Hepatitis B: Clinical Relevance of HBV cccDNA

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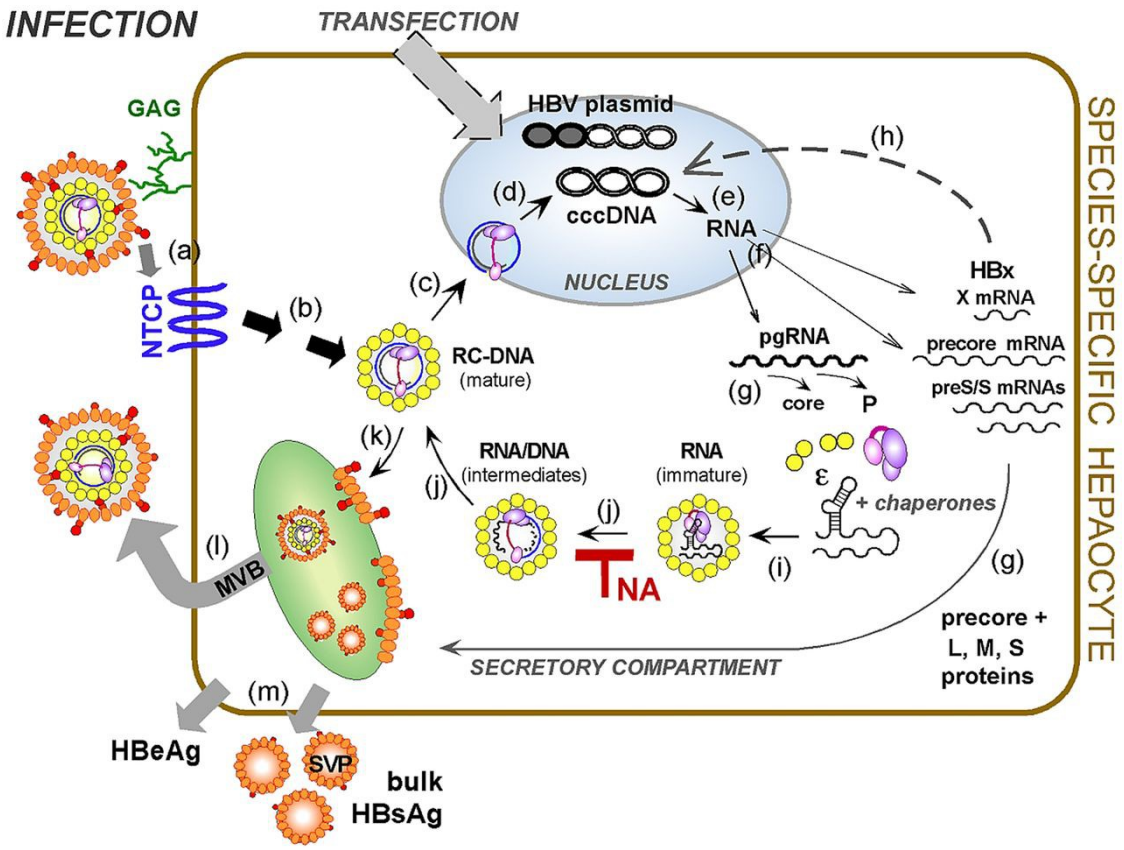
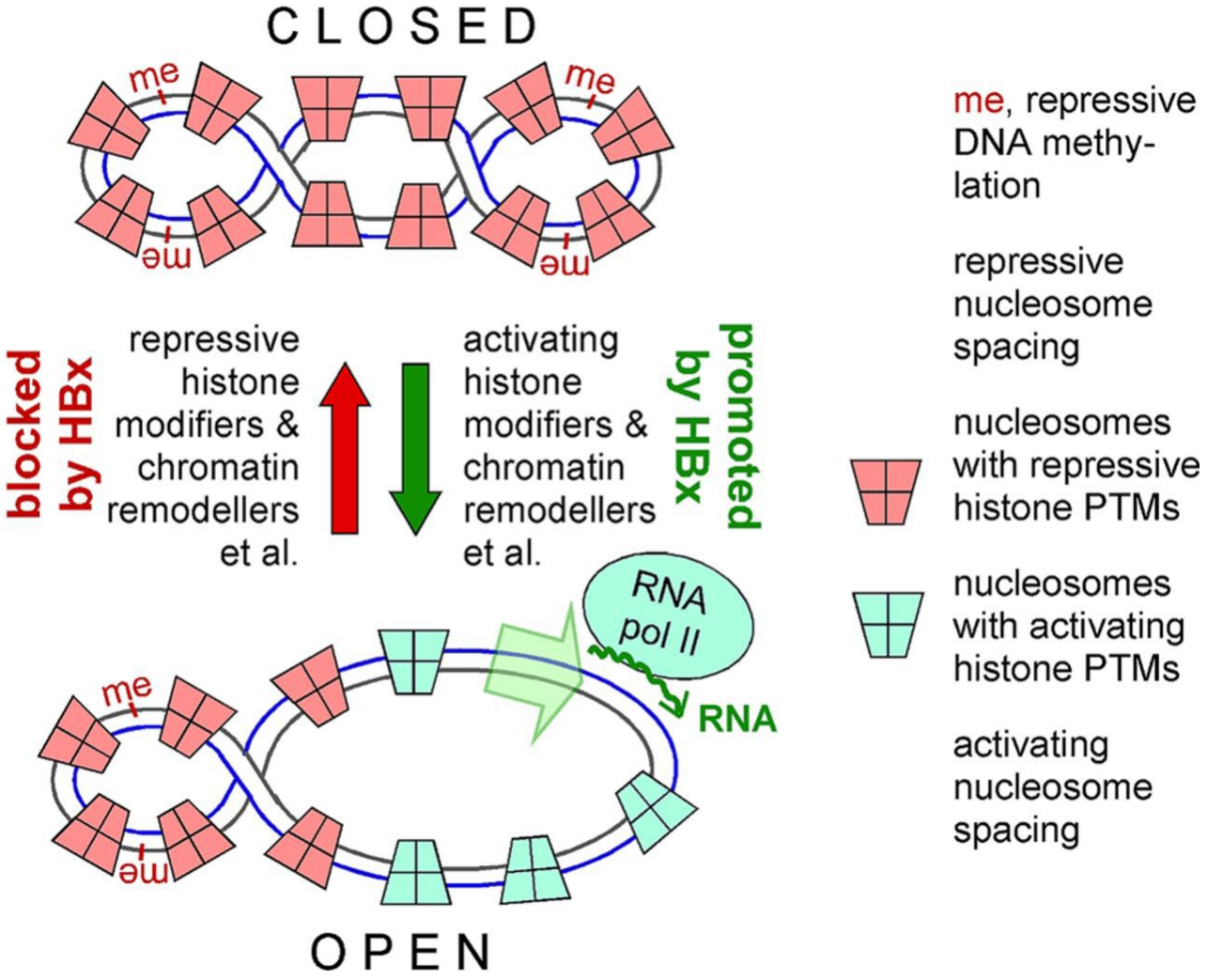
What is cccDNA ?



- Covalently closed circular DNA
- Repair of RC DNA by cellular enzymes
- Wrapped by nuclear chromatin
- « viral minichromosome »
- Template for viral gene expression
- Genetic archiving of mutations
- Responsible for viral persistence at the single cell level
- Repleneshiment by intracellular recycling of viral NC
- Long half-life

