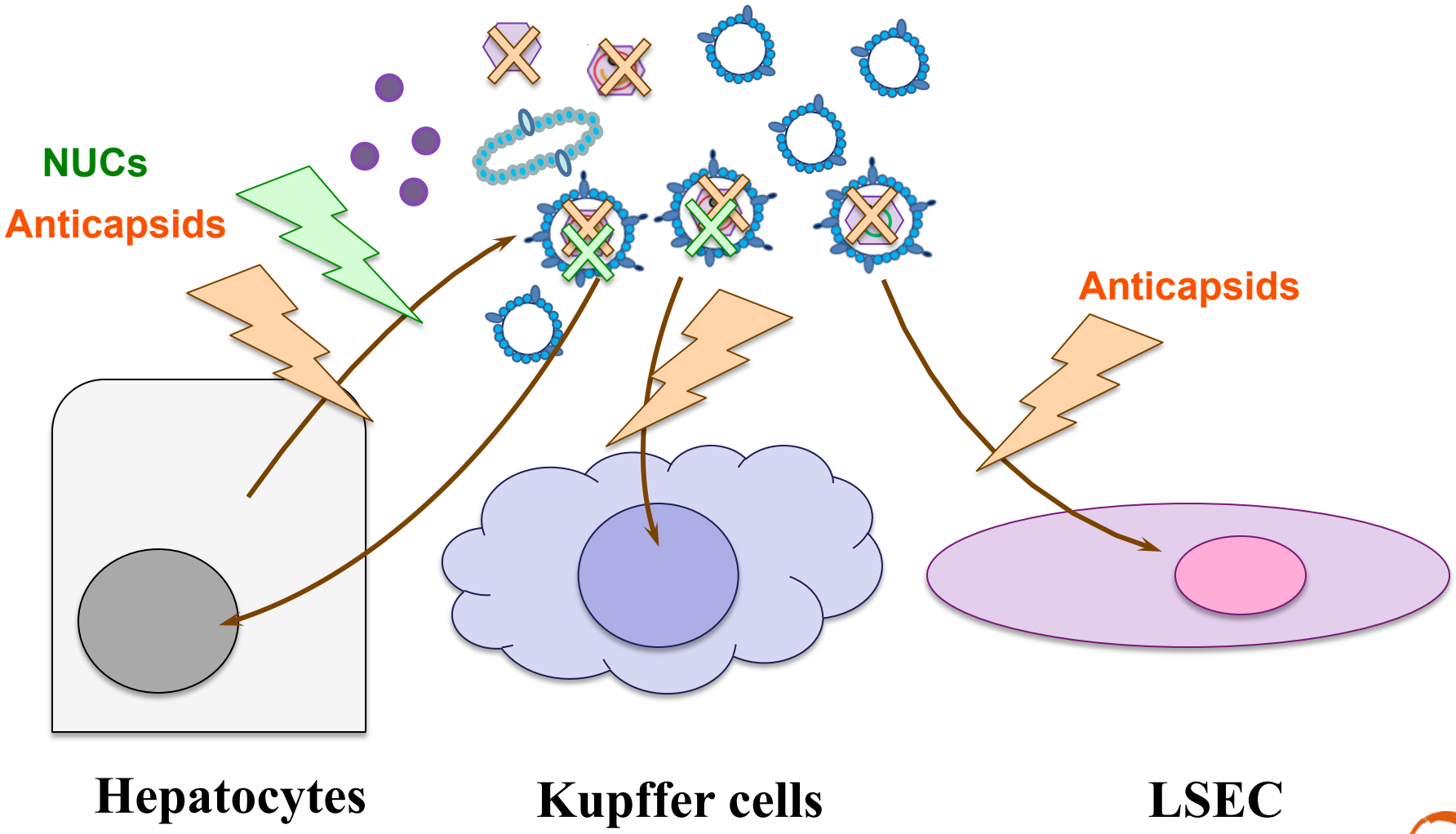
Recycling of nucleocapsid Amplification of cccDNA



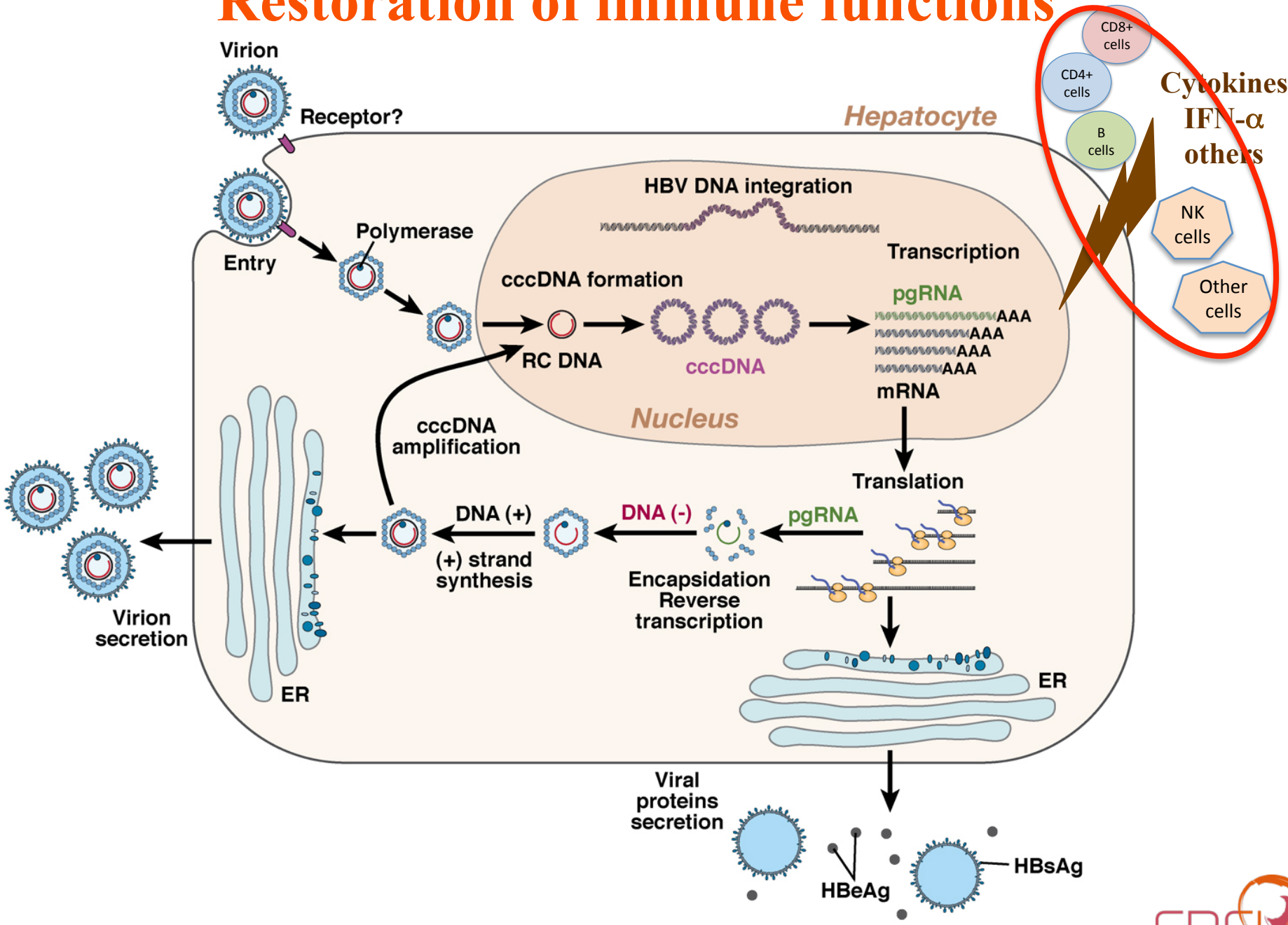
- ✓ Repression of IFNs and ISG expression in hepatocytes and other cells from livermicroenvironment
- ✓ Activation of genes involved in carcinogenesis



