11ème Workshop International ANRS MIE HBV CURE
03 Juillet 2024
Lyon

anrs MALADIES INFECTIEUSES ÉMERGENTES Inserm

IMPACT OF HBV DNA INTERGRATION ON FUNCTIONAL CURE

Professor Patrick Kennedy,

Professor of Translational Hepatology

Barts Liver Centre, Immunobiology, Blizard Institute, Barts and The London SMD,
Queen Mary University of London, UK

Disclosures : Abbott, Aligos, Antios Therapeutics, Assembly Biosciences, Gilead Sciences, Janssen, GlaxoSmithKline, Immunocore, Drug Farm

Endorsed by

HBV cure Task Force

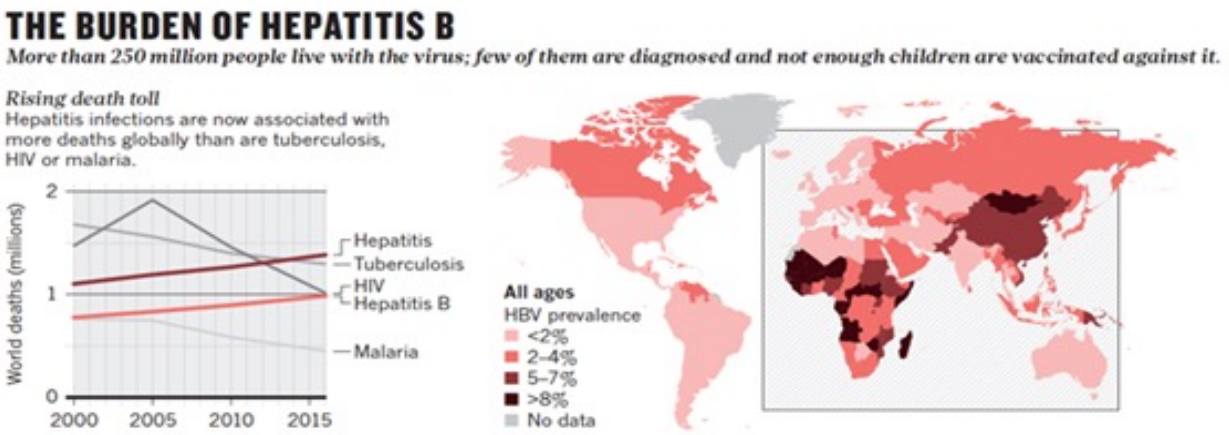
RB-RNA

ICE-HBV International Coalition to Eliminate HBV

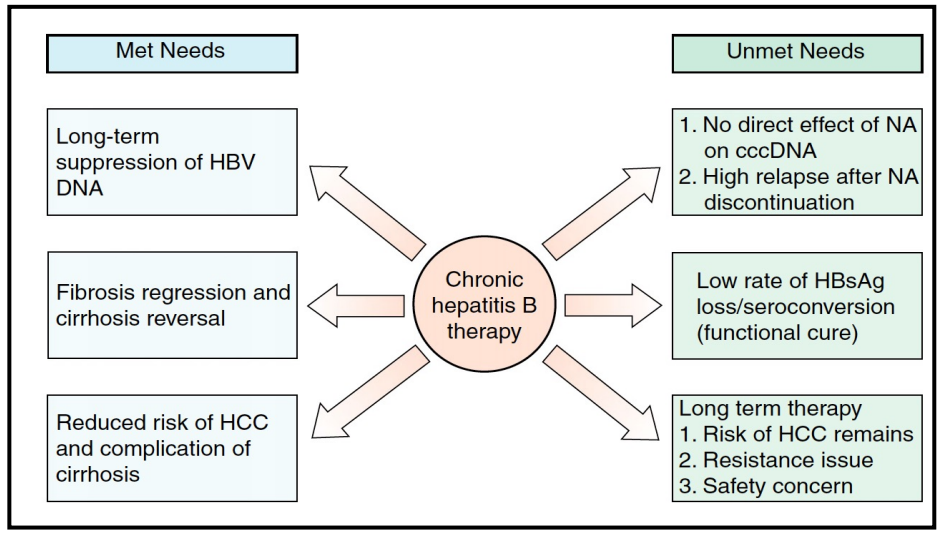
IP-cure-B

LYON HEPATOLOGY INSTITUTE
COMPREHENSIVE LIVER CENTER

Chronic Hepatitis B – unmet needs



Graber-Stiehl, Nature News 2018



Adapted from Lin & Kao, AP&T 2016

Global Health Metrics

Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016–40 for 195 countries and territories

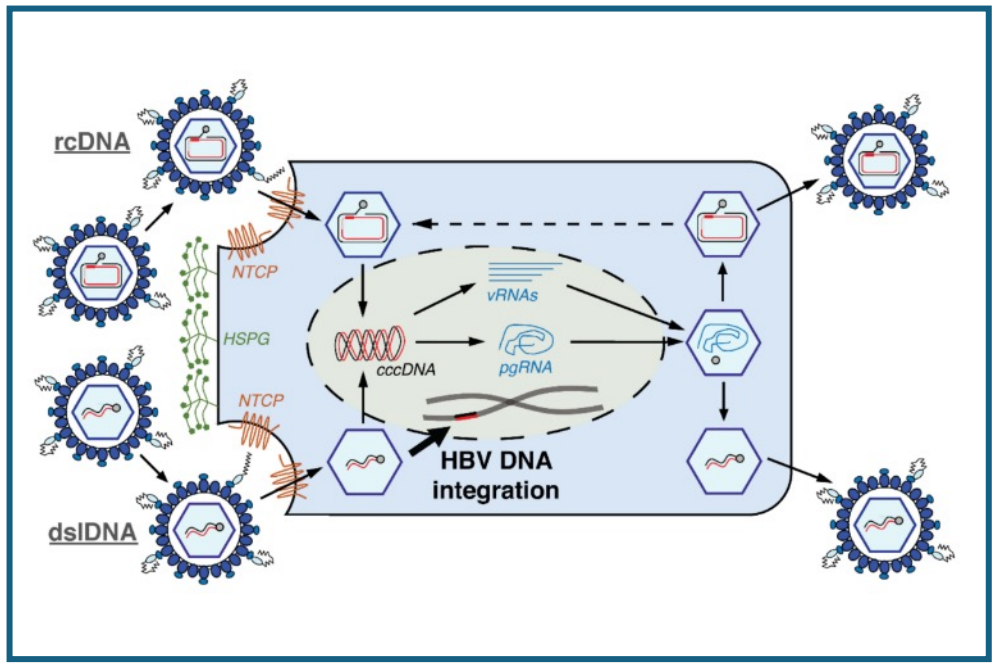
Kyle J Foreman, Neal Marquez, Andrew Dolgert, Kai Fukutaki, Nancy Fullman, Madeline McGaughey, Martin A Pletcher, Amanda E Smith, Kendrick Tang, Chun-Wei Yuan, Jonathan C Brown, Joseph Friedman, Jiawei He, Kyle R Heuton, Mollie Holmberg, Disha J Patel, Patrick Reidy, Austin Carter, Kelly Cercy, Abigail Chapin, Dirk Douwes-Schultz, Tahvi Frank, Falko Goettsch, Patrick Y Liu, Vishnu Nandakumar, Marissa B Reitsma, Vince Reuter, Nafis Sadat, Reed J D Sorensen, Vinay Srinivasan, Rachel L Updike, Hunter York, Alan D Lopez, Rafael Lozano, Stephen S Lim, Ali H Mokdad, Stein Emil Vollset, Christopher J L Murray

Number of global deaths and years of life lost (YLLs) in GBD 2016, and in the 2040 forecast

	YLLs (thousands)				Deaths (thousands)			
	2016	2040 forecast	2040 better health scenario	2040 worse health scenario	2016	2040 forecast	2040 better health scenario	2040 worse health scenario
All causes	1 585 864·98 (1 559 572·96–1 613 799·53)	1 529 212·73 (1 315 165·66–1 744 642·71)	1 188 899·29 (1 021 216·03–1 357 596·47)	2 170 421·82 (1 858 433·76–2 480 811·12)	54 698·58 (54 028·68–55 514·89)	75 263·26 (67 310·21–83 866·49)	62 570·88 (55 675·51–70 026·74)	96 717·00 (86 181·44–107 125·85)
Cirrhosis and other chronic liver diseases	37 283·07 (35 413·31–41 442·98)	48 324·15 (43 655·69–54 757·85)	44 158·29 (39 536·81–50 452·38)	53 144·00 (48 394·75–60 100·36)	1 256·85 (1 197·09–1 376·86)	1903·64 (1760·57–2097·77)	1787·20 (1637·35–1979·96)	2014·49 (1881·85–2228·68)
Cirrhosis and other chronic liver diseases due to hepatitis B	10 846·50 (9787·89–12 777·41)	14 880·49 (12 899·14–17 732·38)	14 308·72 (12 368·22–16 964·03)	15 697·63 (13 716·23–18 707·04)	365·57 (330·81–422·57)	582·17 (514·27–683·44)	572·65 (502·98–670·06)	593·64 (530·13–690·12)
Liver cancer	20 915·71 (20 029·12–21 730·96)	35 487·07 (27 243·22–49 706·06)	32 930·35 (26 712·73–43 698·83)	37 729·38 (28 311·22–55 428·12)	828·94 (796·16–857·96)	1679·63 (1311·17–2297·91)	1602·50 (1328·19–2098·20)	1720·16 (1315·30–2493·46)
Liver cancer due to hepatitis B	9704·02 (8495·14–10 846·75)	15 984·92 (11 742·51–22 690·54)	15 042·06 (11 701·98–20 344·07)	16 334·40 (11 643·45–24 443·45)	349·53 (301·96–391·78)	702·26 (515·29–983·42)	678·35 (529·85–899·84)	688·77 (498·29–1007·57)

Foreman et al., The Lancet 2018

HBV DNA integration plays a key role in HBV-related hepatocarcinogenesis



cccDNA[2–3]
pgRNA[4]

Inflammation

Cirrhosis

HBV DNA integration[5–6]

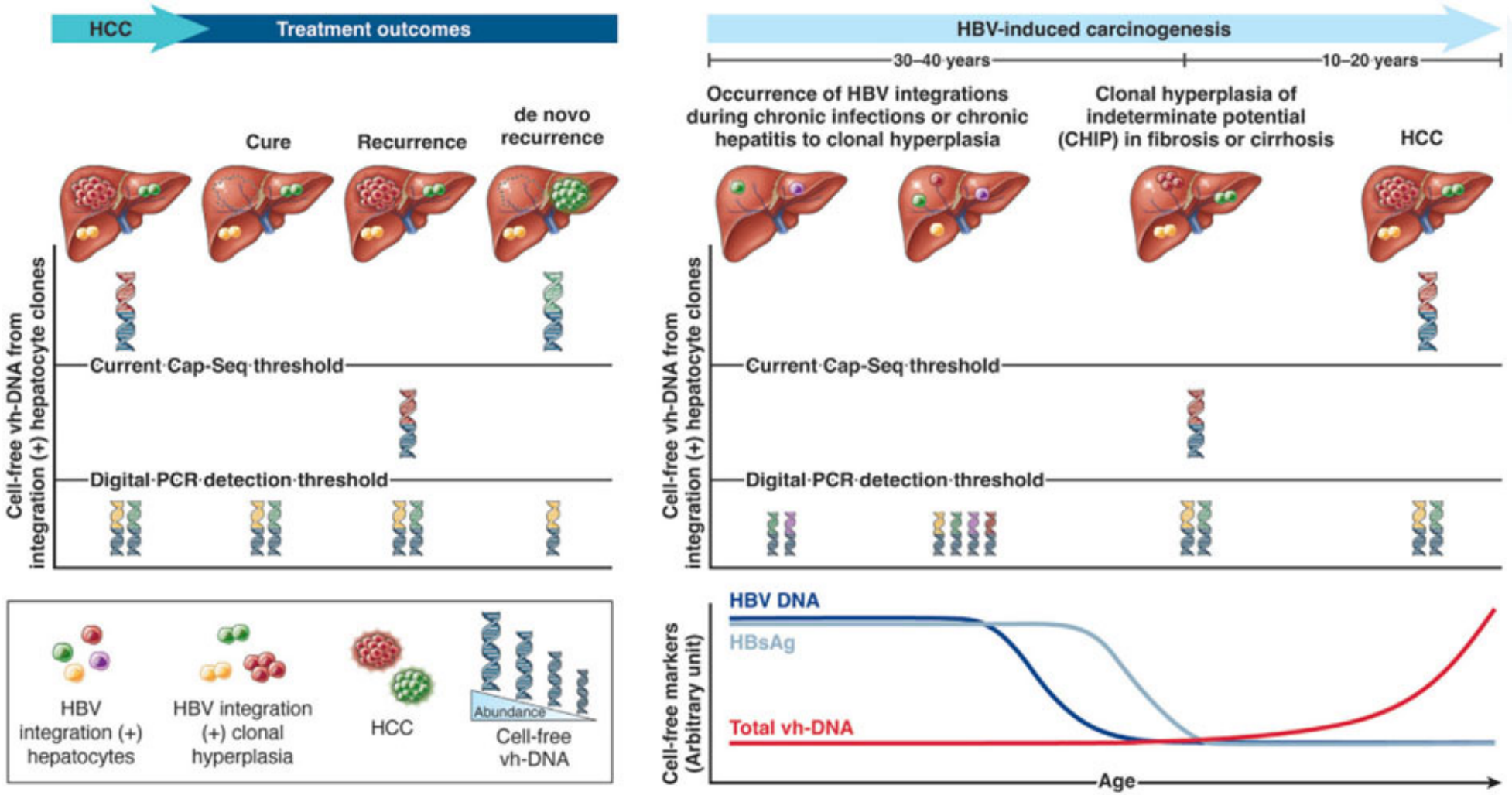
HCC

Intrahepatic HBV transcriptional activity is associated with liver inflammation and disease progression and HBV DNA integration further contributes to hepatocarcinogenesis[2–6]