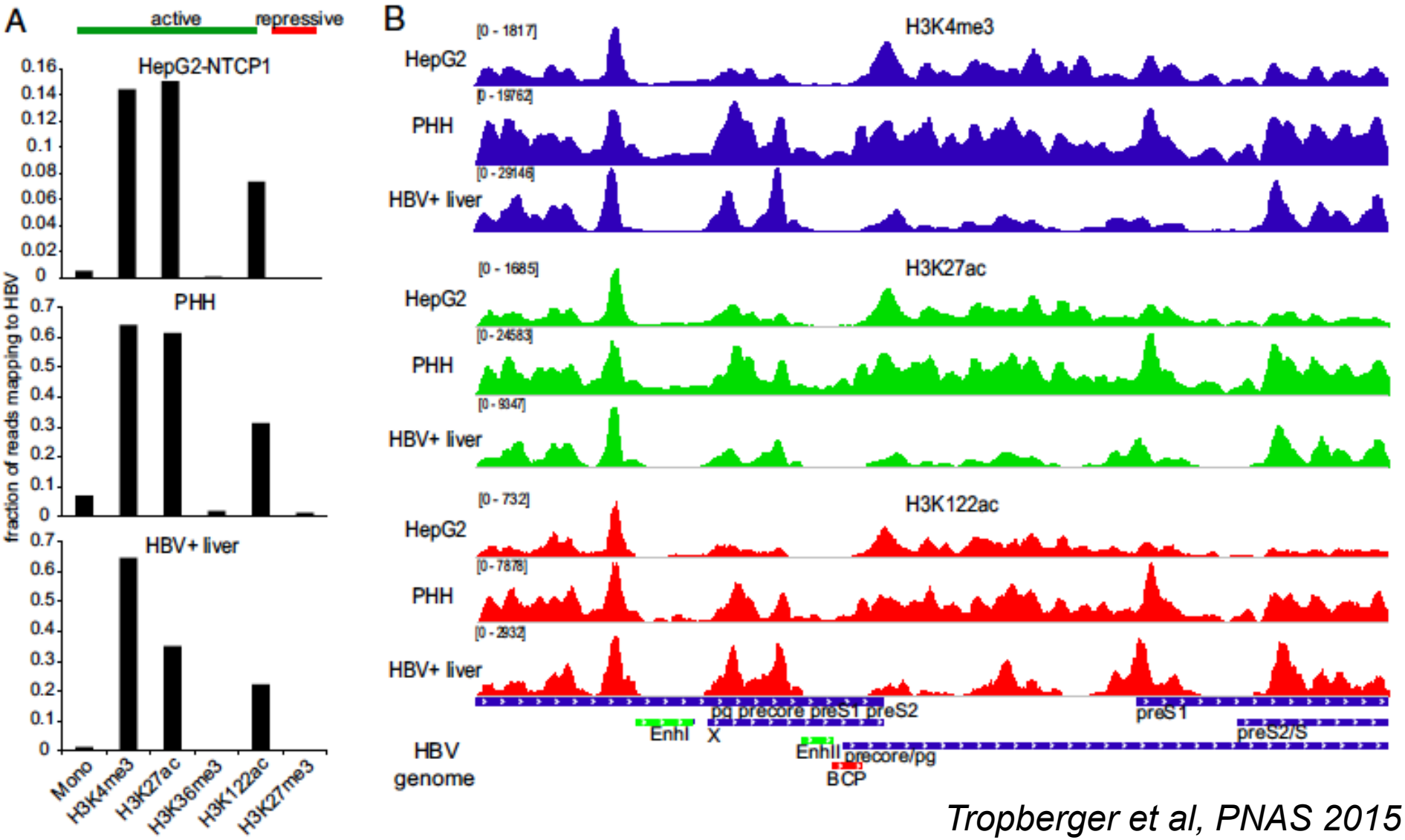
Michael Nassal Gut 2015;64:1972-1984
Levrero et al, J Hepatol 2009; Zoulim J Hepatol 2006

Epigenetics of cccDNA



Michael Nassal Gut 2015;64:1972-1984

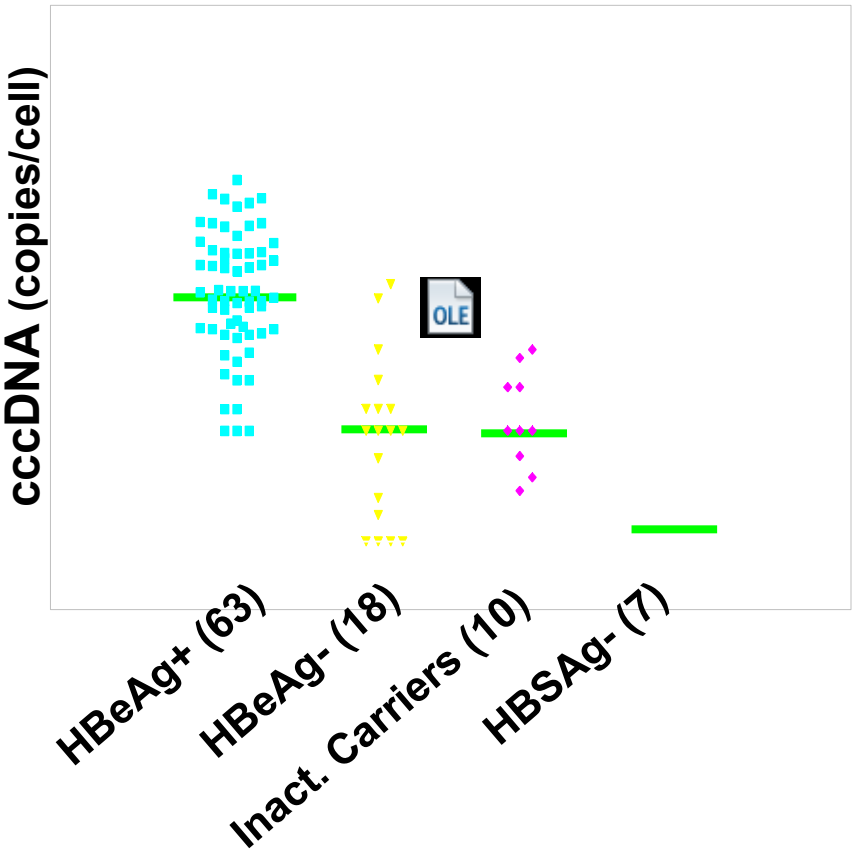
Mapping of histone modifications in cccDNA

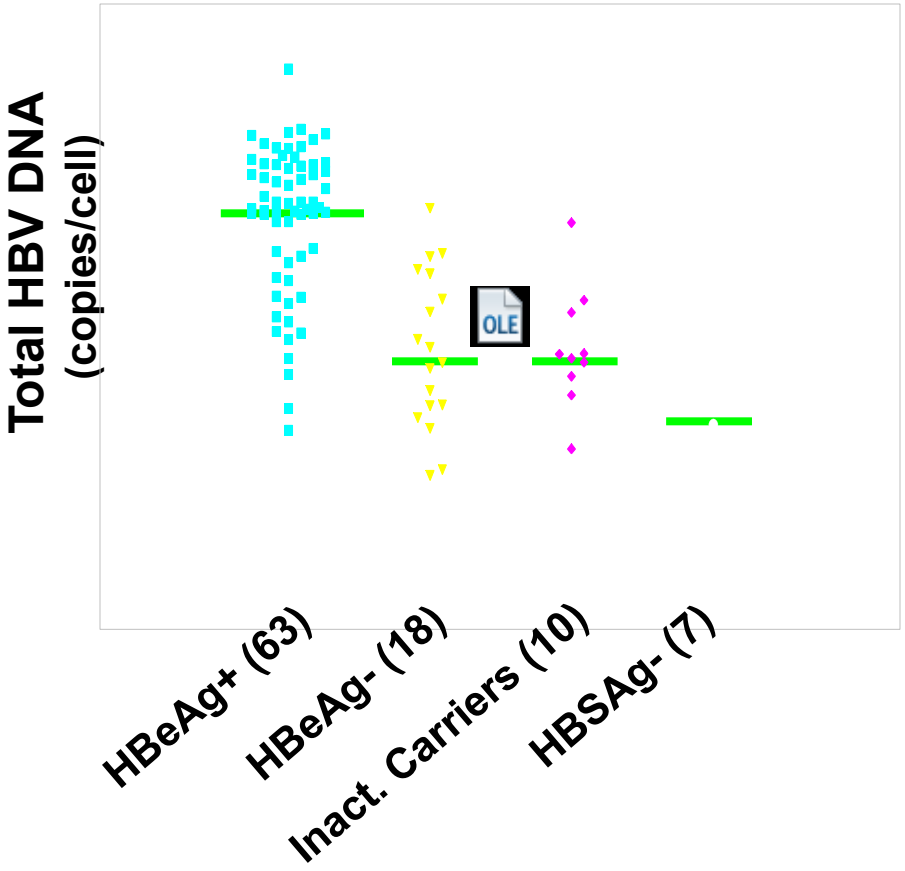


Tropberger et al, PNAS 2015

cccDNA and natural history of CHB

cccDNA levels in the different phases of chronic HBV infection





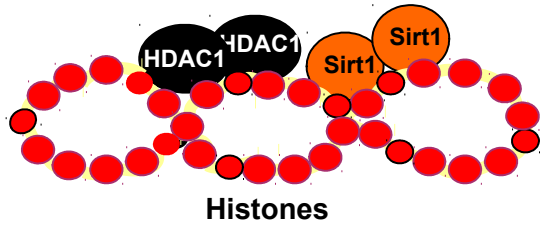
- **HBeAg+ patients had significantly higher cccDNA (90-fold) and total HBV DNA (147- fold) levels compared to HBeAg- patients. (p<0.001, Wilcoxon tests)**

