HBV RNA Assay Updates and Applications

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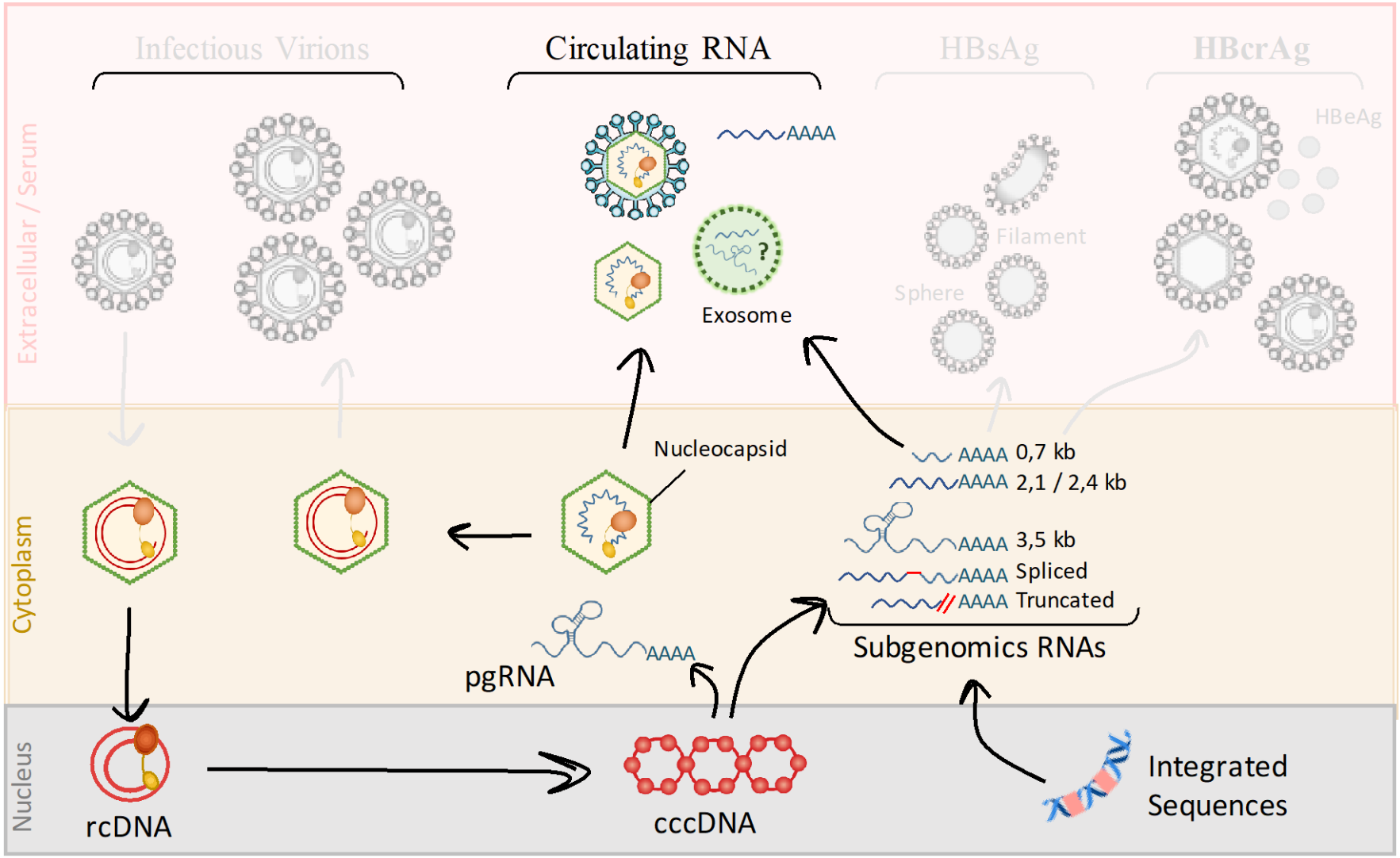
FRANCE 2030