1. Budzinska MA, et al. Genes (Basel) 2018;9:365; 2. Liang L-B, et al. Int J Infect Dis 2016;52:77–82; 3. Larsson SB, et al. Liver Int 2014;34:e238–45; 4. Ding W-B, et al. Hepatology 2021;74:1480–95; 5. 5. Tu T, et al. J Viral Hepat 2015;22:737–53; 6. Zhao LH, et al. Nat Commun 2016;7:12992.

dslDNA: double-stranded linear DNA;
rcDNA: relaxed circular DNA.

Tracking HBV DNA integration: Cell-free vh-DNA from HBV integration sites provides a new biomarker for HBV-related HCC

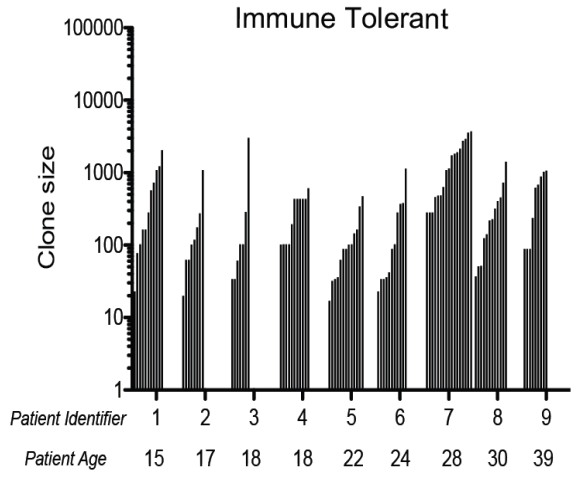


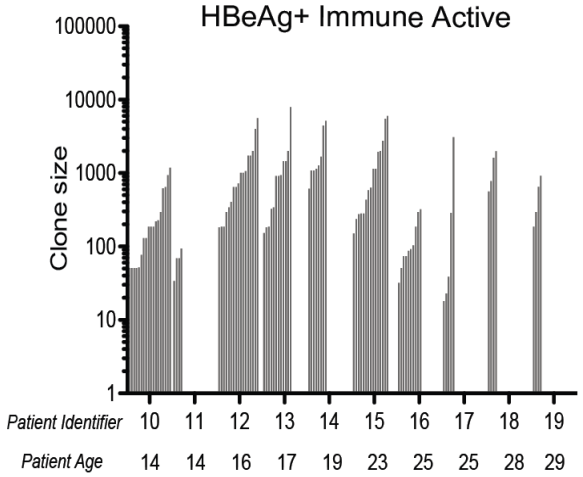
Adapted from *Yeh et al.* Cell Mol Gastroenterol Hepatol 2023; 15:921-929

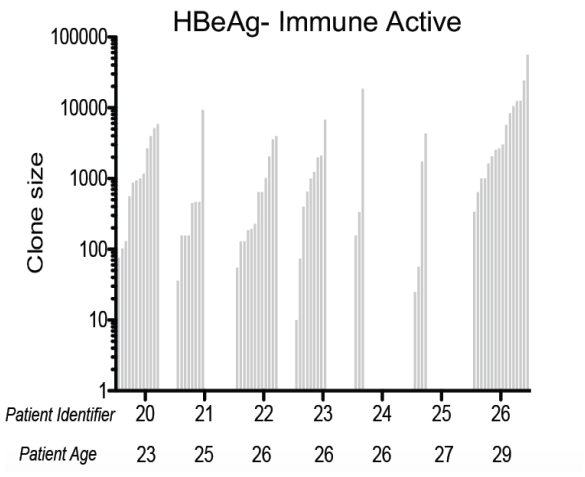
HBV DNA integration Is a barrier to functional cure

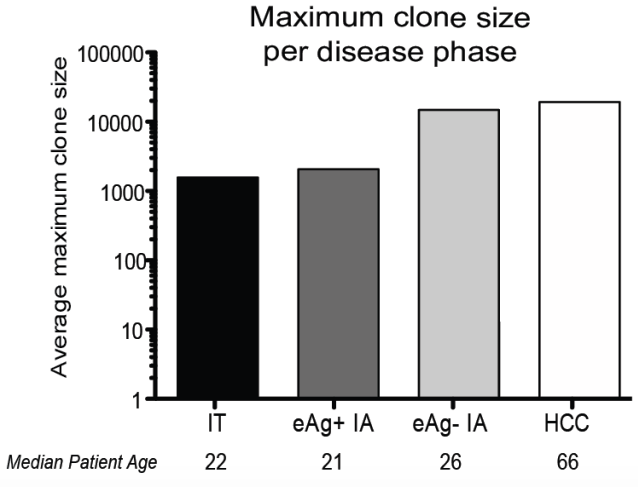
Integration the first step in Hepatocarcinogenesis

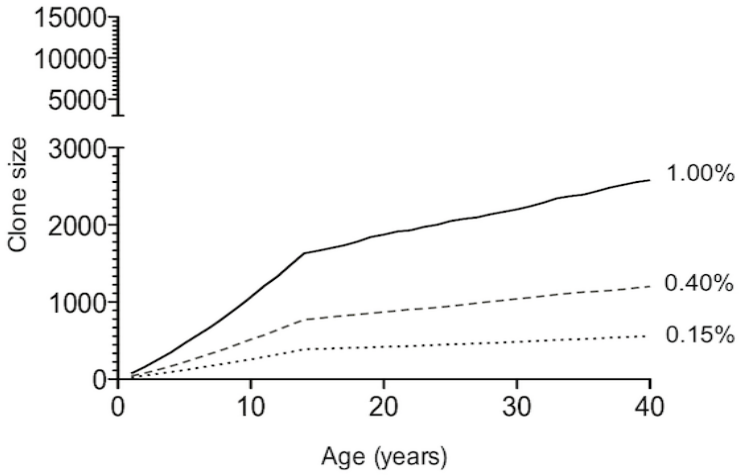
Clonal hepatocyte expansion in 'immune tolerant' patients

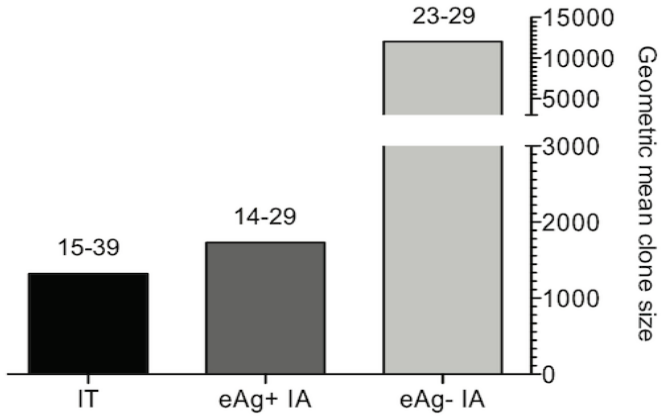






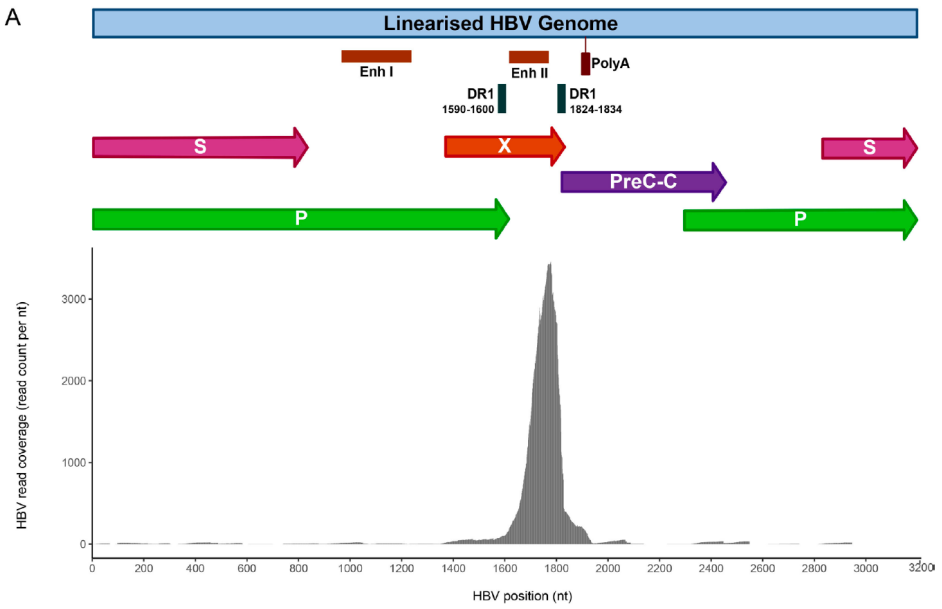






Mason, Gill et al., Gastroenterology 2016

Architecture of HBV integration



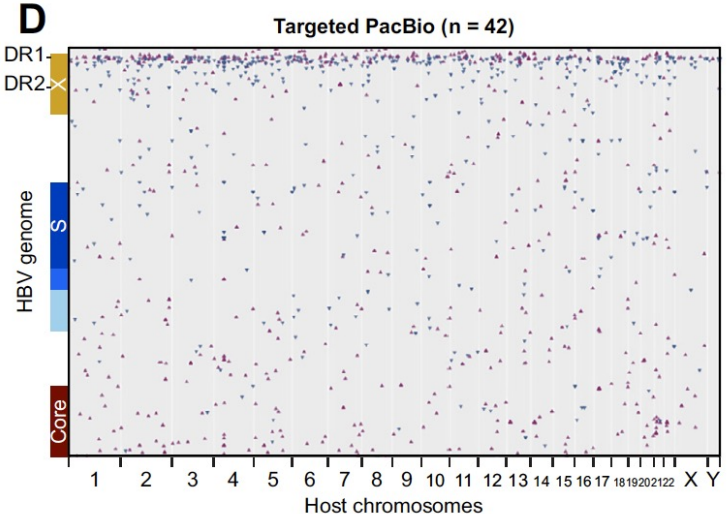
Frequency of active viral integration at each nucleotide position; Yu, Gut 2024

Bill and Summers, PNAS 2004
Mason, Gastroenterology 2016
Tu, JVirol 2018,
Ramirez, J Virol 2021
van Buuren, JHEP Reports 2022
& others…

Scotto, 1983
Lugassy, 1987
Kimbi, 2005
Mason, 2016
Budzinska, 2018
Rydell, 2020
Svicher, Gut 2021
& others…

Integration is found across all CHB phases
(and in acute infections)