Could anticapsids also inhibit HBc function in other cell types?



Restoration of immune functions

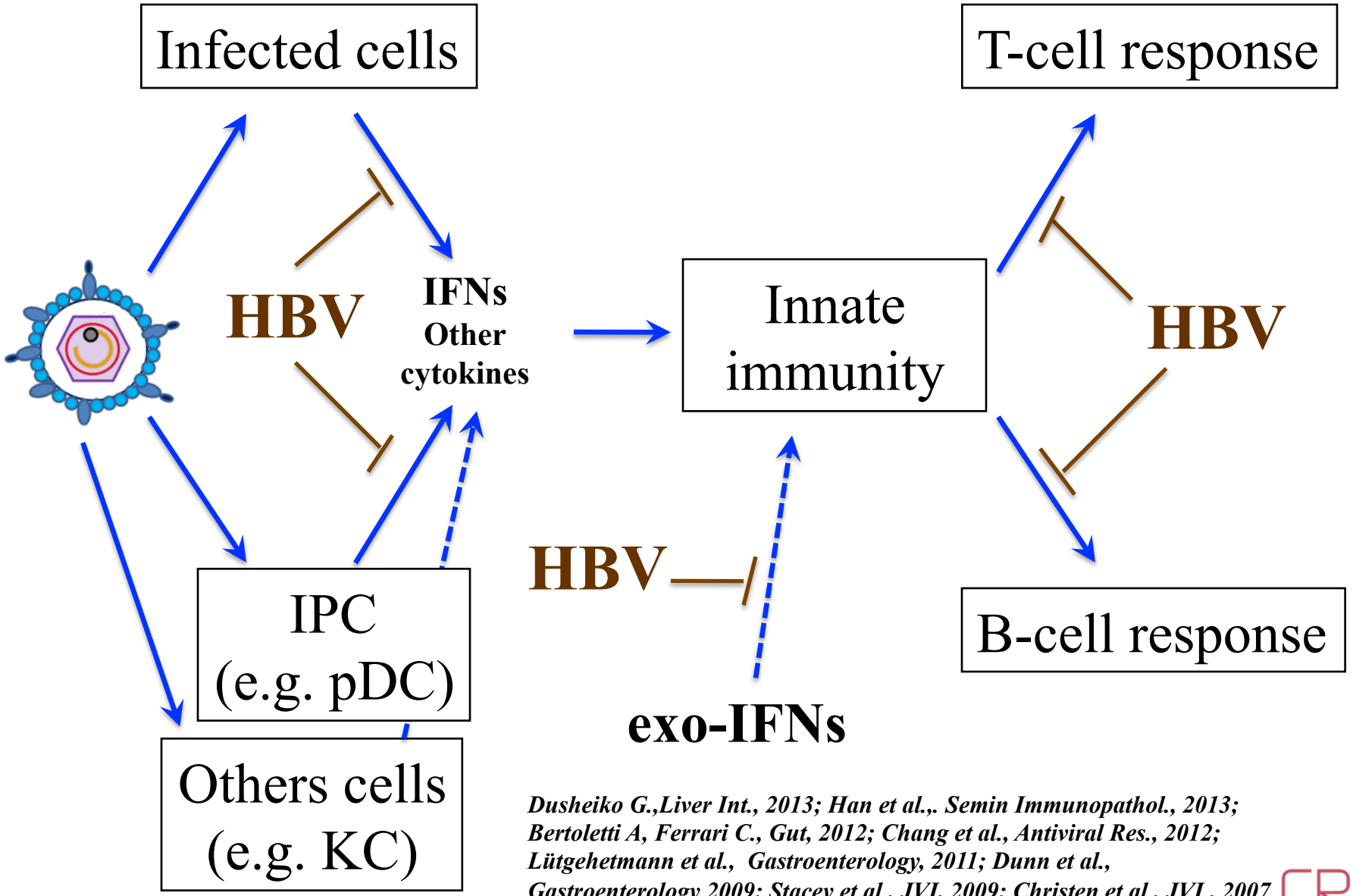


Restoration of immune functions

- **Innate functions**
  - **Stimulation of innate immune cells**
  - **Direct effect on HBV infected haptocytes**

- **Adaptive functions**
  - **Via innate functions restoration**
  - **Direct modulation of inhibitory mechanisms**
  - **Global activation by therapeutic vaccination**

HBV inhibit immune responses
Secreted antigens (HBs, Hbe, HBc) responsible?