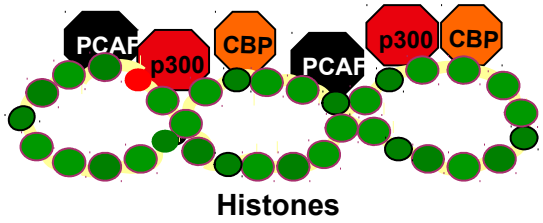
Werle et al, Gastroenterology 2004

cccDNA epigenetics in the different phases of chronic HBV infection

Low-replicative or latent infection

Epigenetic control





ChIP

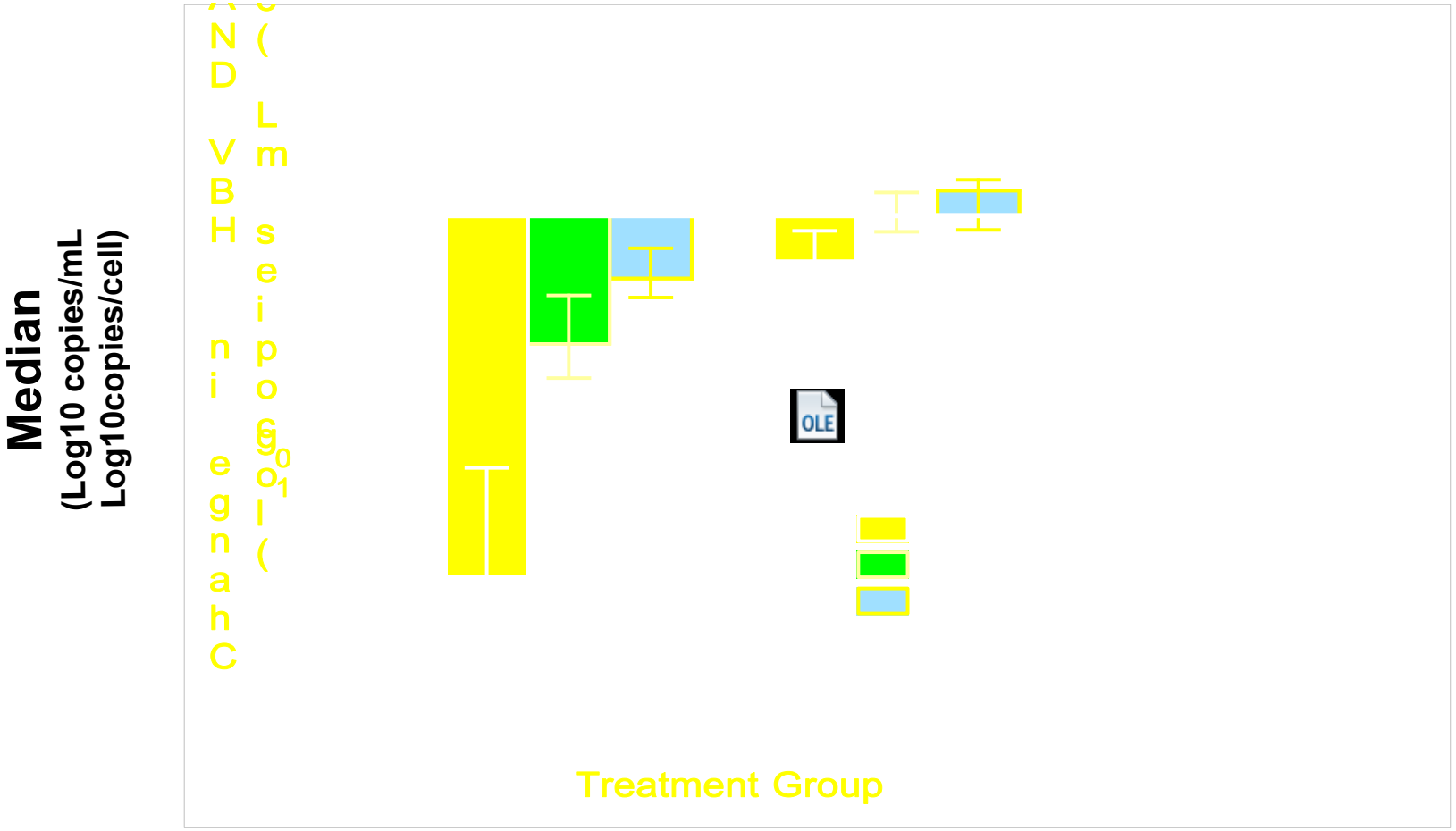
LOW-REPLICATIVE STATE ⟷ **HIGH-REPLICATIVE STATE**

- Spontaneously
- Immunosuppression

Pollicino et al. Gastroenteroplogy 2006
Levrero et al. J Hepatol, 2009

cccDNA and antiviral therapy of CHB

Reductions in Serum HBV DNA, Total Intrahepatic HBV DNA and cccDNA During Adefovir Therapy

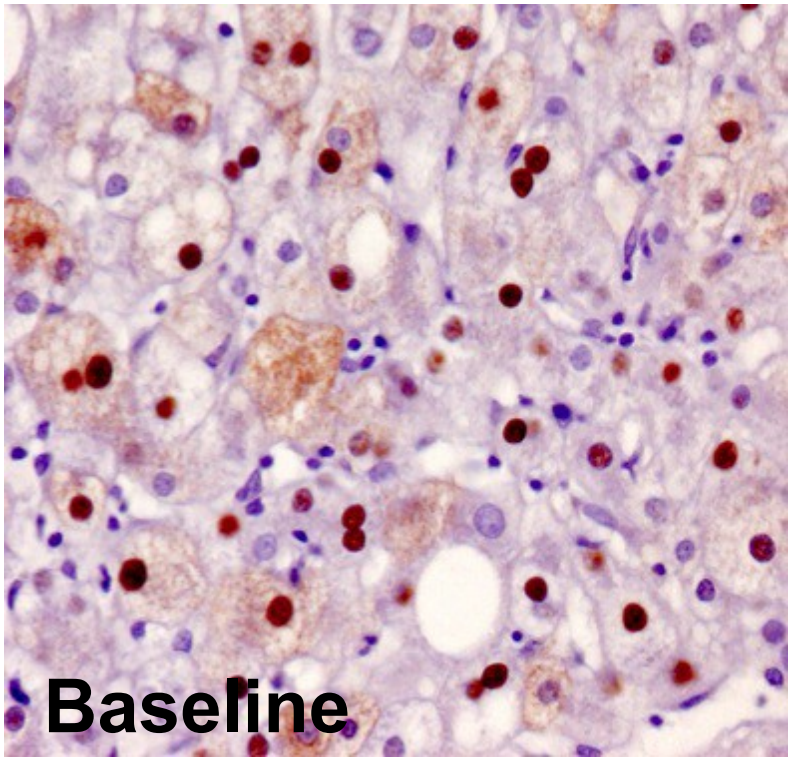


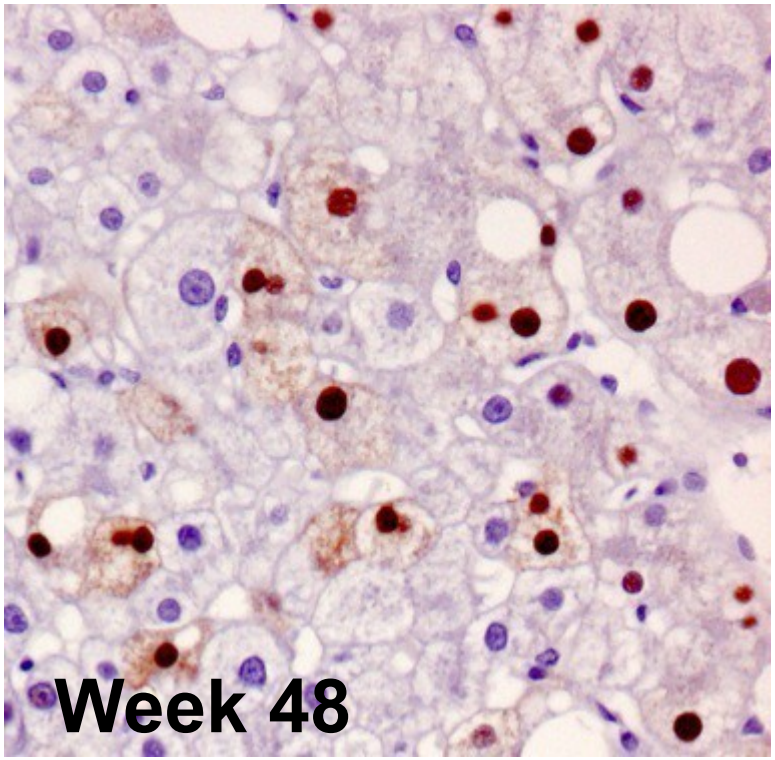
- 48 weeks of ADV resulted in significant reductions in :
  serum HBV DNA > total intrahepatic HBV DNA > cccDNA