Circulating HBV RNAs



Adapted from Testoni, Sem Liver Dis 2017

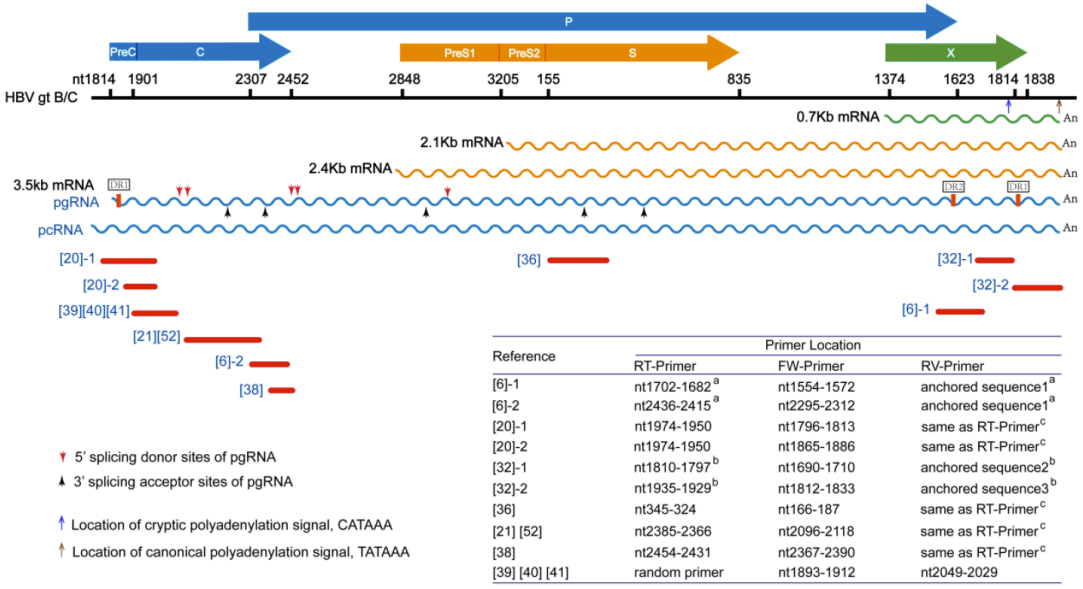
pgRNA is transcribed « only » from cccDNA

Encapsidated pgRNA is the predominant form of circulating HBV RNA

Few studies in CHB patients correlating serum HBV RNA and intrahepatic cccDNA levels/activity

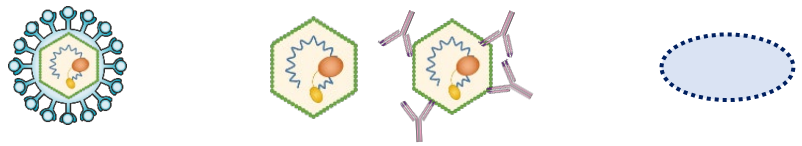
Huang, 2017
Wang, 2021
Testoni, ILC 2021 P-408

Complexity of serum HBV RNAs and assays for their quantification



Reference	Primer Location		
	RT-Primer	FW-Primer	RV-Primer
[6]-1	nt1702-1682[a]	nt1554-1572	anchored sequence1[a]
[6]-2	nt2436-2415[a]	nt2295-2312	anchored sequence1[a]
[20]-1	nt1974-1950	nt1796-1813	same as RT-Primer[c]
[20]-2	nt1974-1950	nt1865-1886	same as RT-Primer[c]
[32]-1	nt1810-1797[b]	nt1690-1710	anchored sequence2[b]
[32]-2	nt1935-1929[b]	nt1812-1833	anchored sequence3[b]
[36]	nt345-324	nt166-187	same as RT-Primer[c]
[21] [52]	nt2385-2366	nt2096-2118	same as RT-Primer[c]
[38]	nt2454-2431	nt2367-2390	same as RT-Primer[c]
[39] [40] [41]	random primer	nt1893-1912	nt2049-2029