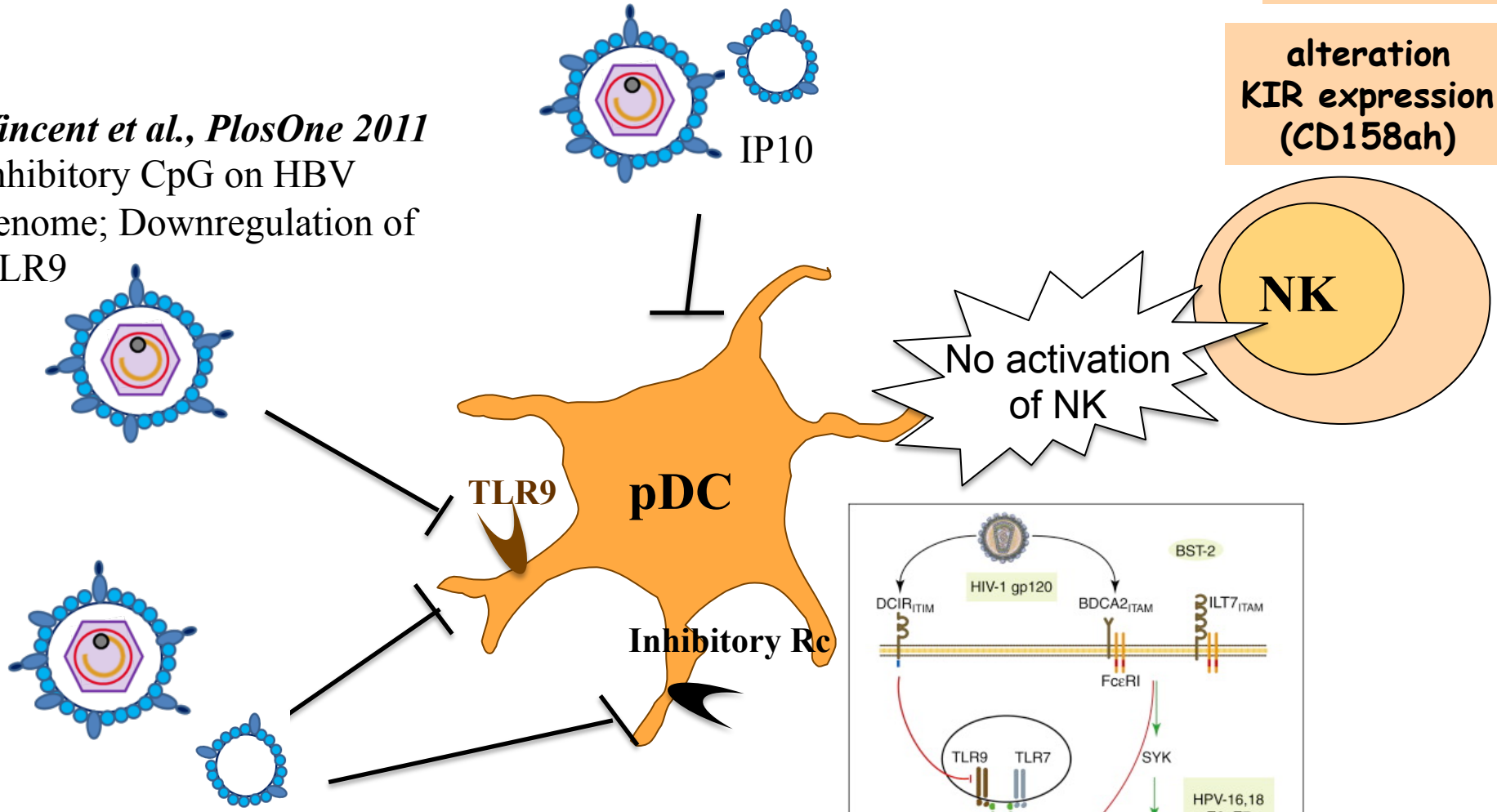
Dusheiko G.,Liver Int., 2013; Han et al.,. Semin Immunopathol., 2013; Bertoletti A, Ferrari C., Gut, 2012; Chang et al., Antiviral Res., 2012; Lütgehetmann et al., Gastroenterology, 2011; Dunn et al., Gastroenterology 2009; Stacey et al., JVI, 2009; Christen et al., JVI., 2007

Martinet et al., Gastro 2013
Downregulation CXCR3 OX40L
and IFNa (other cytokines) prod.

alteration cytotoxicity (CD107,grB)

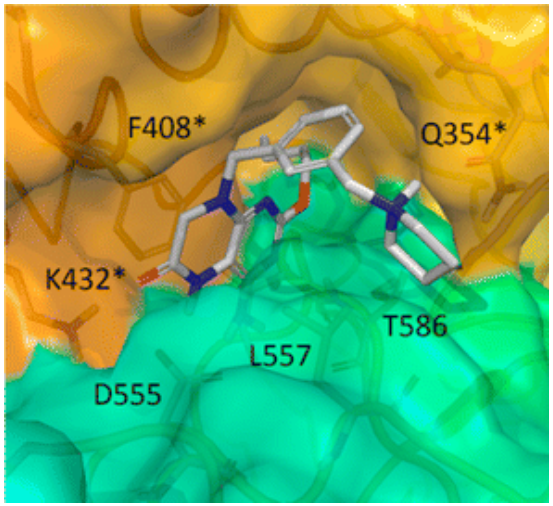
alteration KIR expression (CD158ah)