> 14 years of therapy to clear completely viral cccDNA

Werle et al, Gastroenterology 2004

Immunohistochemical Staining of Patient Biopsies at Baseline and After 48 Weeks ADV Therapy

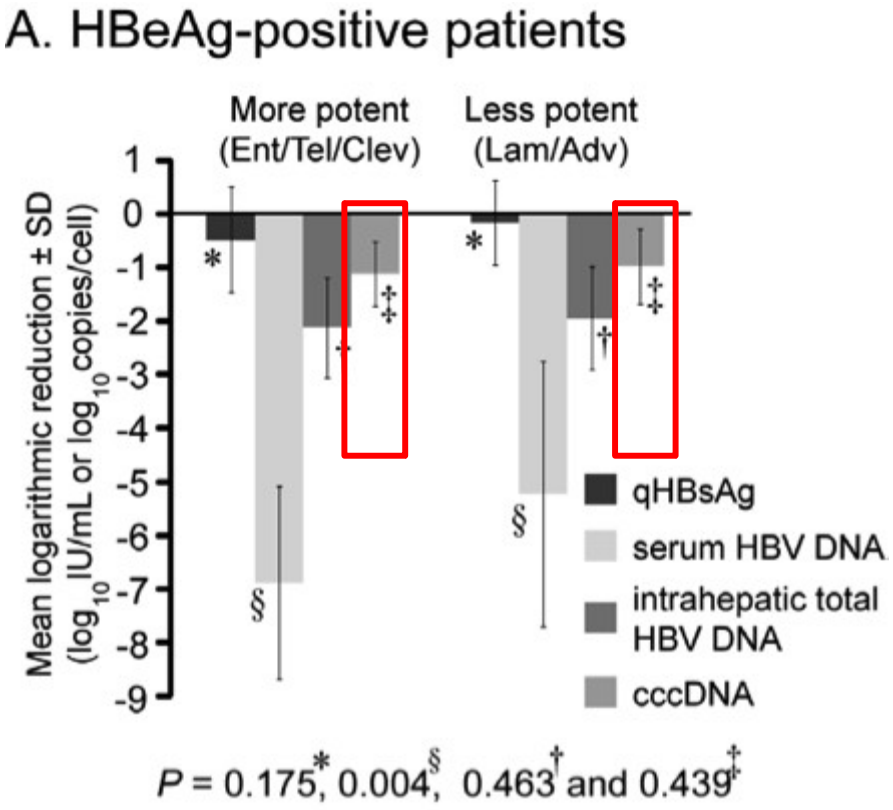


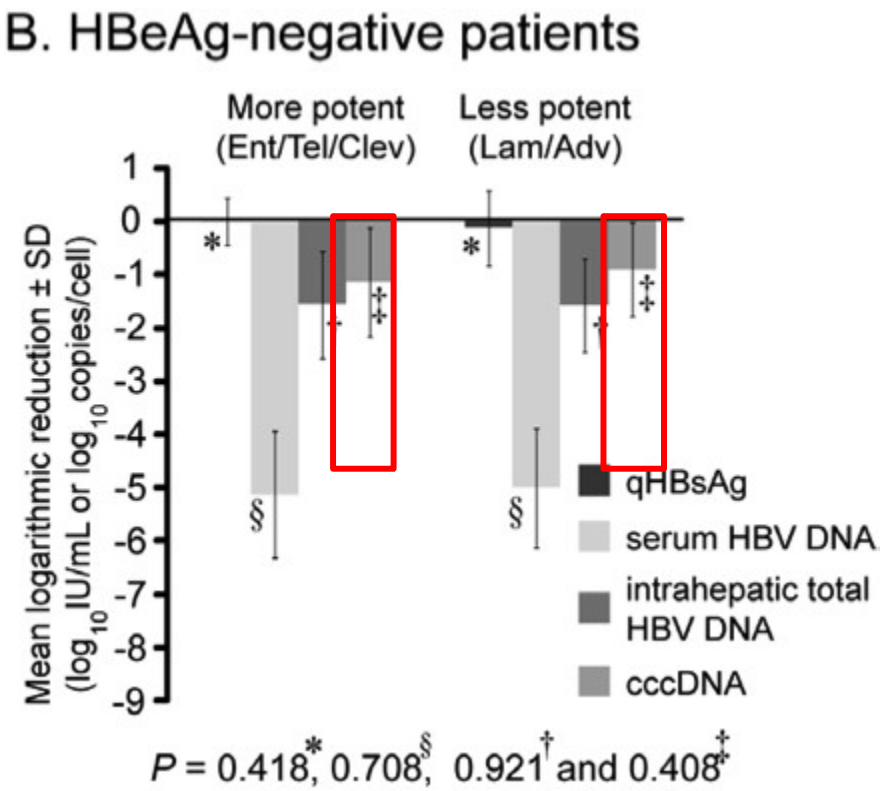


- 0.8 log10 (84%) decline in cccDNA, not paralleled by a similar decline in the number of HBcAg+ cells
- Suggests cccDNA depleted primarily by non-cytopathic mechanisms or new rounds of hepatocyte infection occurred during therapy

Werle et al, Gastroenterology 2004

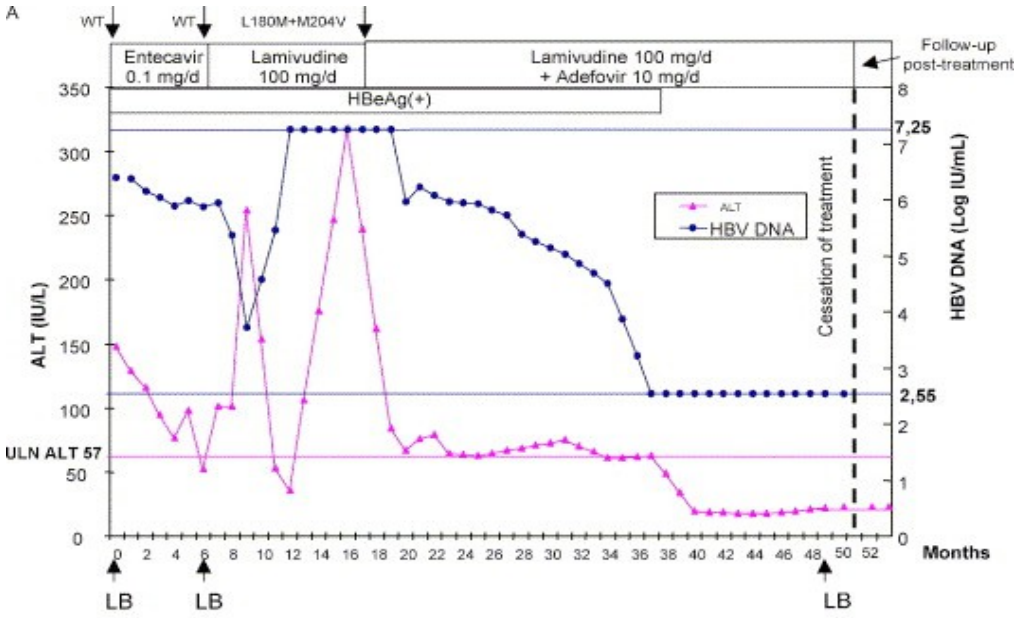
Slow decay of cccDNA and HBsAg during NUC therapy