Liu, Hepatology 2018



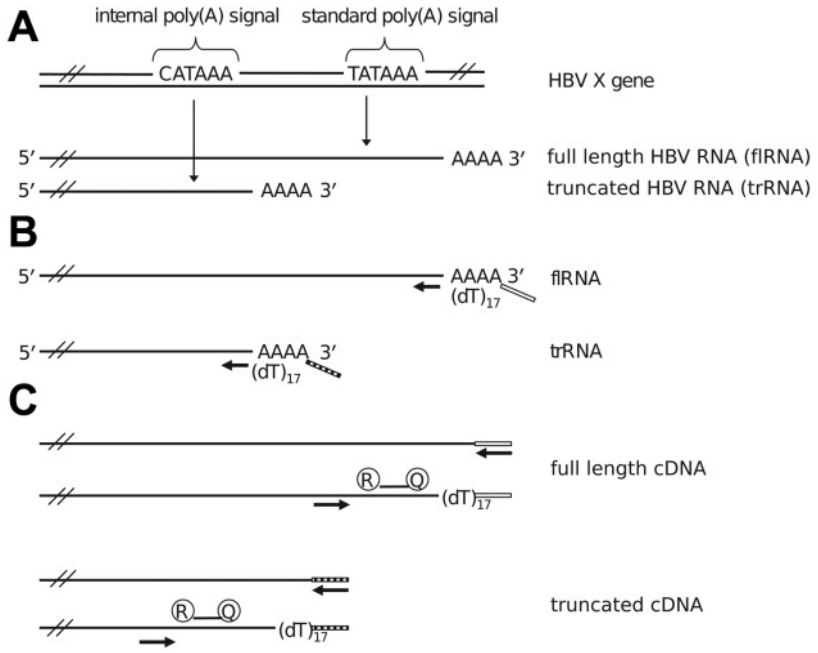
Virion like particles Naked capsids Extracellular Vesicles

Table 1 | **Methods for quantification of HBV RNA in serum**

Method	Details	Reverse transcription primer	Primer sites	LLOQ and LLOD
RT-qPCR	RNA isolation (including DNase treatment) and subsequent PCR method with specific primers either detecting pre-genomic or all HBV RNAs[52,76,165,166]	HBV specific	Precore, X, C or S region	2.55 $\log_{10}$ copies/mL (LLOQ)[10]; 1.85 $\log_{10}$ copies/mL (LLOD)[63] 2.6 $\log_{10}$ copies/mL (LLOD)[75]
Droplet digital PCR	Droplet digital PCR[53,167,168]	HBV specific	all regions	100 copies/mL = 2 $\log_{10}$ copies/mL (LLOD)[79]
3′ Rapid amplification of cDNA ends (RACE)-based	Oligo (dT) primer plus a unique artificial anchored sequence to generate cDNA[63,64,169]	Oligo(dT) primer	Poly(A) tail	2.6–3.4 $\log_{10}$ copies/mL (LLOD)[80,81]
QuantGene assays	Hybridization-based and via branched DNA signal amplification technology–measurement via luminometer[54]	NA	X region	NA
Indirect	HBV (DNA + RNA) minus DNA determined by real-time PCR[170,171] Serum HBV pgRNA minus HBV pcRNA determined by real-time PCR[172]	HBV specific	Precore and C region	2.2–2.3 $\log_{10}$ copies/mL (LLOD)[170–172]
Commercial RNA assays (currently research use only)				
Abbott[a]	Serum HBV RNA, real-time PCR[74]	NA	NA	10 copies/mL (LLOD, V2)
Roche[b173]	Serum HBV RNA, real-time PCR	NA	NA	10 copies/mL (LLOQ); 10–10^9 copies/mL (linear range)