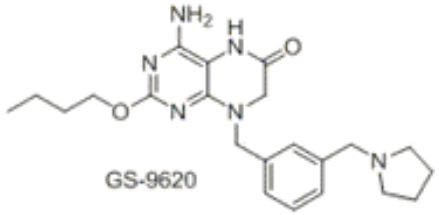
Vincent et al., PlosOne 2011
Inhibitory CpG on HBV genome; Downregulation of TLR9



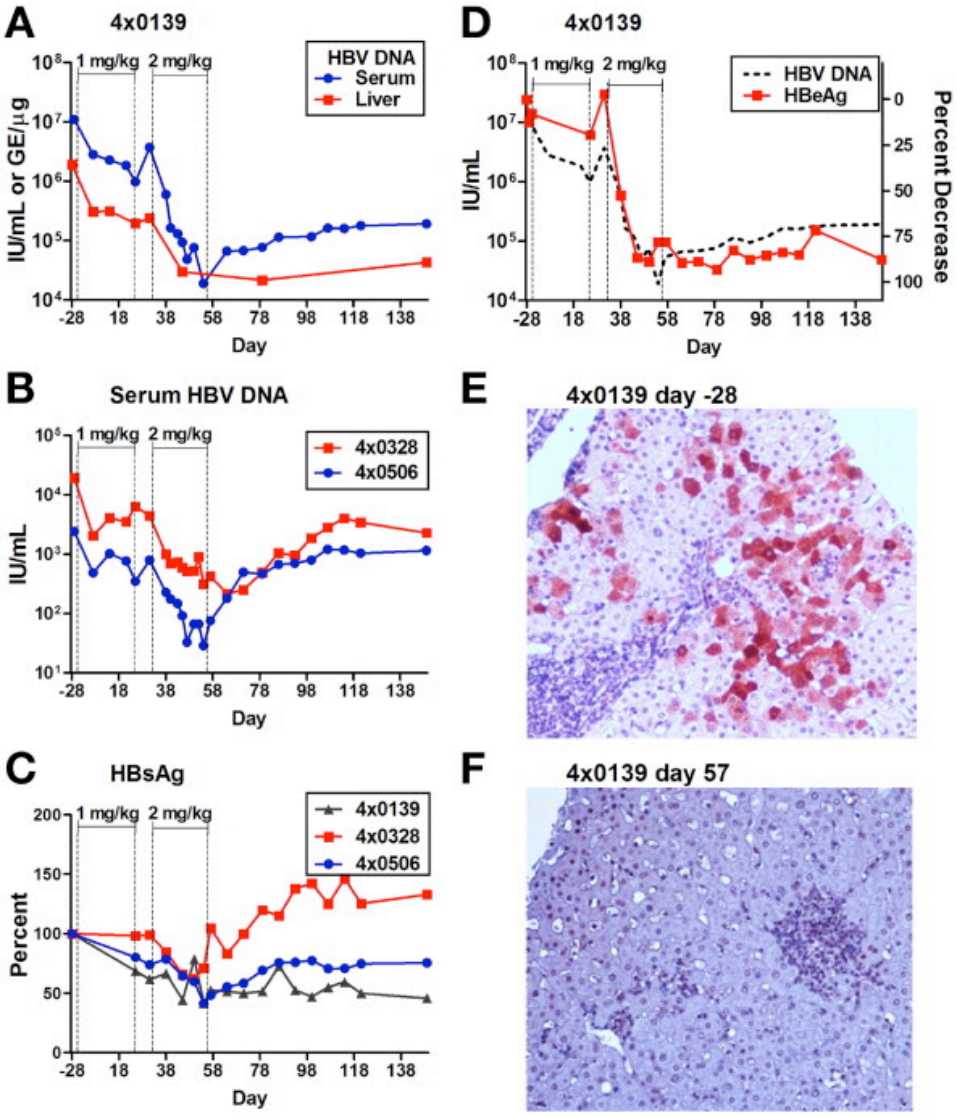
Woltman et al., PlosOne 2011
HBs (and HBe) inhibits TLR9 fonctions and IFN-α prod