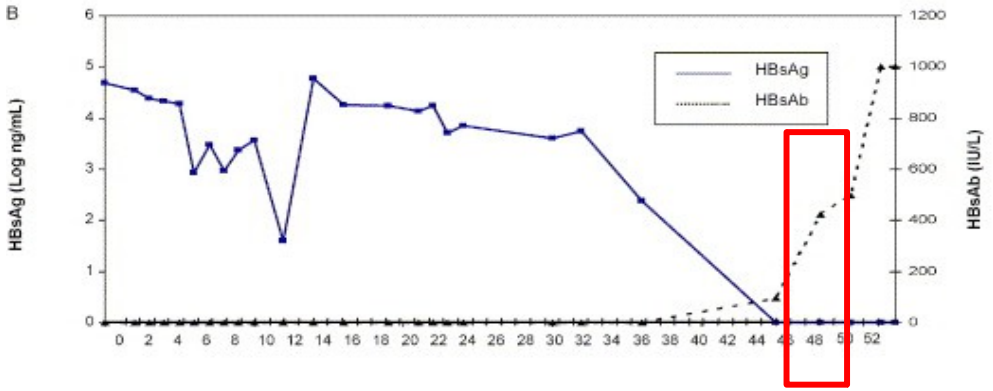


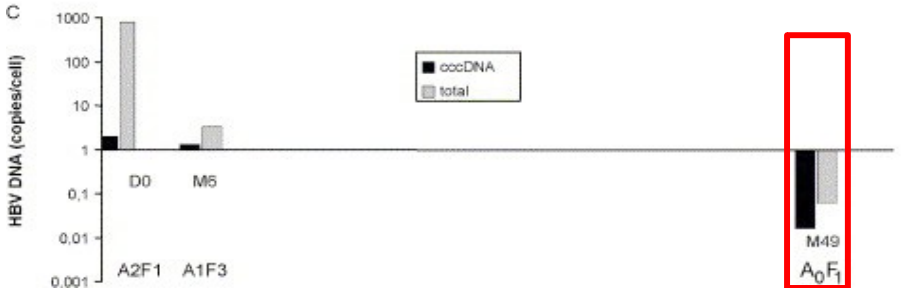


Wong et al, Clin Gastroenterol Hepatol 2013
Werle et al, Gastroenterology 2004

Persistence of cccDNA after HBs seroconversion

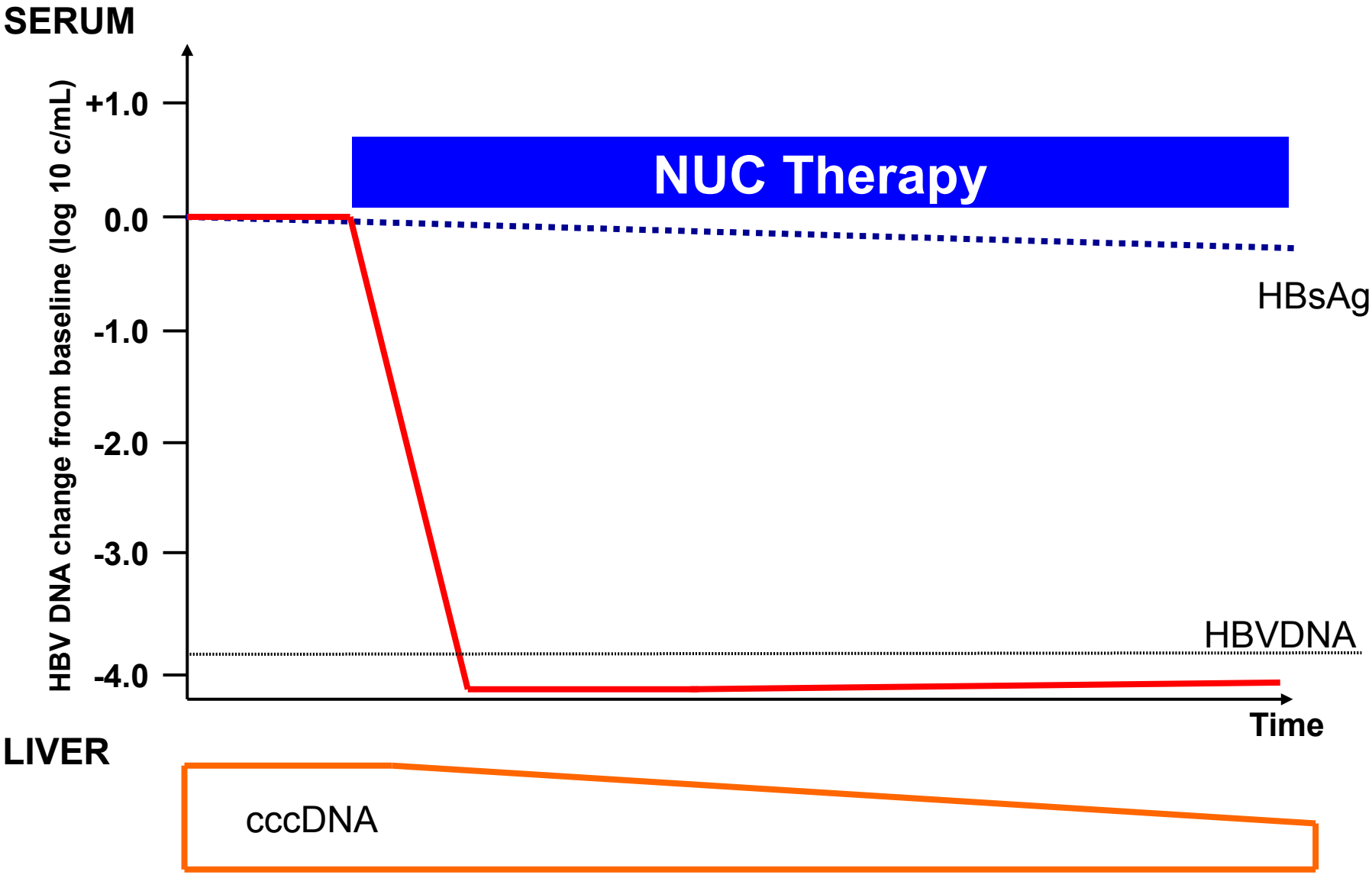






Maynard et al, J Hepatol 2005

Long-term therapy is required to maintain viral suppression



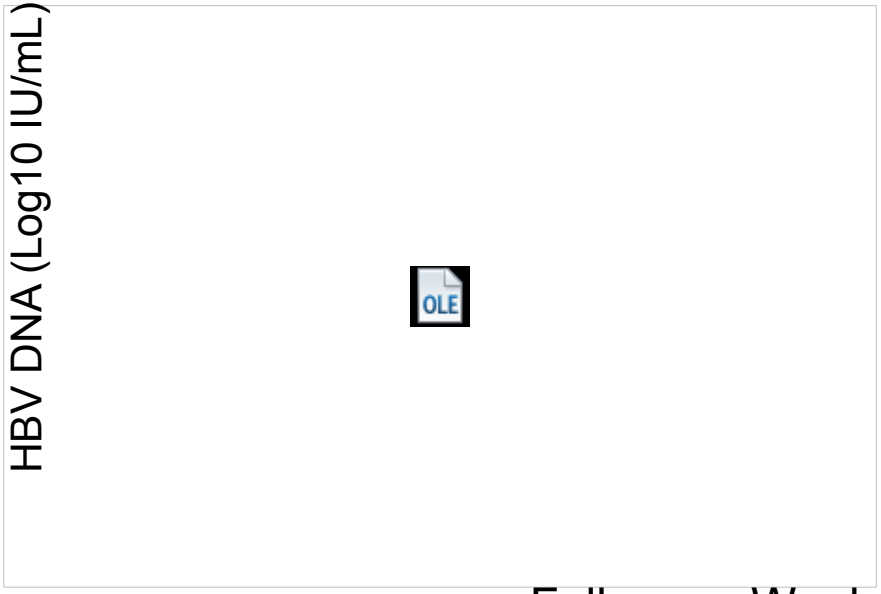
Stopping TDF therapy after long-term viral suppression

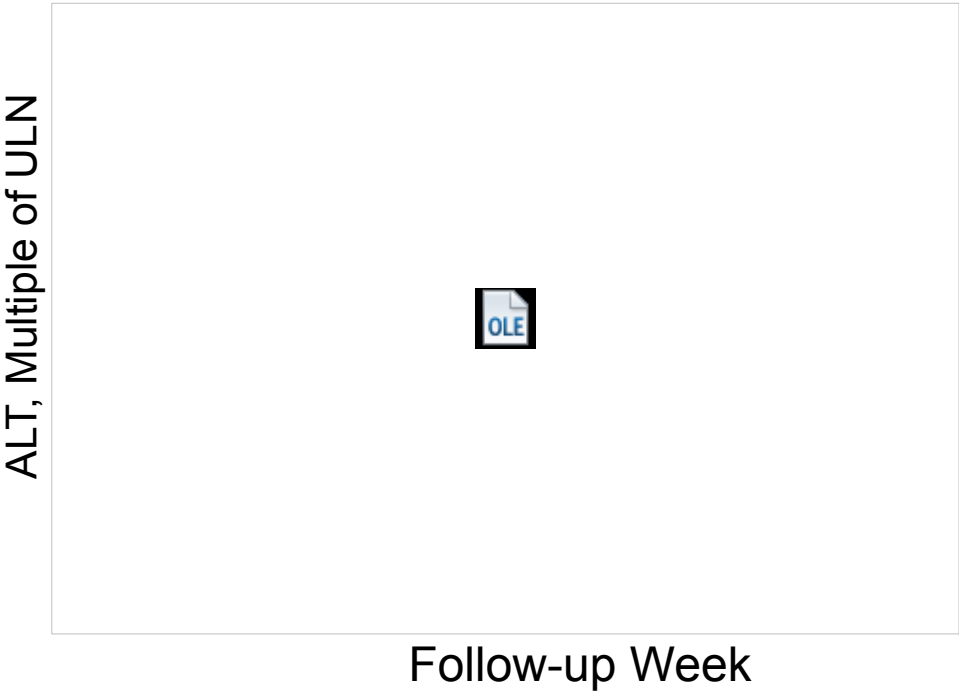
High rates of viral replapse & ALT elevations
3 patients with HBsAg loss out of 41