PacBio DNA Seq



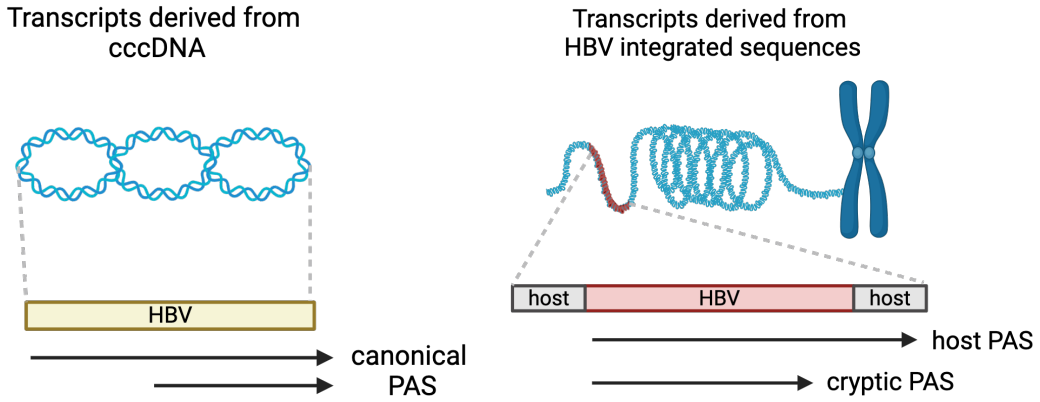
Chimeric reads from all 42 **CHB patients** (GS-US-174-0149)

Baseline Liver Bx samples

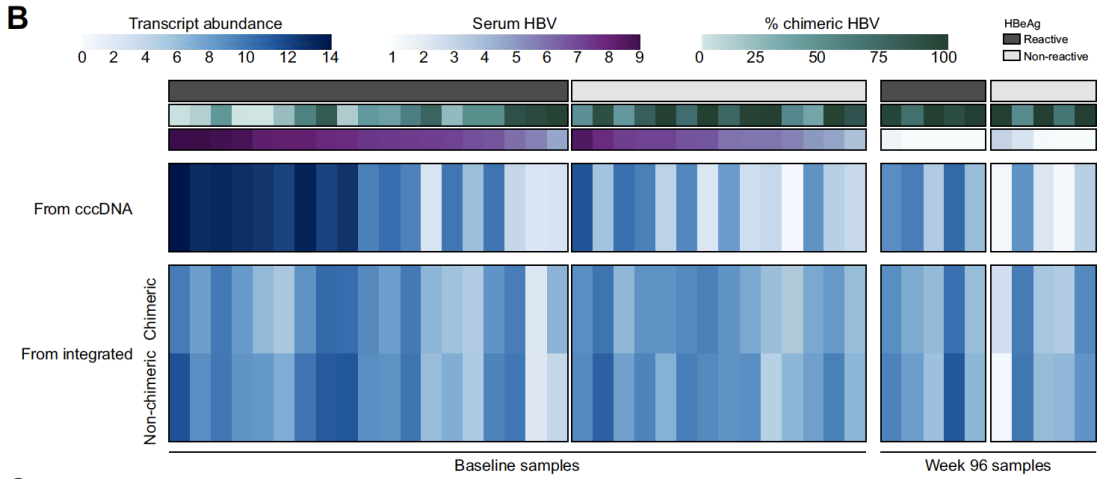
No two patients had identical integration events or patterns shared

Van Buuren, JHEP Reports 2022

HBV transcripts heterogeneity in patients



Iso-Seq RNA sequencing



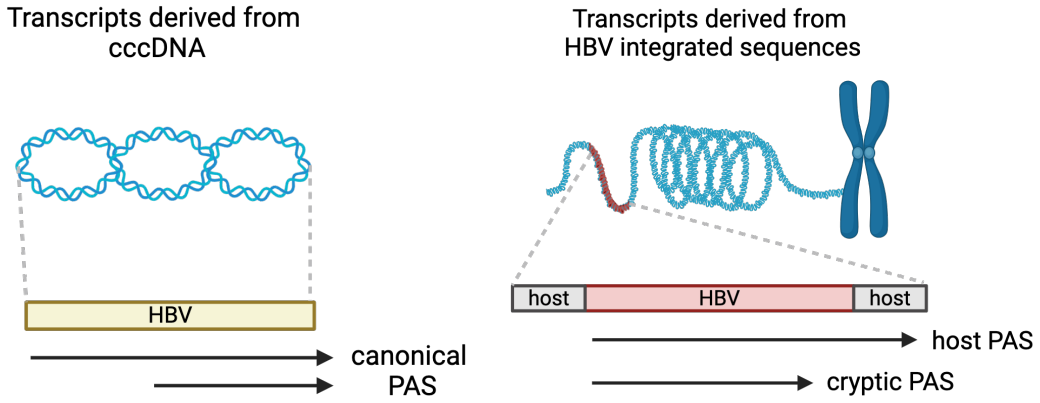
42 **CHB patients** from study GS-US-174-0149
(TDF ± PEG-IFNa for 96 weeks)

Lower transcription* from cccDNA in HBeAg(-) pts

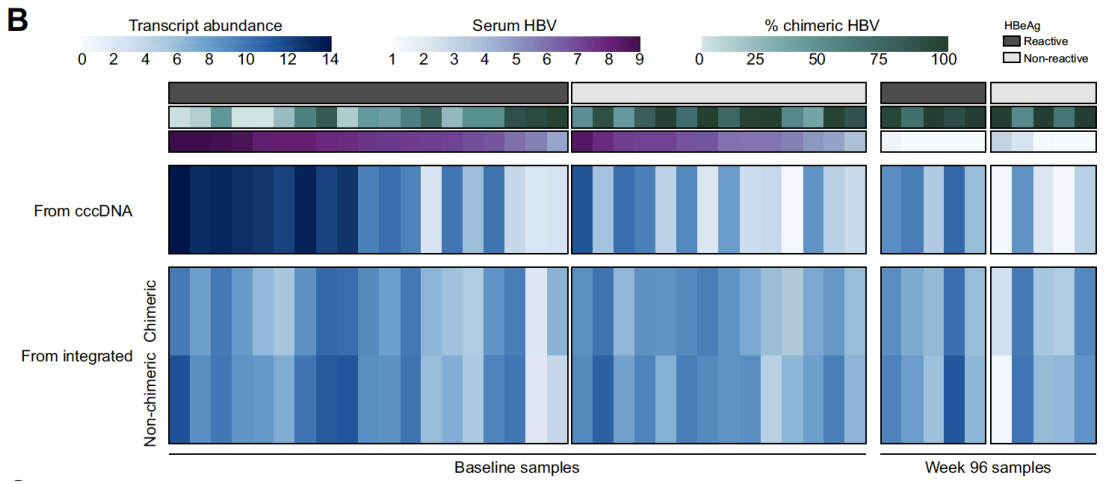
the amount of transcription* from integrations similar between HBeAg(+) and HBeAg(-) pts

**correlated to serum HBV DNA as well as the ratio of chimeric to non-chimeric HBV sequences obtained from targeted DNA-Seq.*

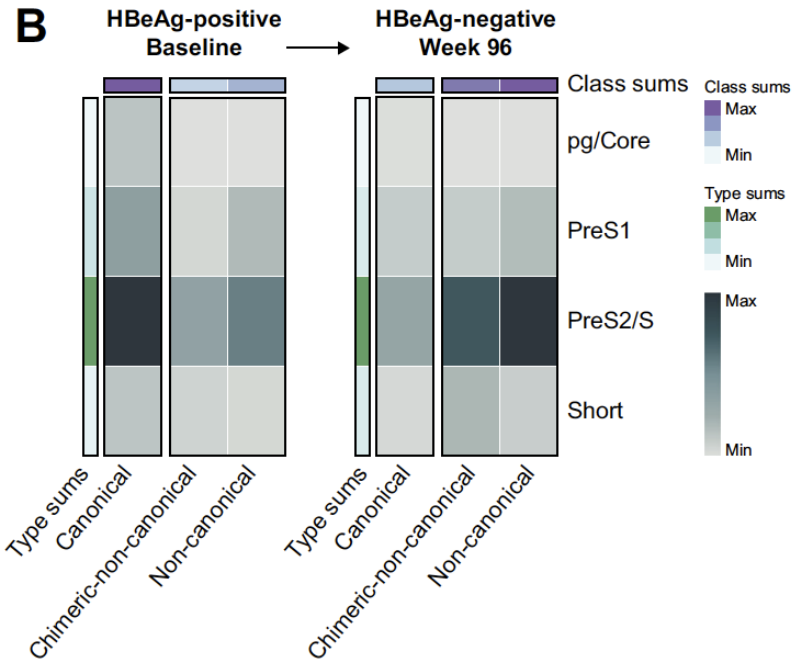
HBV transcripts heterogeneity in patients



Iso-Seq RNA sequencing



Van Buuren, JHEP Reports 2022

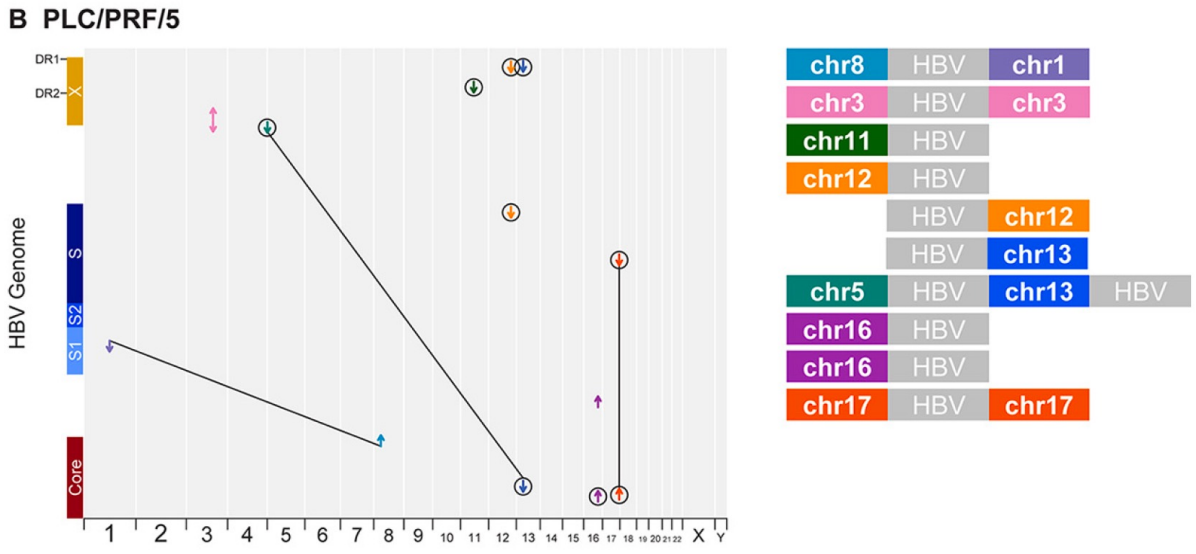


Continuous HBsAg production from integration

Van Buuren, JHEP Reports 2022
Meier, J Hepatol 2021
Svicher, Gut 2021
Grudda, JCI 2022