Antiviral activity of a TLR7 agonist (GS-9620) in HBV infected chimpanzees



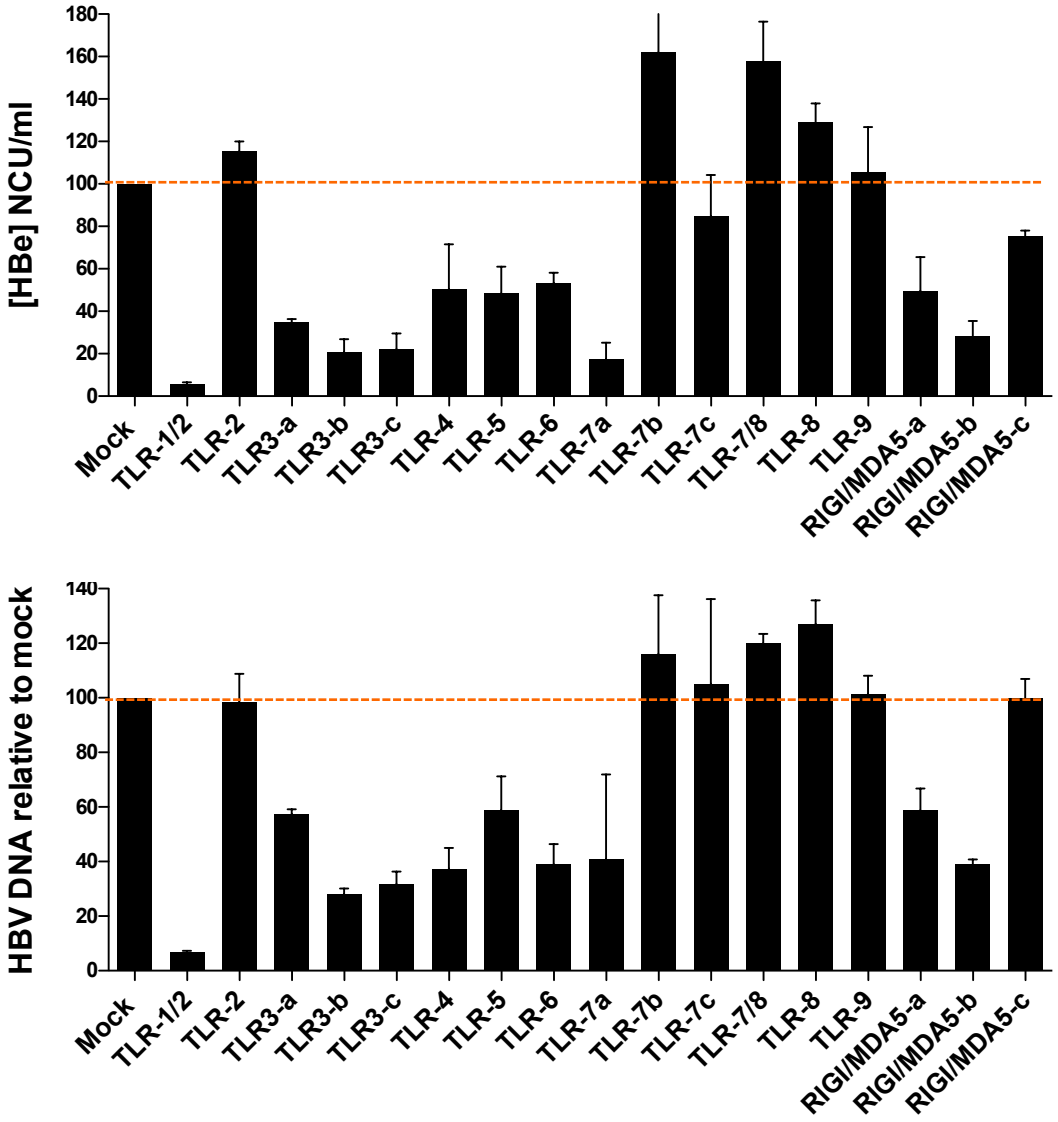


Safety studies in human also done and reported



Lanford et al, Gastroenterology 2013
Lawitz et al. Antivir Ther. 2014

Antiviral activity of other PRR agonists *in vitro*



TLR-7a = equi. of GS-9620

Floriot et al, in prep.

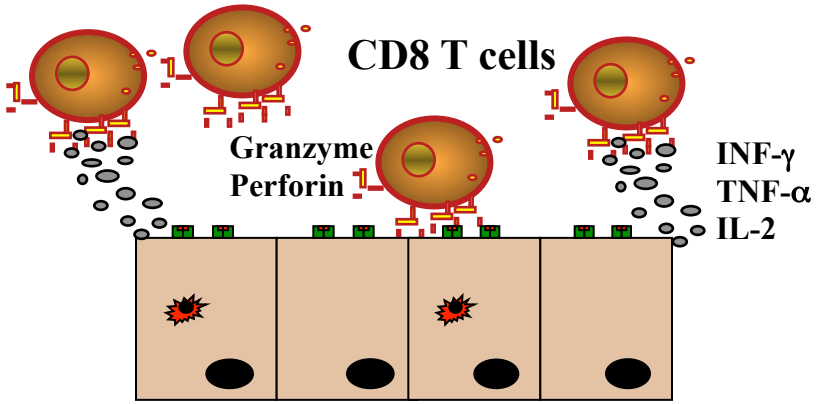
Restoration of immune function

- Innate functions
  - Stimulation of innate immune cells
  - Direct effect on HBV infected haptocytes

- **Adaptive functions**
  - **Via innate functions restoration**
  - **Direct modulation of inhibitory mechanisms**
  - **Global activation by therapeutic vaccination**

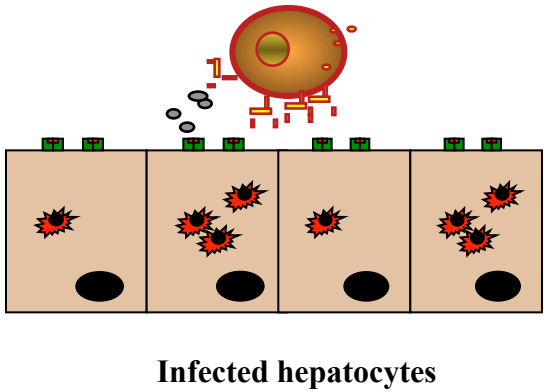
Restoration of defective T-cell immune control

Patients who have resolved HBV



Effective T-cells control virus

Patients with chronic HBV



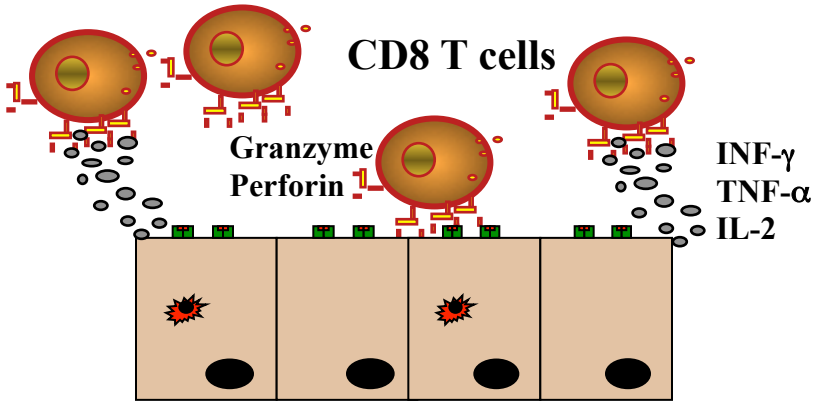
Exhausted T-cells lose control of virus

Can an effective antiviral T-cell response be recovered?