24-week TFFU Completers (n=41)

HBeAg positive (n=4)

HBeAg negative (n=37)





Buti et al AASLD 2015

Challenges with cccDNA (1)

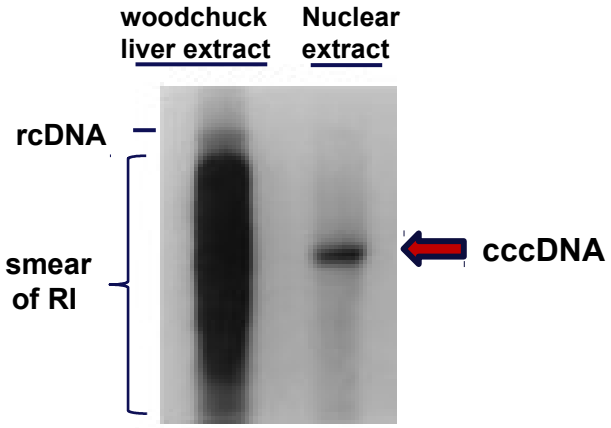
- **Harmonization of the detection and quantification methods**
  - Southern blot analysis
  - dPCR assays

- **Analysis of cccDNA epigenome**
  - Need for sensitive methodologies

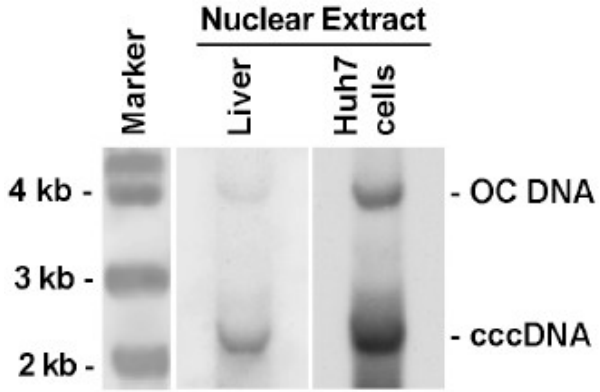
- **Surrogate markers for non invasive cccDNA evaluation in patients**
  - qHBsAg: relevant in cell culture systems
  - But not *in vivo*: many confounding factors (cccDNA levels, epigenetic status, number of infected cells, integration etc…)

Methods for the detection of cccDNA

- **1990ies and before: Southern Blot analysis of HBV replicative intermediates and cccDNA selectively extracted from liver tissues or HBV-transfected cells**



(Dandri, Hepatology 2000)



Pollicino et al., Gastroenterology, 2006

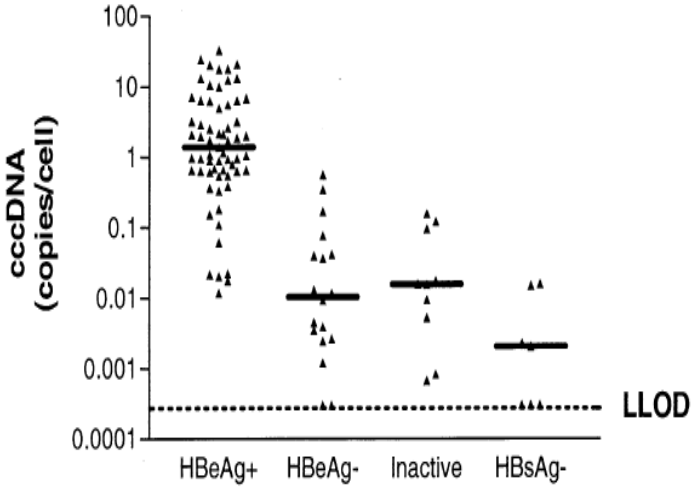
Pros: you see it
Cons: detection limits

- **real-time PCR method for the detection of relaxed circular and cccDNA in frozen liver biopsies**

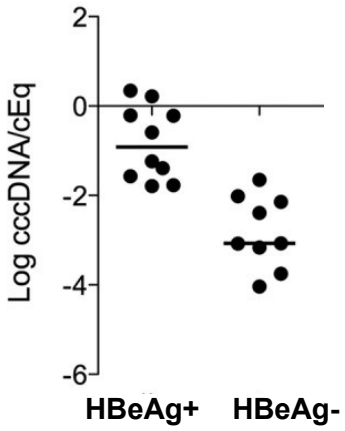
(Werle-Lapostolle B, et al. *Gastroenterology* 2004)

The method was validated in 3 different labs: Zoulim (France)
Locarnini (Australia)
Petersen (Germany)