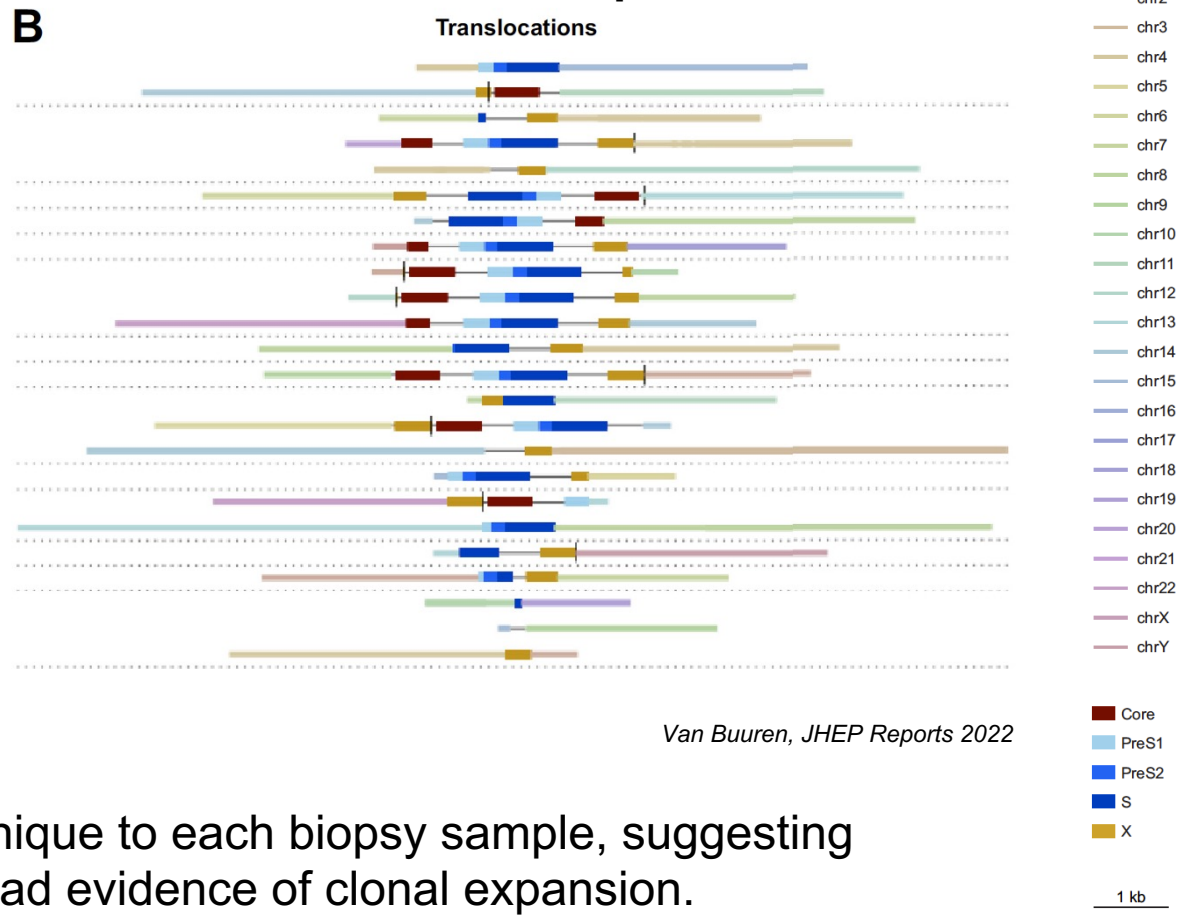
Architecture of HBV integration

In vitro



Ramirez, J Virol 2021

In CHB patients



Van Buuren, JHEP Reports 2022

In CHB patients, chromosomal translocations are unique to each biopsy sample, suggesting that each originated randomly, and in some cases had evidence of clonal expansion.

Van Buuren, JHEP Reports 2022

HBV integration – Impact on host genome

Feature in Which Integration Occurs	Enrichment in HCC	Enrichment in Non-Tumour Tissue
Specific HCC driver genes	Yes, but minority of HCCs (TERT, MLL4)	FN1
Telomeres	Yes	No
CpG islands	Yes	Slight (~2-fold greater than expected)
Repetitive regions (e.g., LINEs and SINEs)	No, except one report [54]	No
Transcriptionally-active sites	Yes	No
Exons and Introns	Yes	Slight
Fragile sites	Yes	No
Promoter regions	Yes	Slight

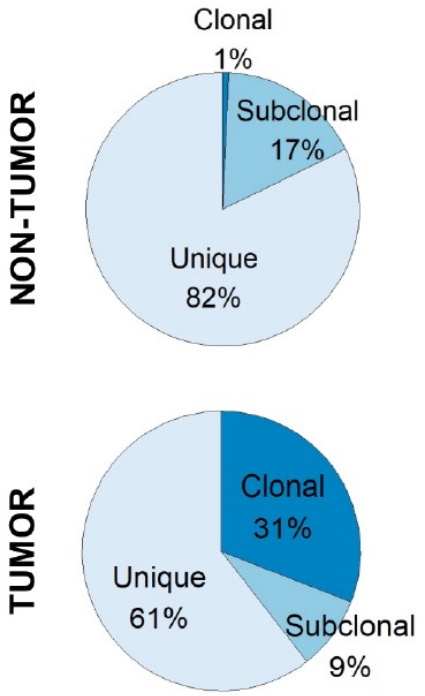
HCC, Hepatocellular Carcinoma; LINEs, Long Interspersed Nuclear Elements; SINEs, Short Interspersed Nuclear Elements.

Adapted from Budzinska, Genes 2018

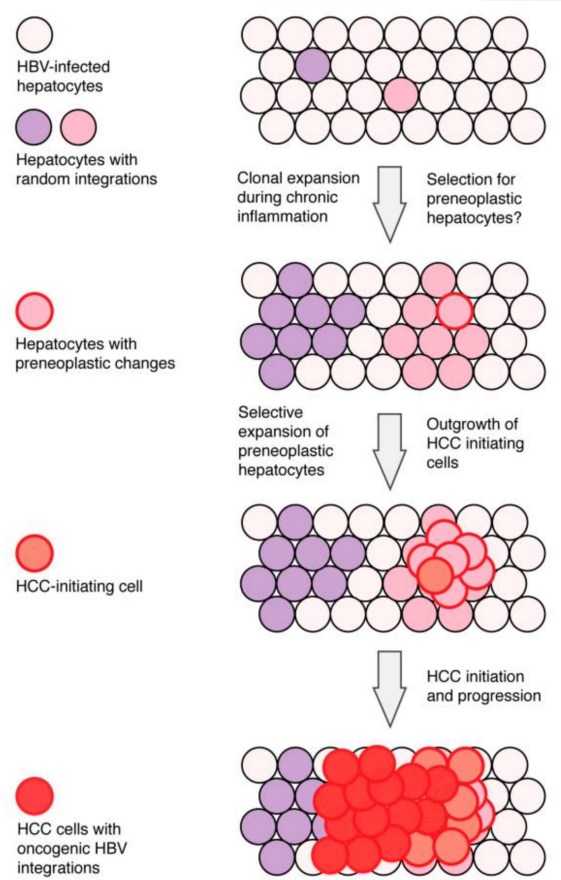
HBV DNA integration sites in HCC tumour tissue are enriched in particular genetic regions (particularly cancer associated genes)

Budzinska, Genes 2018
Svicher, Gut 2021
& others…

Repartition of clonality



Adapted from Péneau, Gut 2021



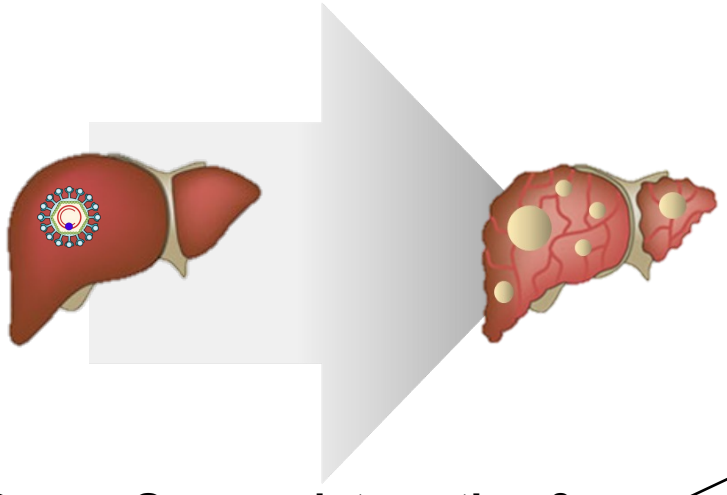
Adapted from Budzinska, Genes 2018

Role of HBV in liver pathogenesis

Continuous balance with immune responses → chronic liver inflammation

High HBV replication

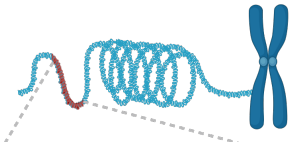
High antigen burden



Impact on transcription of host genes

(Paterlini-Bréchot et al., 2003; Murakami et al., 2005; Saigo et al., 2008; Sung et al., 2012; Kawai-Kitahata et al., 2016; Tang et al. 2020; Sze et al, 2021; Zhao et al, 2016; Cui et al. 2020; Svicher et al, 2021; Peneau et al, 2022)

Genome integration & chromatin interaction



Disruption of chromosomal stability → re-arrangements

(Bailey and Murnane, 2006; Feitelson and Lee, 2007; Zhao et al., 2016; Yu et al., 2017; Álvarez et al., 2021; Bousali et al., 2021; Péneau et al., 2022; Ramirez et al., 2021)

Altered HBx and HBs functions

(Shamay et al., 2001; Tu et al., 2001; Wang et al., 2003, 2006, 2010; Ning-Fang et al., 2008; Warner and Locarnini, 2008; Sze et al., 2013; Ng et al., 2016; Salpini et al., 2017; Zhang Y. et al., 2021; Hu et al, 2020; Jang et al, 2020)

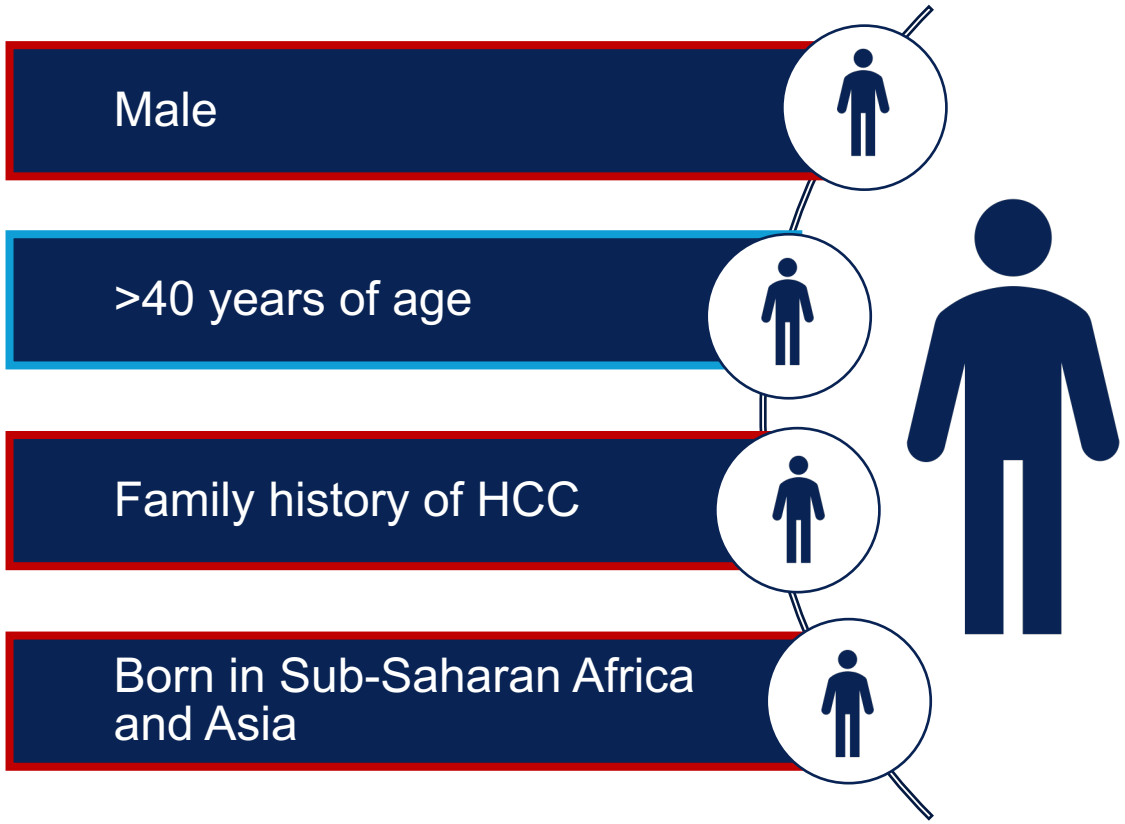
Generation of novel fusion proteins with altered properties

(Heikenwalder and Protzer, 2014; Lau et al., 2014; Liu et al., 2021; Zhang et al, 2021)