Figure adapted from Nebbia G, et al. Q J Med 2012;105:109–13 and Freeman G, et.al. J Exp Med 2006;203(10):2223–7

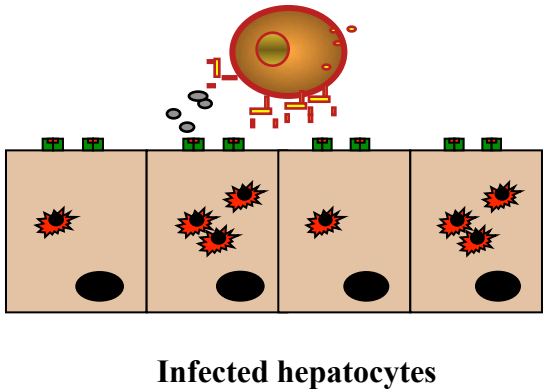
Restoration of defective T-cell immune control

Patients who have resolved HBV



Effective T-cells control virus

Patients with chronic HBV



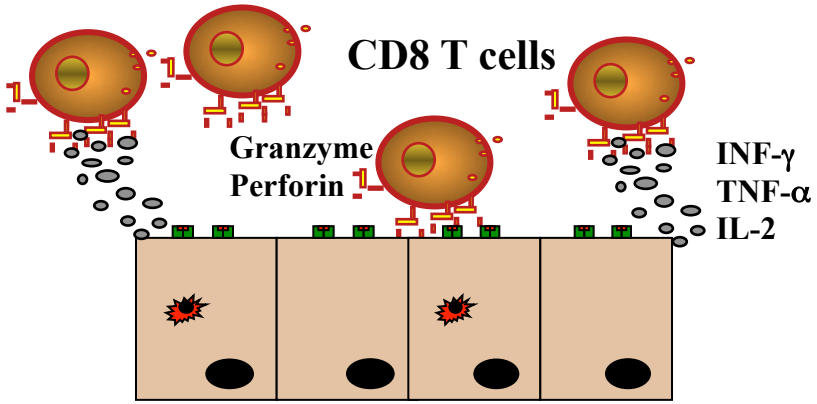
Exhausted T-cells lose control of virus

Prolonged nucleotide therapy in a subset of patients[1]

[1]Boni C, et al. Gastroenterology 2012;143:963–73

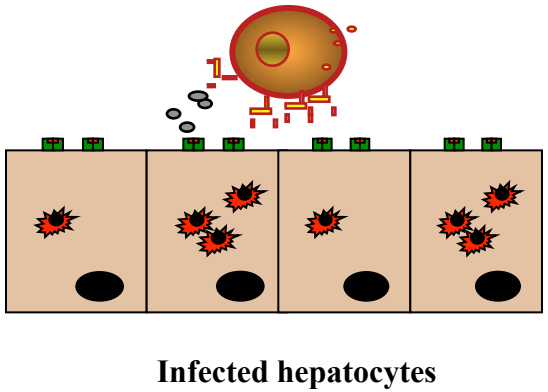
Restoration of defective T-cell immune control

Patients who have resolved HBV



Effective T-cells control virus

Patients with chronic HBV

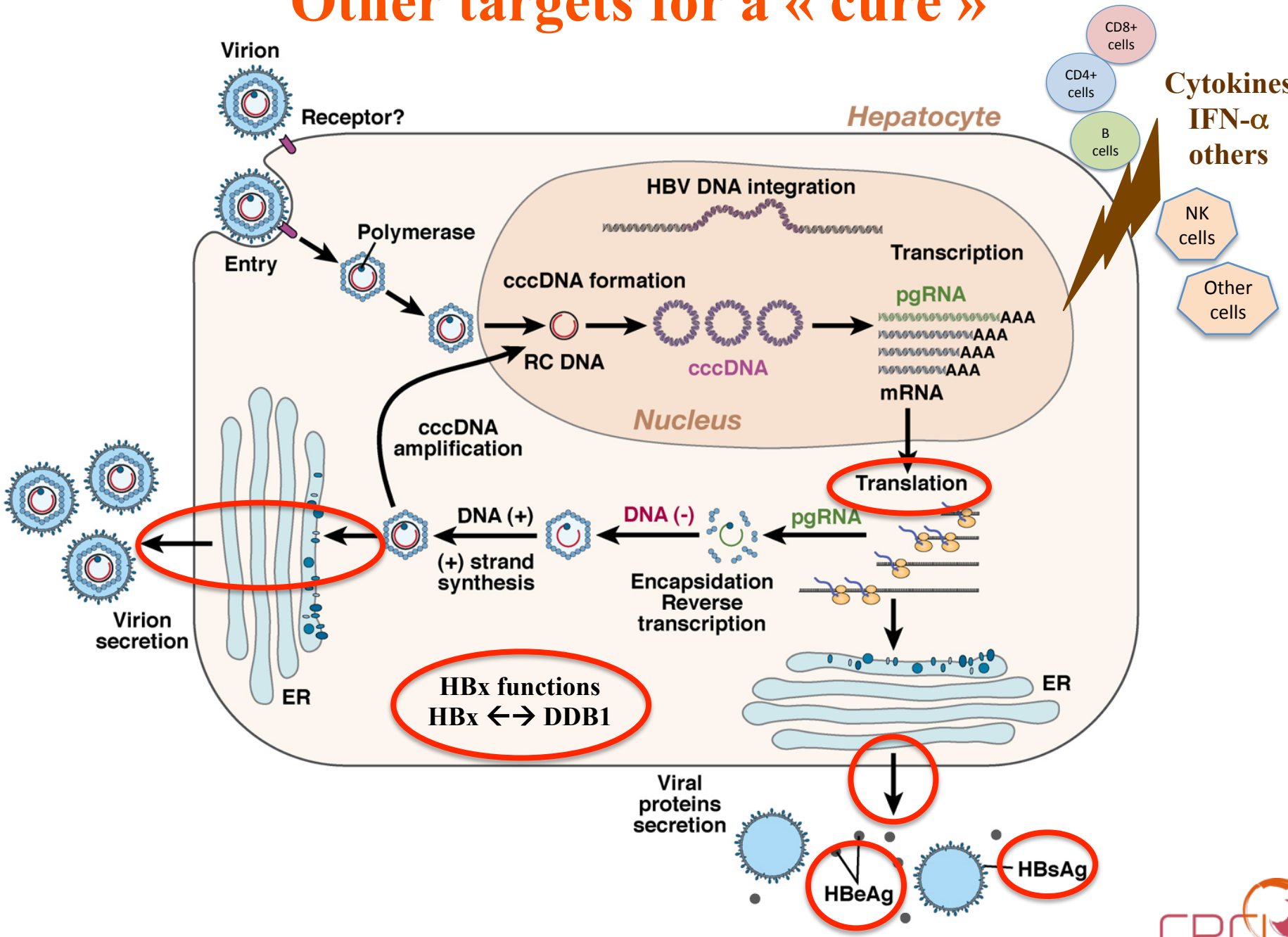


Exhausted T-cells lose control of virus

Specific immunomodulation of existing T-cells e.g. PD-1 blockade[1,2]