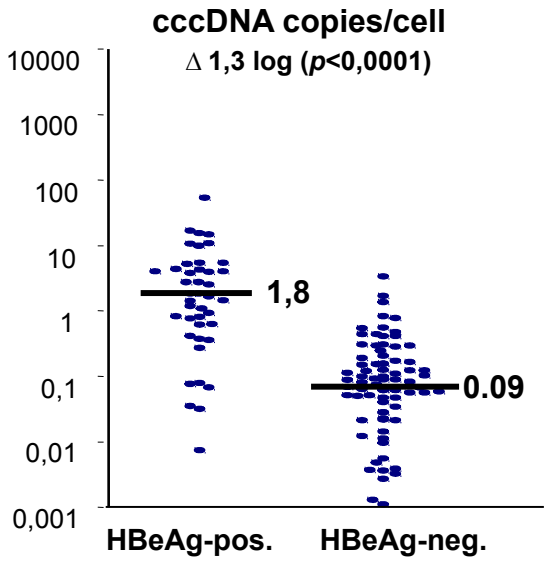
cccDNA quantification by real time PCR



Werle-Lapostolle B, et al. *Gastroenterology* 2004



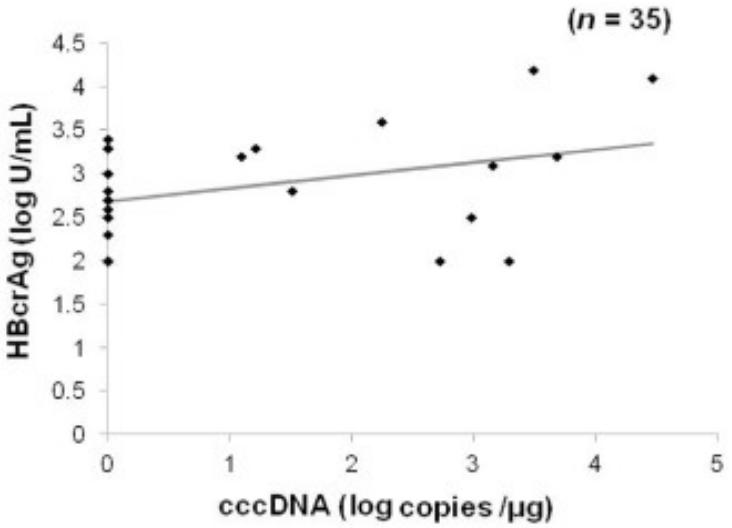
Malmström, et al. *PLOS ONE 2012*

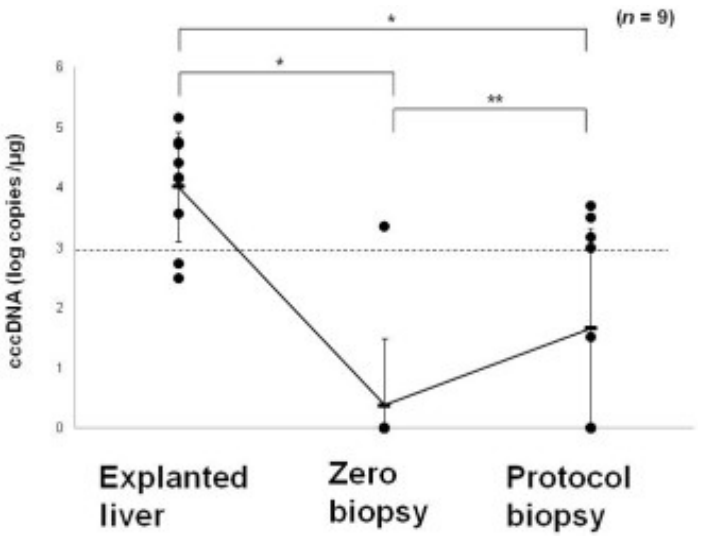


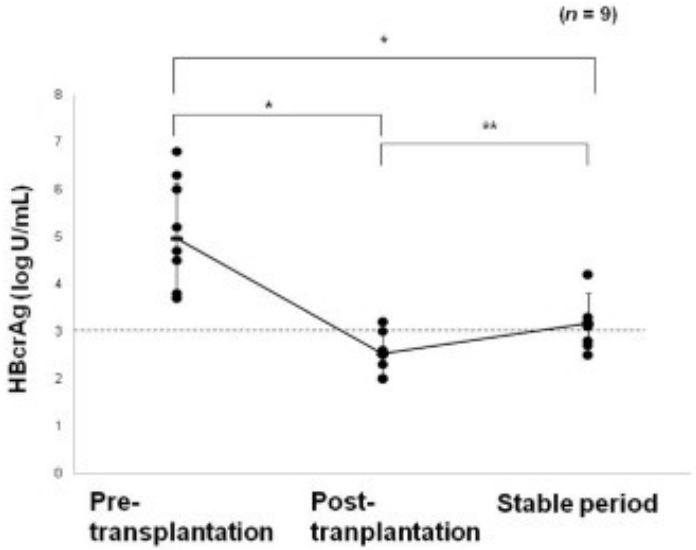
Volz, et al. *Gastroenterology 2007*

- Many studies have been published!
- cccDNA measurements both using liver tissues and cell culture systems
- But no real standardization of the methods: DNA extraction, cccDNA enrichment, primer sequences etc…
- Need for harmonization of the technologies

Correlation between HBcrAg and cccDNA







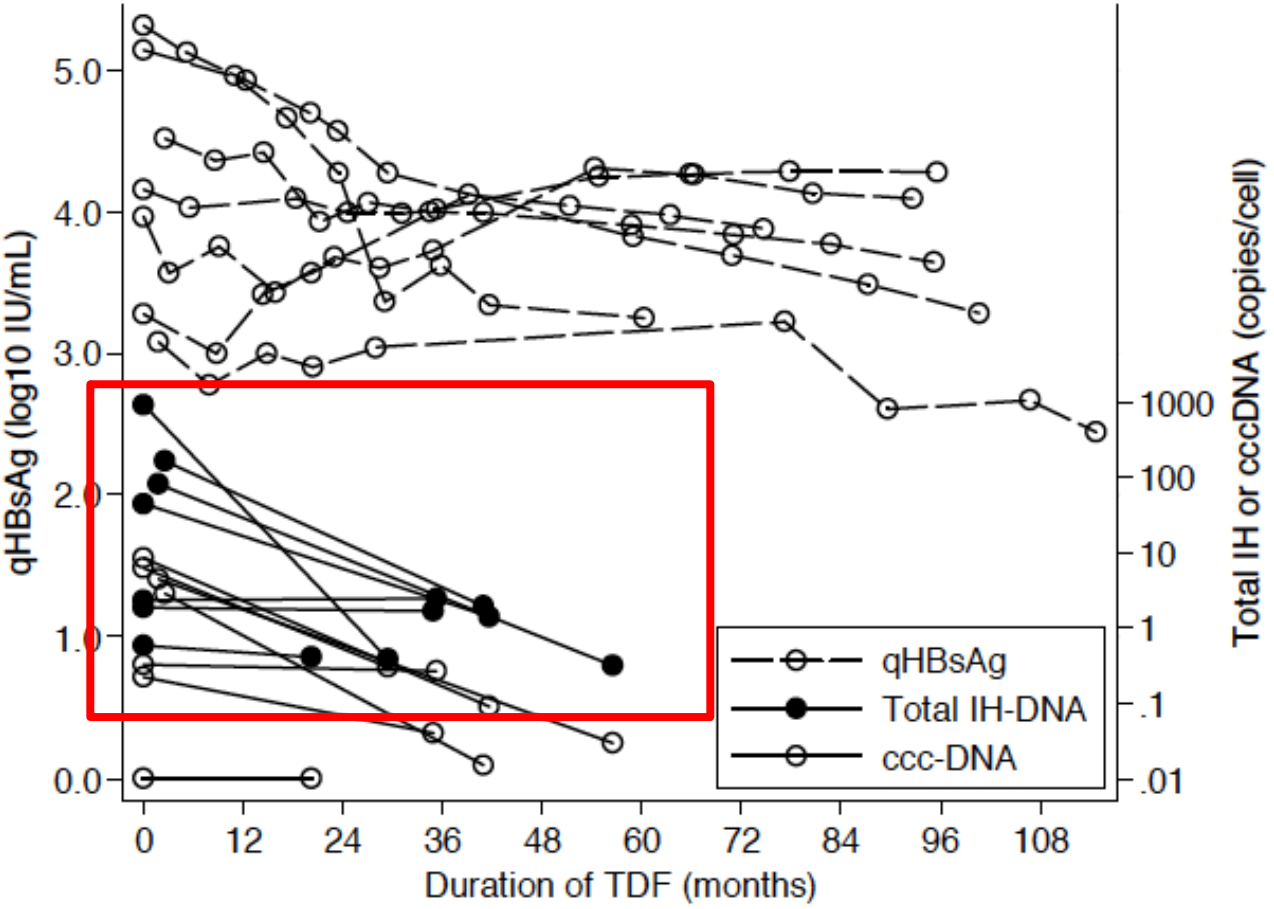
Matzusaki et al, Journal of Gastroenterology and Hepatology 2013

Challenges with cccDNA (2)

- **Can we target cccDNA to improve the rate of functional cure with antiviral therapy ?**
  - Improved viral suppression to deplete the cccDNA pool
  - cccDNA degradation: is the whole pool of cccDNA susceptible to degradation ? will all infected cells be susceptible ?
  - cccDNA silencing: targeting virus-specific mechanisms to avoid safety issues, i.e. HBx and/or HBc
  - Hepatocyte turn-over to dilute cccDNA but exposes to the risk of clonal selection of hepatocytes

Persistence of intrahepatic viral DNA synthesis during Tenofovir therapy

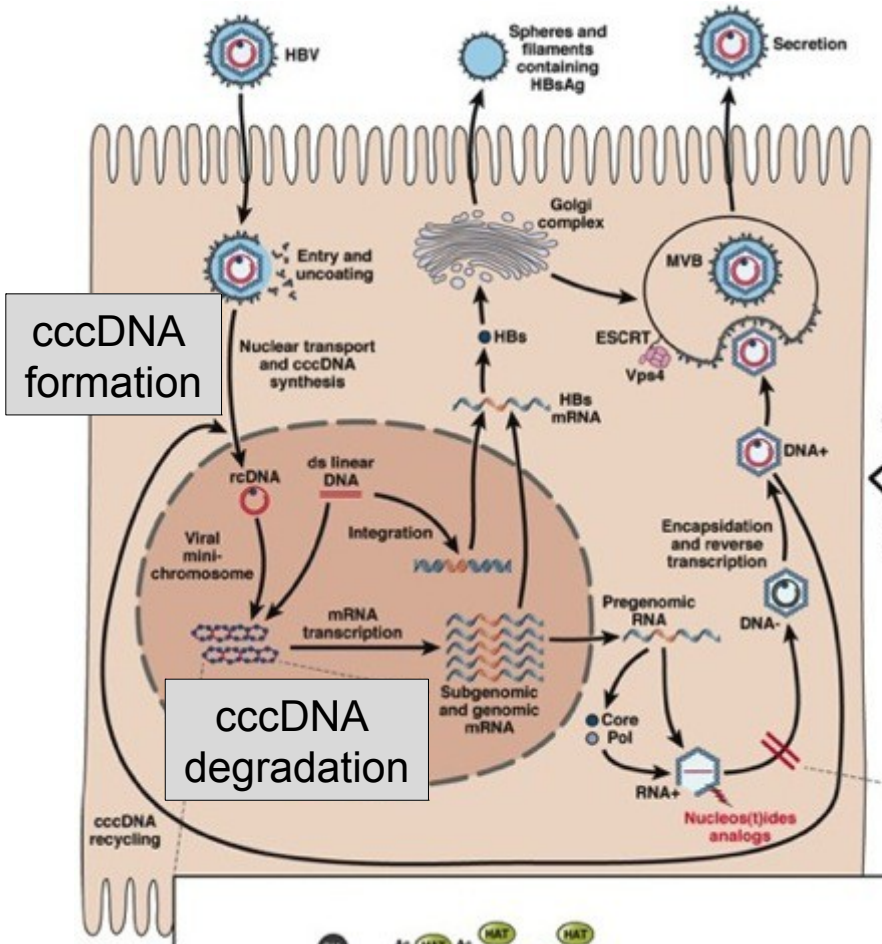
(HIV HBV cohort)