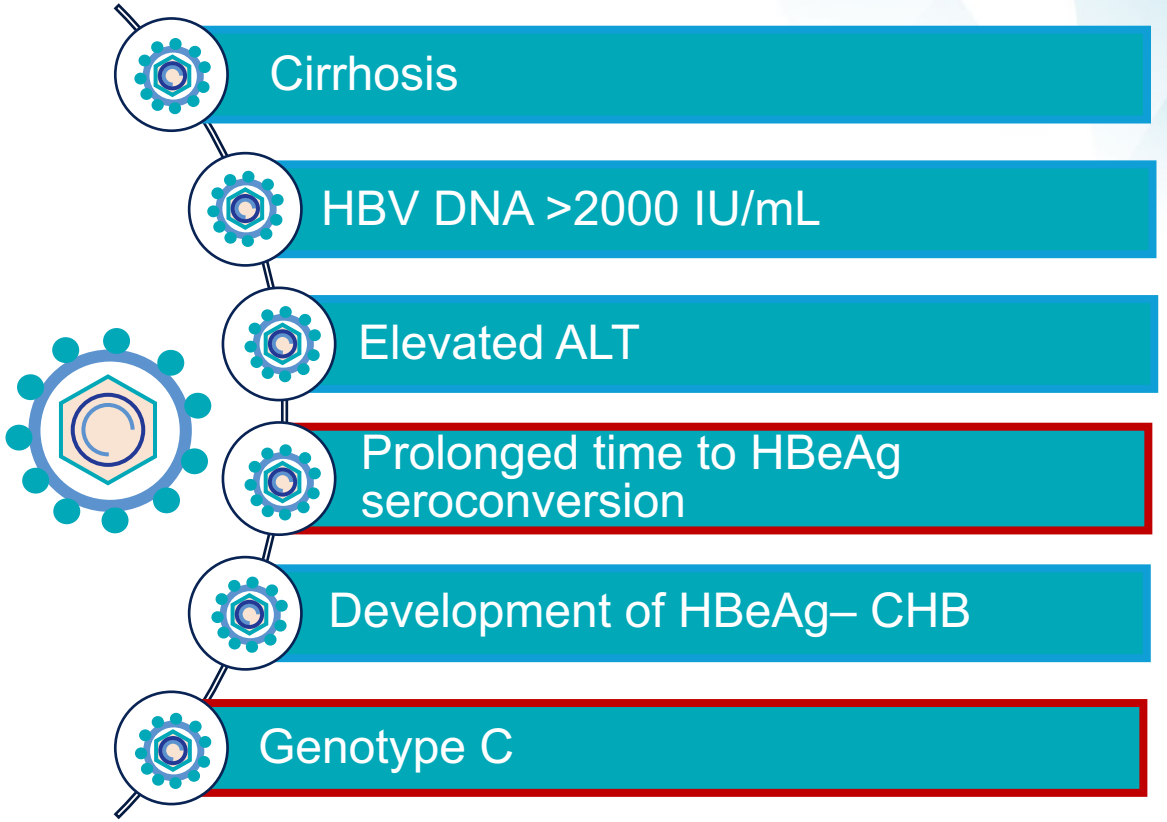
EARLY TREATMENT: a strategy to overcome HBV DNA INTEGRATION

Untreated CHB and HCC risk

Host factors



Viral and disease factors

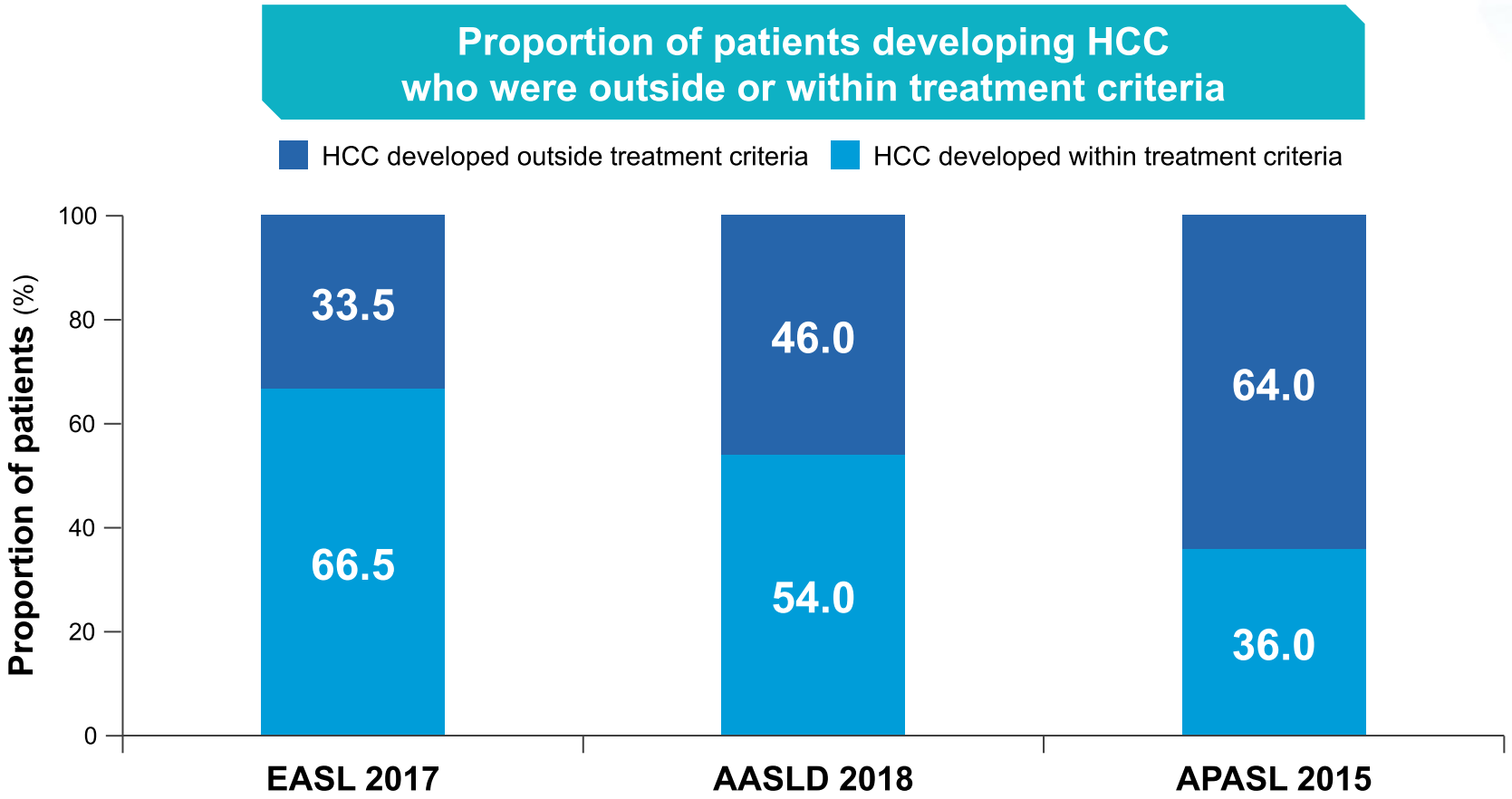


Modifiable factors Non-modifiable factors

- Chen CJ, et al. JAMA 2006;295:65–73; Iloeje UH, et al. Gastroenterology 2006;130:678–86; Wong G, J Hepatol 2018;69:793–802; Tong M, et al. Aliment Pharmacol Ther 2018;47:1181–200; Yip CF, APASL 2018; Oral YIA-C-05; Terrault NA, et al. Hepatology 2018;67:1560–99; Yu MW, et al. J Natl Cancer Inst 2005;97:265–72; Yang HI, et al. NEJM 2002;347:168–74.

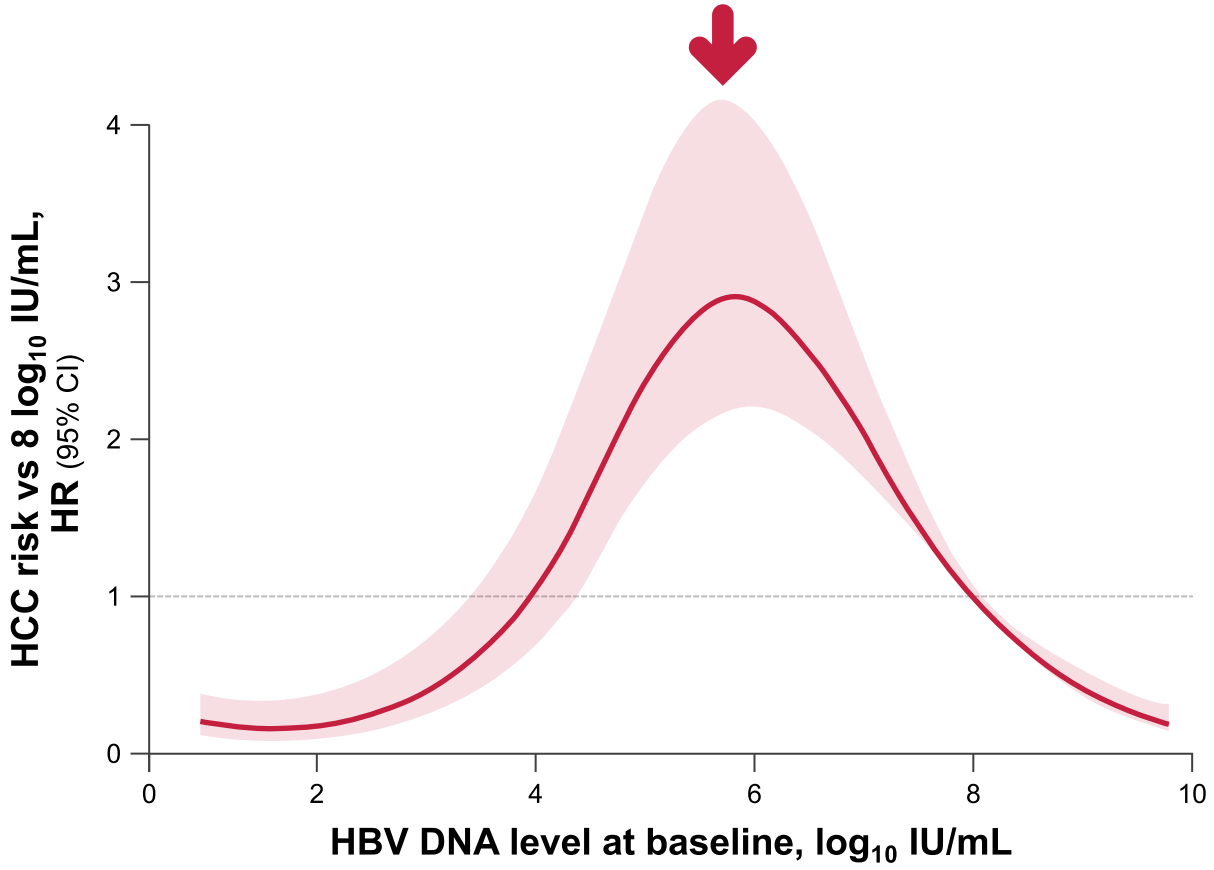
- ALT: alanine aminotransferase; CHB: chronic hepatitis B;
- HBeAg: hepatitis B ‘e’ antigen; HCC: hepatocellular carcinoma.