[1]Fisicaro P, et al. Gastroenterology 2010;138:682–93. [2]Fisicaro P, et al. Gastroenterology 2012;143:1576–85

Other targets for a « cure »



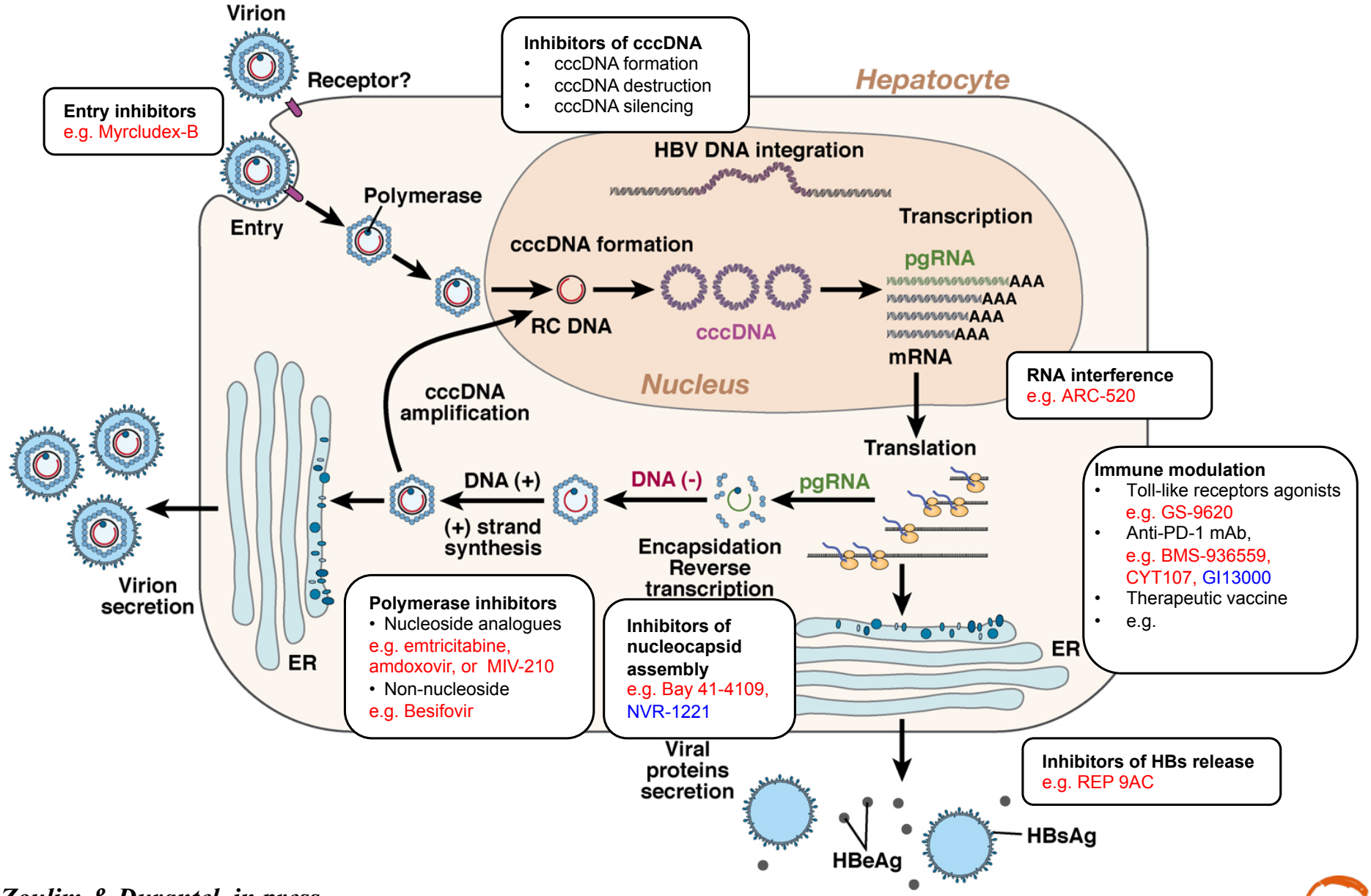
The concept of combination therapy

Viral targets → **Immune modulation**

- ✓ **Entry inhibition**
- ✓ **cccDNA**
  - **formation**
  - **stability / destruction**
  - **epigenetic regulation**
- ✓ **Viral core functions**
- ✓ **Other viral targets (HBx)**

- ✓ **Stimulating innate responses**
- ✓ **Specific ligands**
- ✓ **Stimulating adpative responses**
- ✓ **Co-inhibitory signals**
- ✓ **Co-stimulatory signals**
- ✓ **Therapeutic vaccination**

Functional cure / control
Real cure ?