New round of infection and/or replenishment of the cccDNA pool occur despite « viral suppression »

Boyd et al, in revision

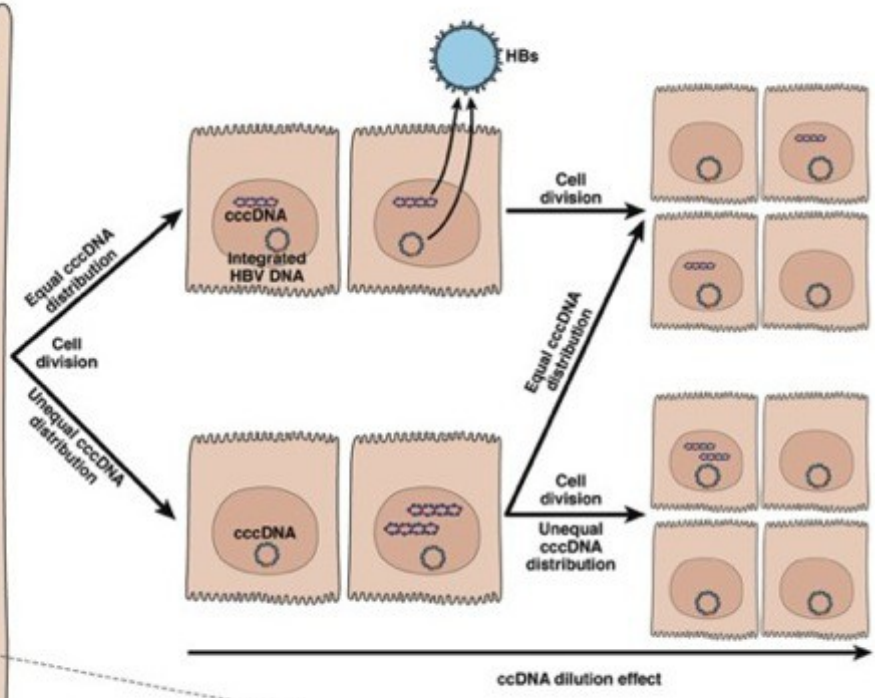
Targeting cccDNA

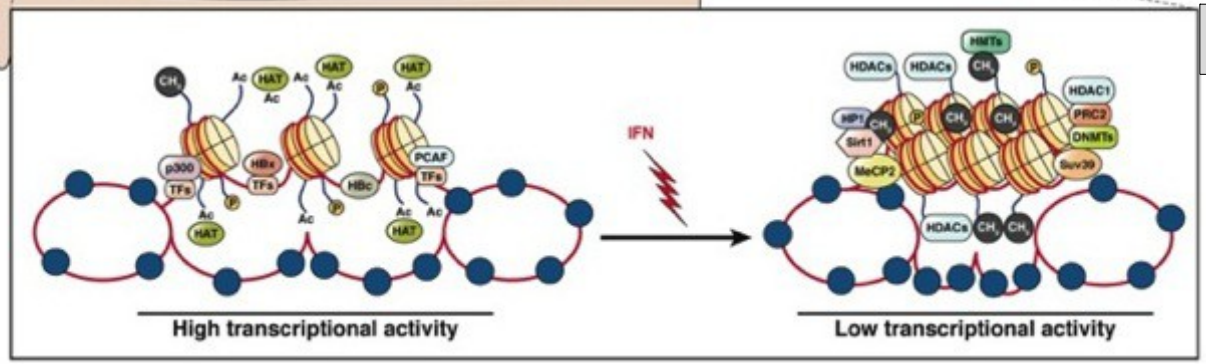


cccDNA formation

cccDNA degradation

Hepatocyte turn-over

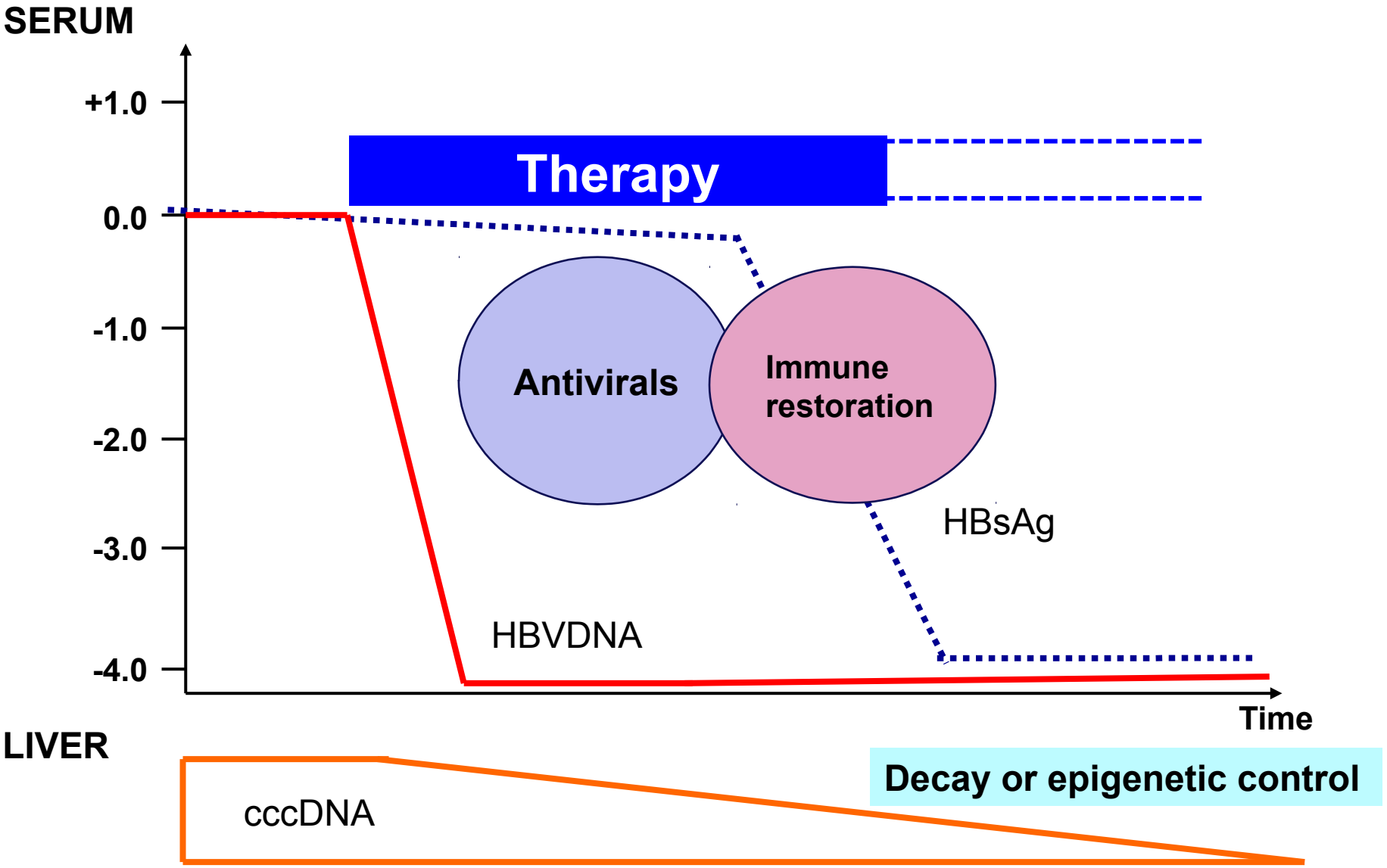




cccDNA silencing

Lucifora et al, **Science** 2014
Zoulim, et al, **Clin Gastroenterol Hepatol** 2013
Belloni et al, **JCI** 2012
Koeniger etal, **PNAS** 2014
Tropberger et al, **PNAS** 2015

New treatment concepts for a functional cure of HBV infection



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"Save the date"

Third ANRS "HBV cure" Workshop
HBV pathobiology and target discovery

Scientific coordination: Fabien Zoulim

Tuesday, May 31st, 2016
Union internationale des chemins de fer (UIC)
16, rue Jean Rey - 75015 PARIS

HBV cure 2014: Zeisel, M. B. *et al.* Towards an HBV cure: state-of-the-art and unresolved questions-report of the ANRS workshop on HBV cure. *Gut*, doi:10.1136/gutjnl-2014-308943 (2015).

HBV cure 2015: http://www.anrs-hbvcure2015.com/