CHB patients develop HCC outside current international treatment criteria



P-value not reported.
EASL, European Association for the Study of the Liver; **APASL,** Asian Pacific Association for the Study of the Liver.
Adapted from Sinn *et al.* J Viral Hepat 2019;26:1465–1472

Non-Linear Parabolic Association between HBV DNA Levels and HCC Risk

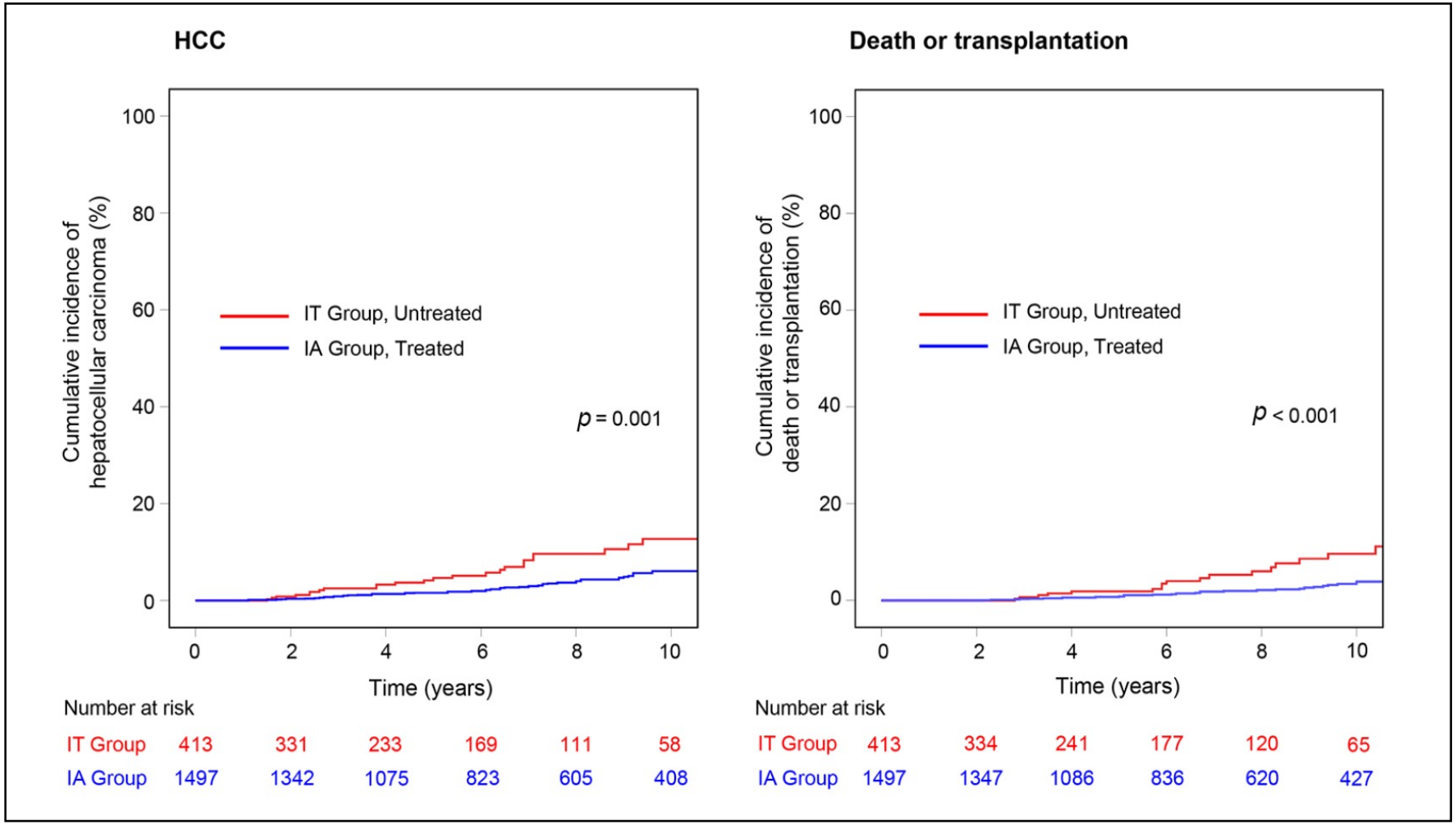


Definition of IT Phase CHB by Guidelines			
Guidelines	**HBV DNA**	**ALT**	**Tx**
APASL 2016	**>2 x 7 $\log_{10}$ IU/mL**	normal	No
EASL 2017	**>7 $\log_{10}$ IU/mL**	normal	No (may consider Tx if age >30)
AASLD 2018	**>6 $\log_{10}$ IU/mL**	Normal or mildly elevated	No

Highest risk of HCC with HBV DNA level around 6 $\log_{10}$ IU/mL

Kim GA, Lim YS, *et al.* Aliment Pharmacol Ther. 2020;51:1169–1179.

Earlier treatment improves disease outcomes



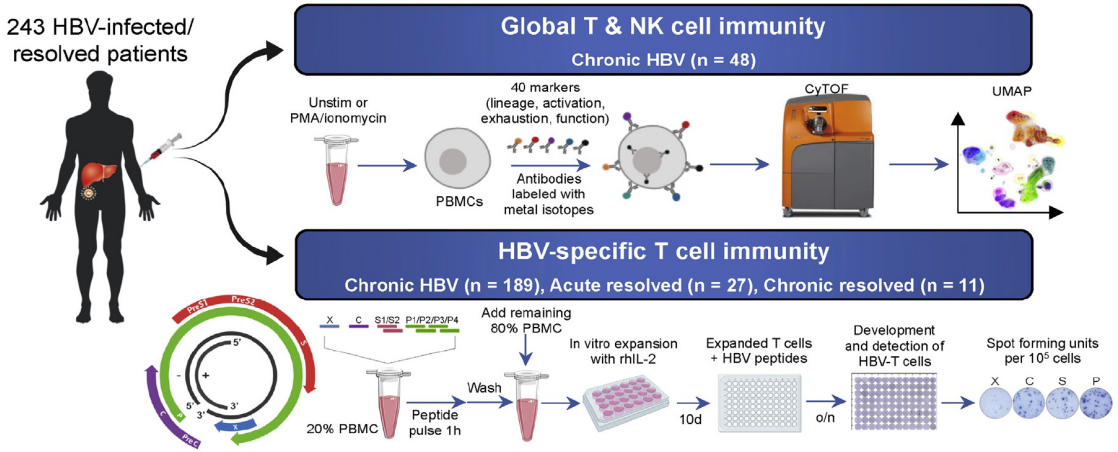
Kim et al., Gut 2018

Age-related immune changes in CHB

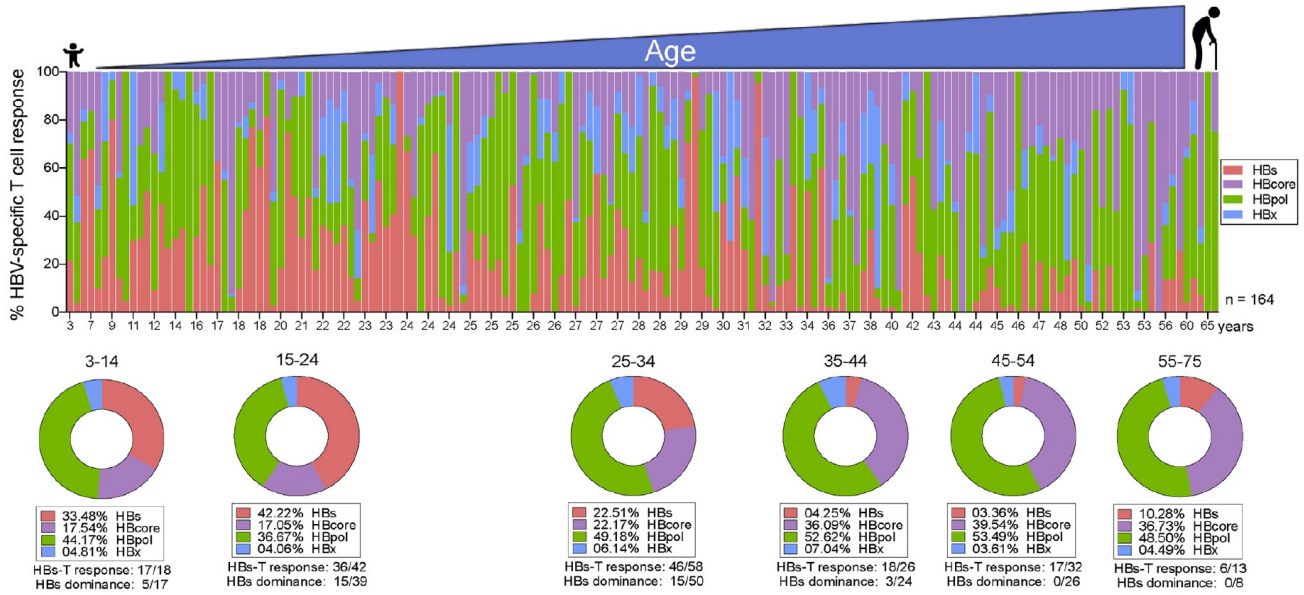
Gastroenterology 2020;159:652–664

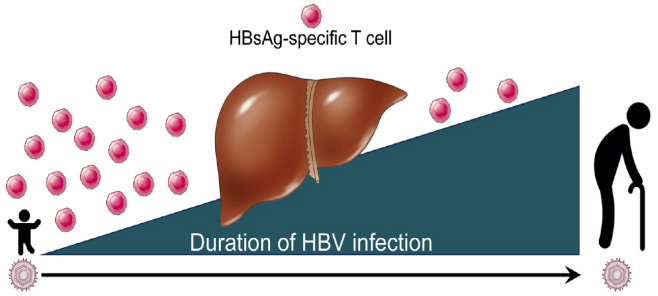
Effects of Hepatitis B Surface Antigen on Virus-Specific and Global T Cells in Patients With Chronic Hepatitis B Virus infection

Nina Le Bert,[1,*] **Upkar S. Gill**,[2,*] **Michelle Hong**,[1,*] Kamini Kunasegaran,[1] Damien Z. M. Tan,[1] Raidah Ahmad,[1] Yang Cheng,[3] Charles-A. Dutertre,[1,3] Andreas Heinecke,[4] Laura Rivino,[1,5] Anthony Tan,[1] Navjyot K. Hansi,[2] Min Zhang,[6] Sujuan Xi,[6] Yutian Chong,[6] Stefan Pflanz,[7] Evan W. Newell,[3] Patrick T. F. Kennedy,[2,§] and Antonio Bertoletti[1,3,§]



HBs-specific T cells reduce based on duration of infection, rather than HBsAg quantity





- *HBV-specific T cells ↓ with age*
- *Earlier treatment may be beneficial for HBsAg loss`*

Le Bert, Gill*, Hong* et al., Gastroenterology 2020*

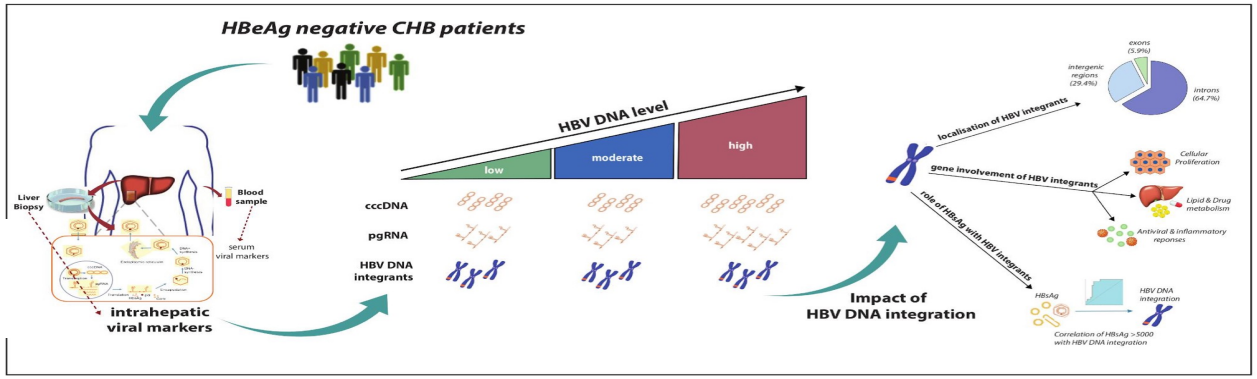
HBV integration in immune control CHB

Hepatology

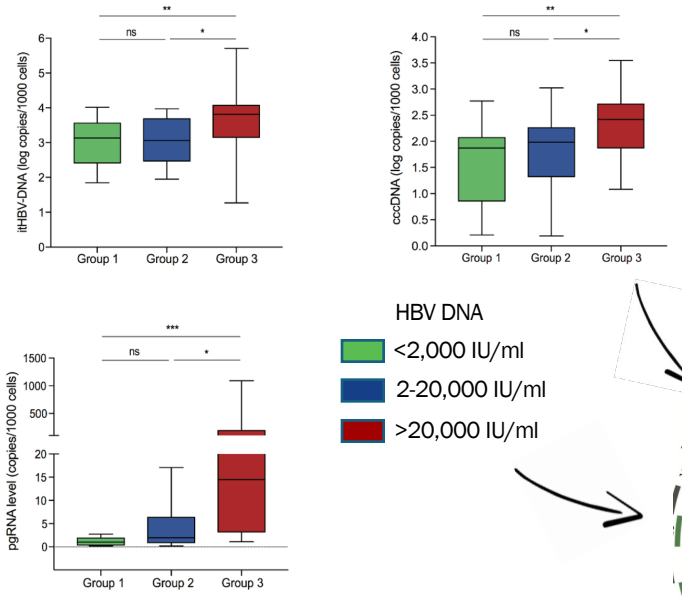
Original research

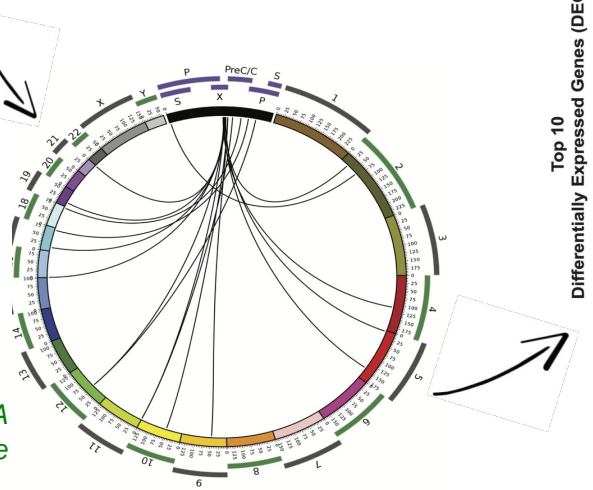
Whole exome HBV DNA integration is independent of the intrahepatic HBV reservoir in HBeAg-negative chronic hepatitis B