Zoulim & Durantel, in press
HBF Drug Watch, available at: http://www.hepb.org/professionals/hbf_drug_watch.htm

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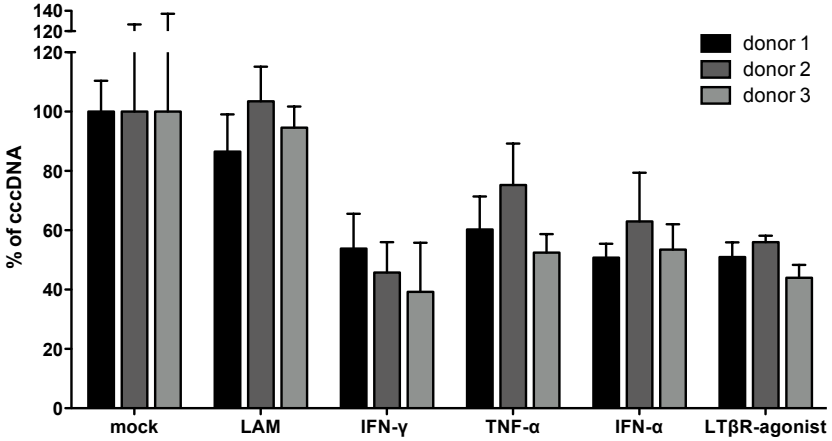
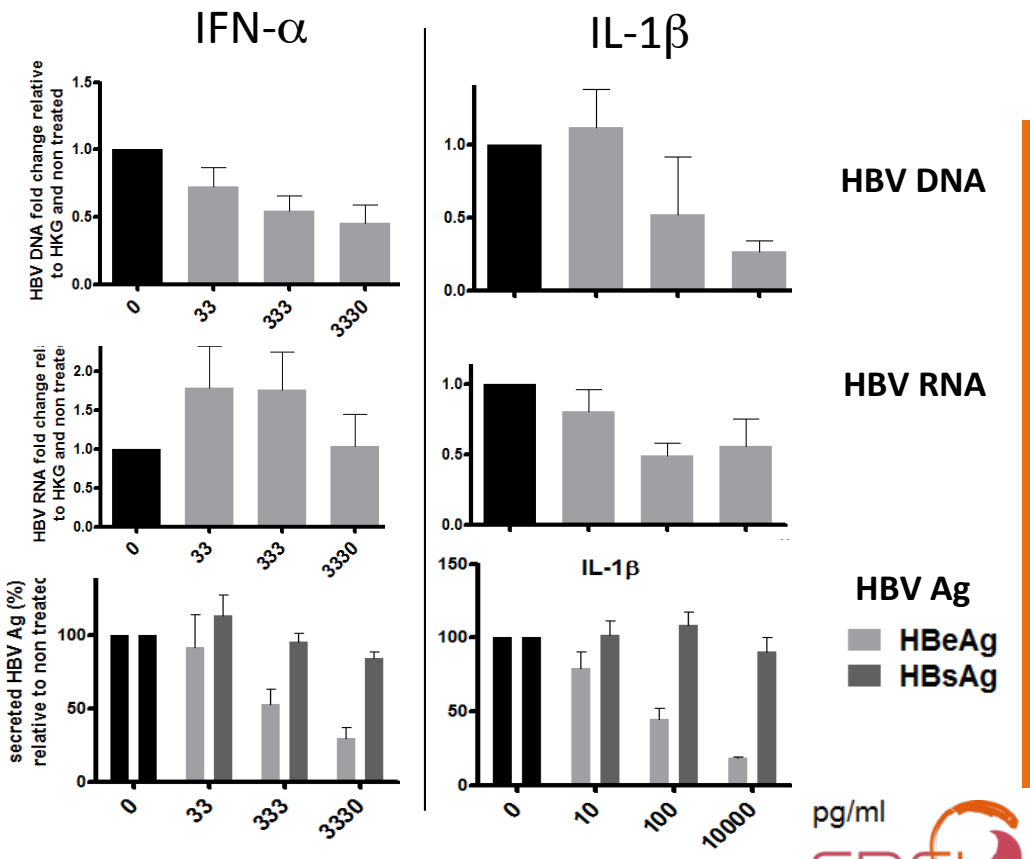
cccDNA degradation via induction of « intra-cellular » effectors: an ubiquitous mechanisms ?

Effect of various cytokines on cccDNA in HBV infected PHH cells



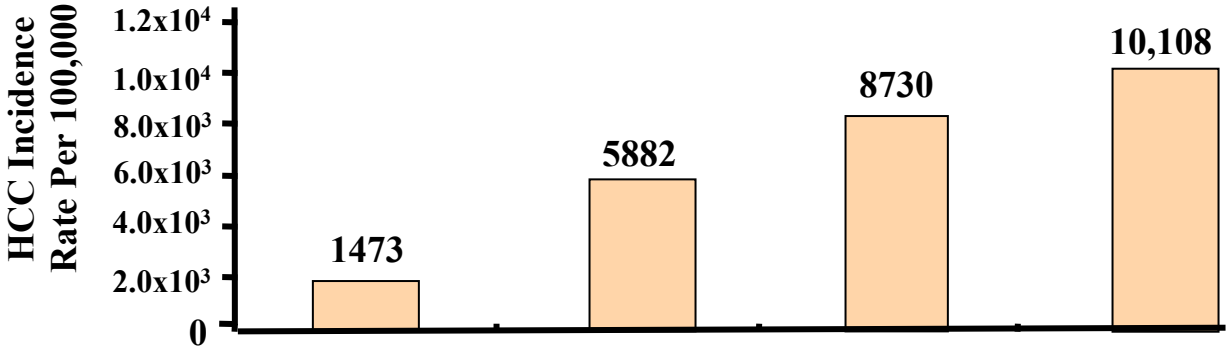
Antiviral properties of IL1-b and IFN-a in the model of HBV infected HepaRG cells



Xia et al., submitted (Protzer's lab)
Isorce et al., in prep.

Relationship Between Persistent Viremia and HCC: Argument For Antiviral Therapy

- Persistent replication associated with greater risk of HCC
- Decreased risk when viral replication declines



Baseline HBV DNA, (copies/mL)	< 10^4	≥10^5	≥10^5	≥10^5
Follow-up HBV DNA, copies/mL	---	< 10^4	10^4 to <10^5	≥10^5
Adjusted RR (95% CI)	1.0 (ref)	3.6 (1.7-7.6)	6.9 (3.4-13.8)	9.1 (5.8-14.1)
P Value	--	< 0.001	< 0.001	< .001

Chen et al; JAMA 2006