Valentina Svicher,[1] Romina Salpini,[1] Lorenzo Piermatteo,[1] Luca Carioti,[1] Arianna Battisti,[1,2] Luna Colagrossi,[1,3] Rossana Scutari,[1] Matteo Surdo,[4] Valeria Cacciafesta,[4] Andrea Nuccitelli,[4] Navjyot Hansi,[2] Francesca Ceccherini Silberstein,[1] Carlo Federico Perno,[5] Upkar S Gill,[2] Patrick T F Kennedy [2]



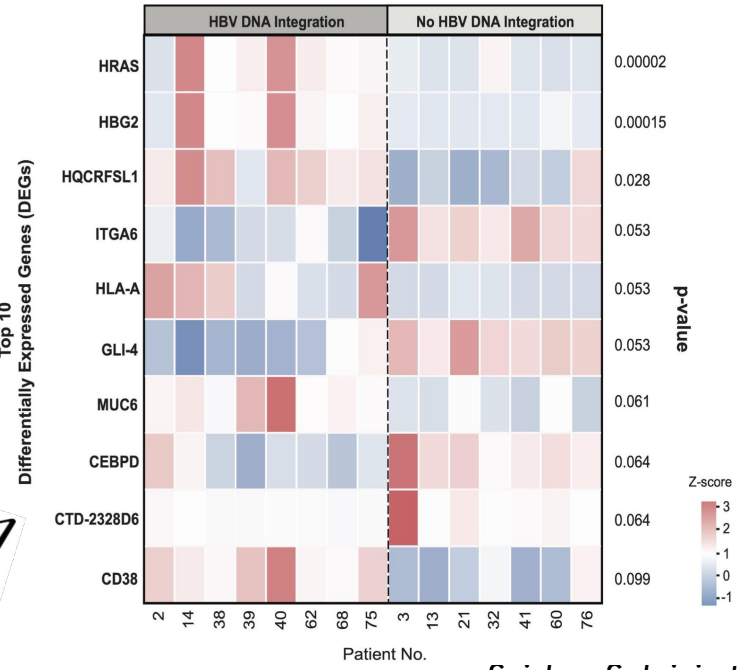
Differential expression of intrahepatic viral markers





Visualisation of HBV DNA integrations within the viral genome

Differentially expressed genes in relation to HBV DNA integration

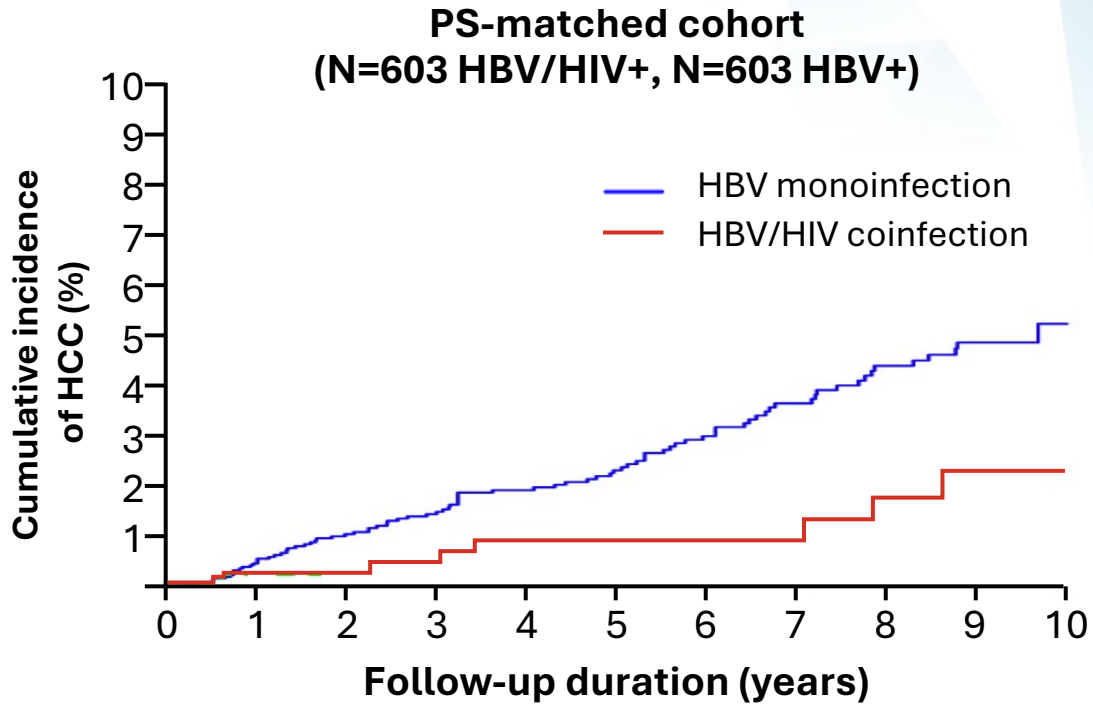


Svicher, Salpini et al., Gut 2020

Treatment as a bridge to FUNCTIONAL CURE

HIV/HBV co-infected patients have a lower risk of HCC compared with treated HBV mono-infected patients

- HIV/HBV and HBV mono-infected patients from Hong Kong from 2000 to 2017*
  - Primary outcome was HCC
- Patients were PS-matched in a 1:5 ratio
- Patient characteristics
  - 85% were male
  - Mean (±SD) age was 42±12 years
  - 4.5% had cirrhosis at baseline
- Weighted Fine–Gray model showed that HIV infection was associated with a lower risk of HCC
  - Subdistribution hazard ratio: 0.39 (95% CI, 0.16–0.94, p=0.036)



Number at risk

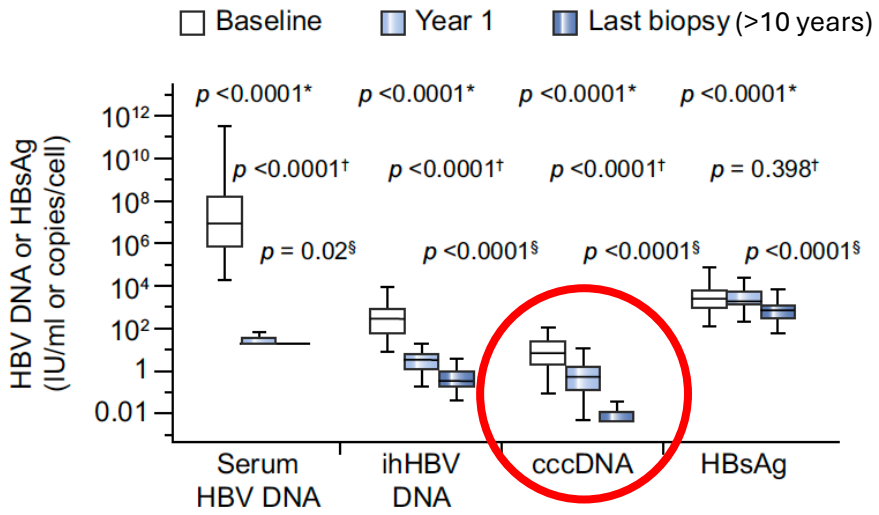
HBV	603	558	486	427	379	325	275	228	188	153	116
HBV/HIV	603	560	494	446	396	353	298	263	214	183	161

HIV/HBV patients start antiviral treatment immediately compared with HBV mono-infected patients

Adapted from Lui G, et al. Open Forum Infectious Diseases 2019;6:S188.

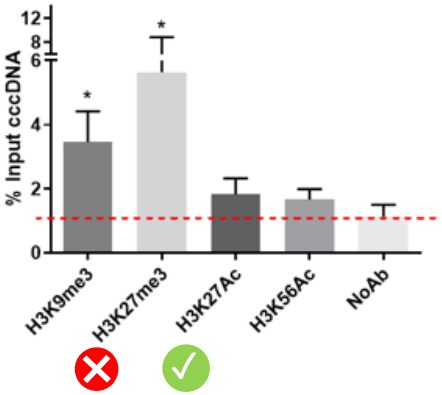
*Patients were excluded if they had HCV, had HCC diagnosed within 6 months, had a follow up of <6 months;
HIV: human immunodeficiency virus; PS: propensity score; SD: standard deviation.

NUCs can reduce cccDNA



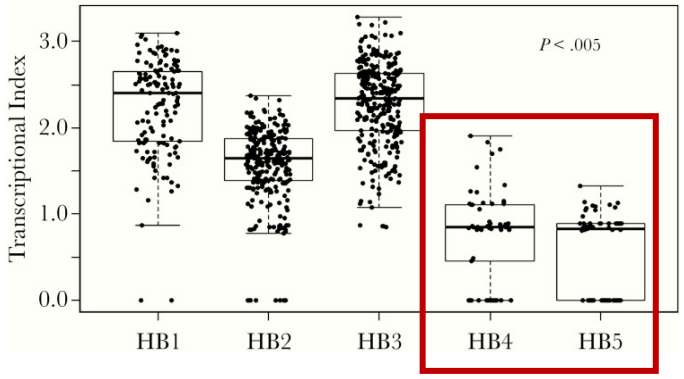
Lai, 2016 & 2020

cccDNA ChIP qPCR



Lebossé, Sci Rep 2020

cccDNA transcriptional index



Balagopal, J Infect Dis 2020
Balagopal, JCI Insight 2020

Decrease in cccDNA pool, but no complete clearance
(incomplete blockade of intrahepatic HBV replication?)

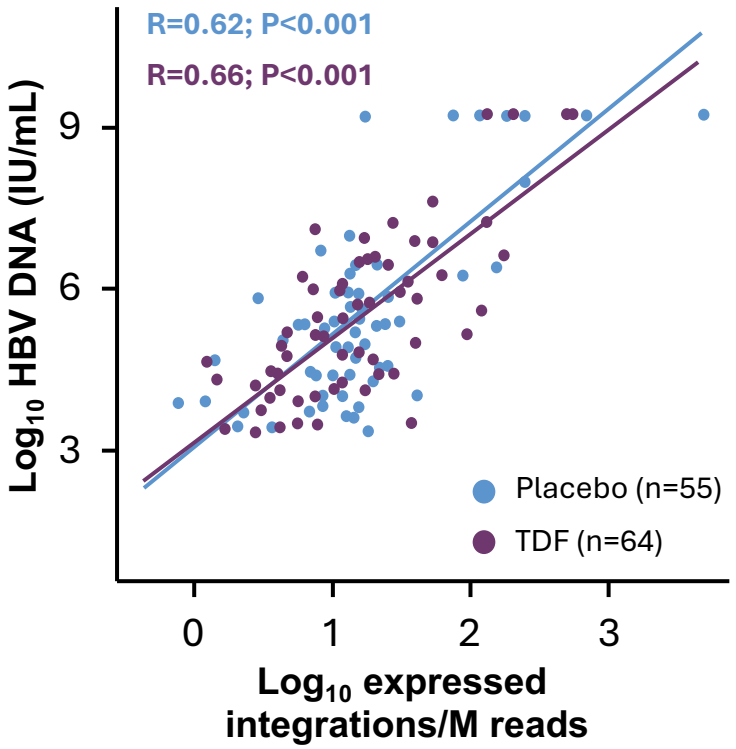
Werle-Lapostolle, Gastroenterology 2004
Boyd, 2016
Lai 2016 & 2020
Lebossé, Sci Rep 2020

Decrease in cccDNA transcriptional activity?
(unknown mechanism so far)

Blockade of viral replication (→ de novo infections)

NA treatment reduces the number of transcriptionally active viral integrations in chronically infected HBV patients

HBV integrations correlate with baseline viral markers



Cancer-related genes are often dysregulated

In **44.1%** (105/238) of samples **≥1 cancer-related gene dysregulated**

NA treatment significantly reduces the number of viral integrations

3.28-fold decrease in number of viral integrations

P=0.037*

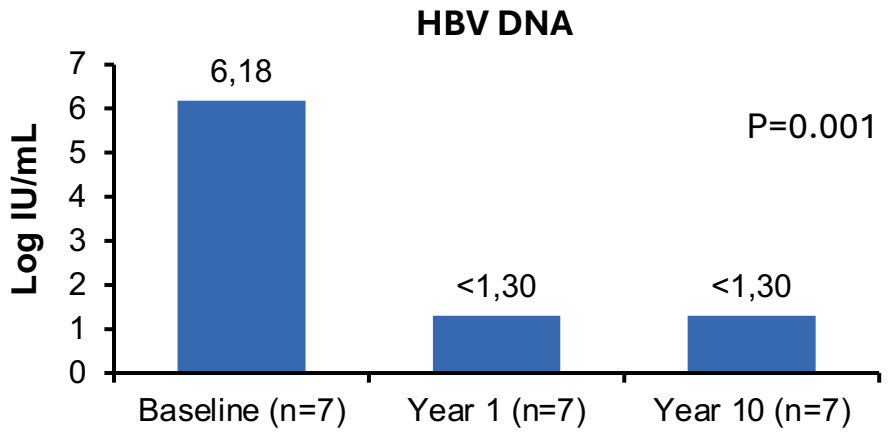
Adapted from Hsu Y-C, et al. Gastroenterology 2022;162:1160–70.

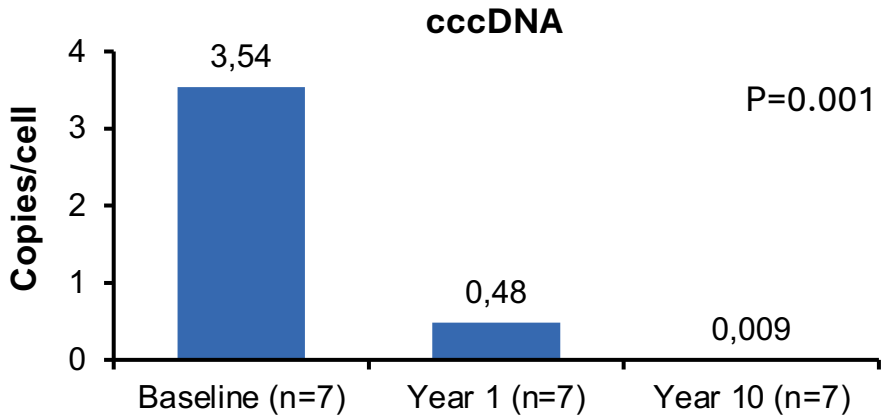
Patients (n=119) who had serum ALT 1–2×ULN, HBV DNA HBV DNA >2000 IU/mL and no clinical liver cirrhosis or decompensation allocated TDF or placebo for 3 years and given had paired liver biopsies.
*Between baseline and Year 3 of treatment compared to the placebo group.
M: million.

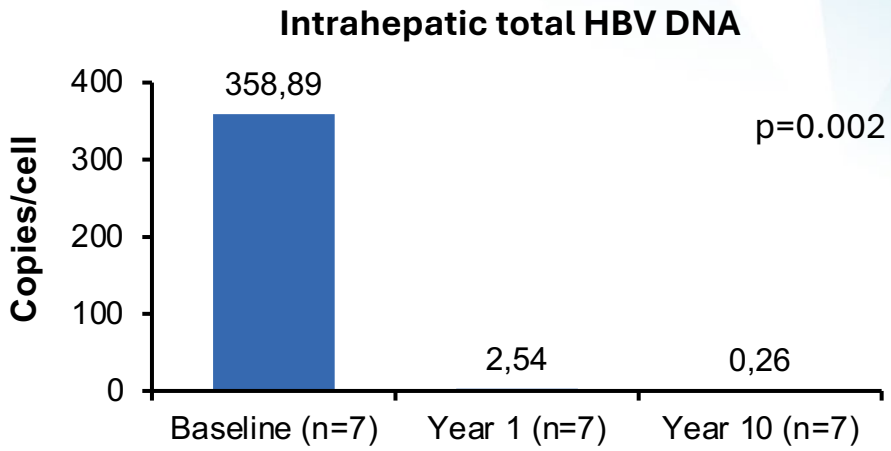
NA treatment significantly reduced the extent of HBV DNA integration and clonal expansion of hepatocytes

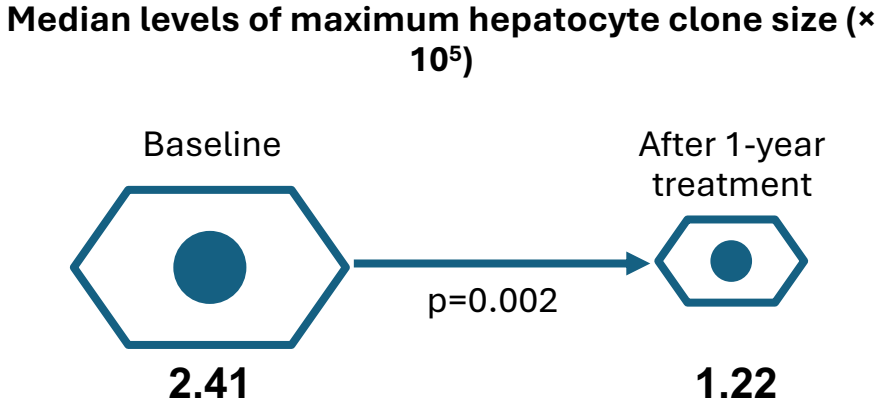
HBV DNA integration analysed from paired liver biopsies before and after treatment in 28 patients

Baseline characteristics	n=28
Male, n	21
HBeAg+, n	14
Mean age, years	45
NA, n LAM LdT ETV	 11 7 10
Median HBV DNA, log_{10} IU/mL	6.4 (5.8–9.0)







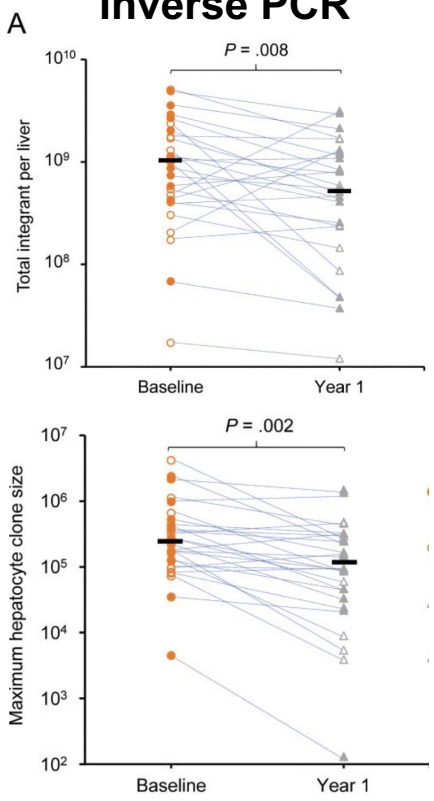


Chow N, et al. Clin Infect Dis 2022;76:e801–9.

All had liver biopsies at baseline and 1 year after treatment. Seven had a third liver biopsy at year 10.
ETV: entecavir; LAM: lamivudine; LdT: telbivudine.

NUCs can also impact HBV integration

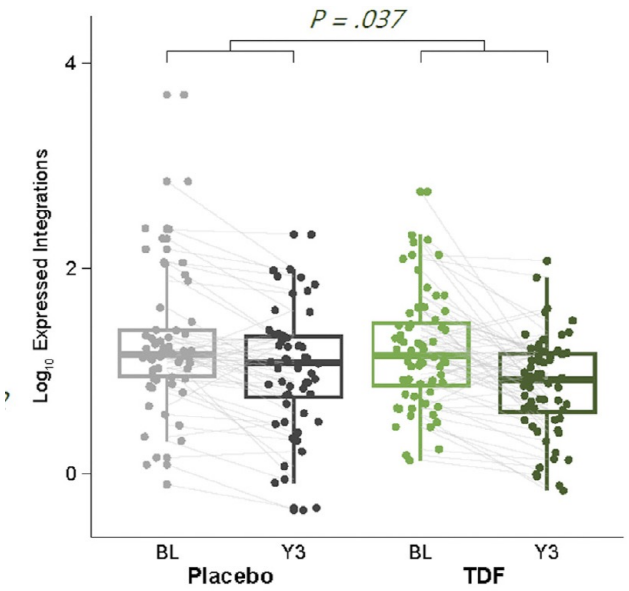
Inverse PCR



Longitudinal biopsies from 28 pts before/after 1 year NUCs

Chow, CID 2022

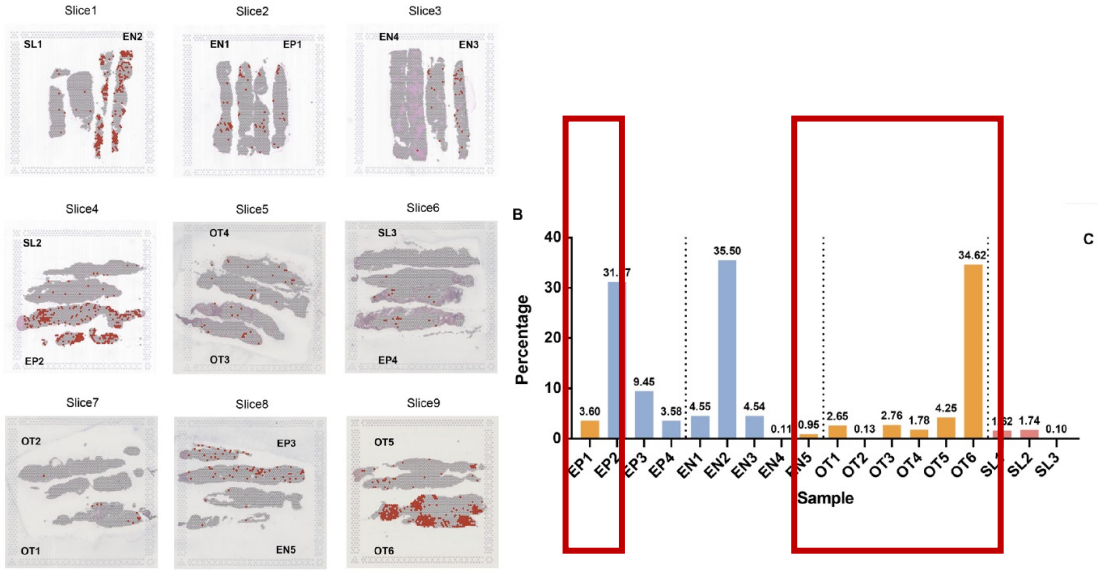
RNA- Sequencing



Longitudinal placebo controlled study on 119 CHB patients before/after 3 years NUCs (TORCH-B)

Hsu, Gastroenterology 2022

Spatial transcriptomics



Cross-sectional study on 18 CHB patients (untreated, NUC-treated, HBsAg loss)

Yu, Gut 2024

Decrease in integration burden: (decrease in number and extent of transcriptionally active events on NUCs)

CONCLUSIONS

- HBV integration is a barrier to functional cure
- Molecular and environmental determinant of HBV integration largely unknown
- NA treatment has been shown to reduce the extent of HBV DNA integration and clonal expansion of hepatocytes
- Expanding treatment criteria will reduce the number of cases of HCC and the complications of CHB
- Early treatment is cost effective and should be employed as a bridge to Functional Cure