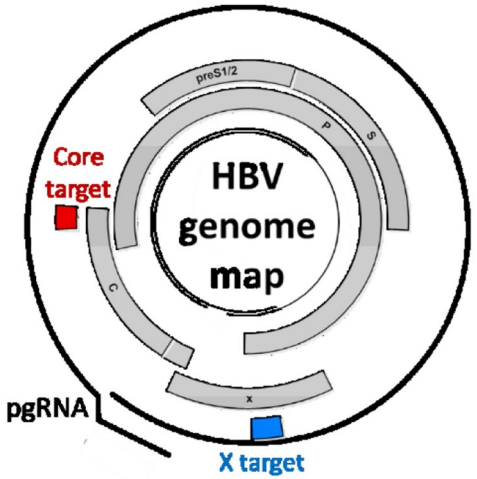
Kramvis, Nat Rev Gastroenterol Hepatol 2022

HBV RNA PCR assays (RUO)



3’ RACE PCR assay

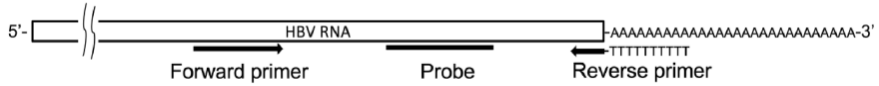
Van Bommel F et al, Hepatology, 2014



Abbott real time PCR assay

Primers and probes are designed to conserved regions within the 5’ end of the X gene and the 3’ end of the core gene
Targets are independently detected

Butler, Hepatology 2018;
Anderson, CID 2021
Anderson, Hepatol Commun 2023



Roche cobas PCR assay

Primers and probes located across 3’ end canonical polyadenylation signal (lost in integrated HBV DNA)

Scholtès, J Clin Virol 2022
Jackson, J Med Virol 2022

ANR-17-RHUS-0003

Journal of Clinical Virology 150-151 (2022) 105150

Contents lists available at ScienceDirect

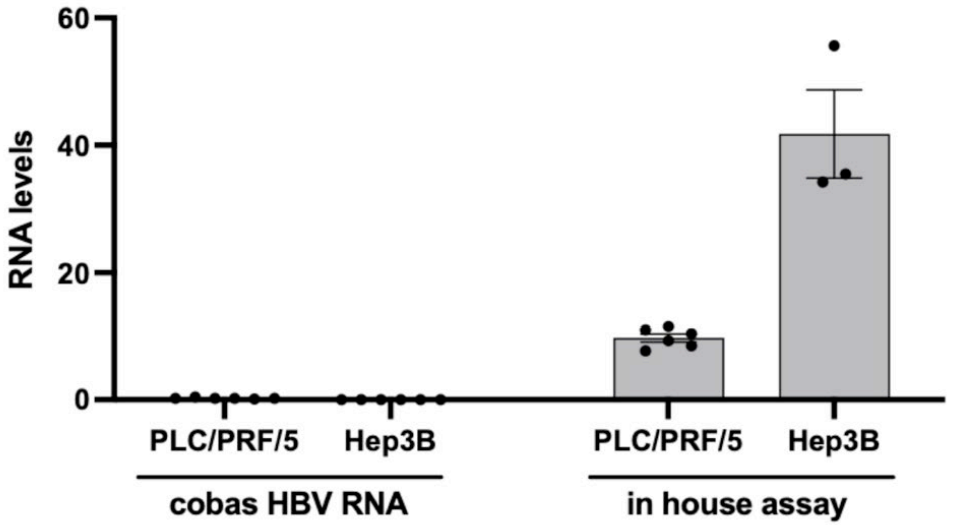
Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Performance of the cobas® HBV RNA automated investigational assay for the detection and quantification of circulating HBV RNA in chronic HBV patients

- Target: 3' end of HBV RNAs
- Linearity between 10 and 10^7 copies/mL in clinical samples, and up to 10^9 copies/mL with synthetic RNA.
- HBV genotype inclusivity.
- Excellent precision and reproducibility: standard deviation < 0.15 $\log_{10}$ copies/mL ; coefficients of variation < 5%.
- LOD 5 copies/mL, LLOQ 10 copies/mL.
- Minimal impact of HBV DNA (<0.3 $\log_{10}$ copies/mL) on HBV RNA quantification at DNA:RNA ratios of up to 10^6.
- cirB-RNA concentrations approximately 200-fold lower than HBV DNA.

Scholtès, J Clin Virol 2022
Jackson, J Med Virol 2022

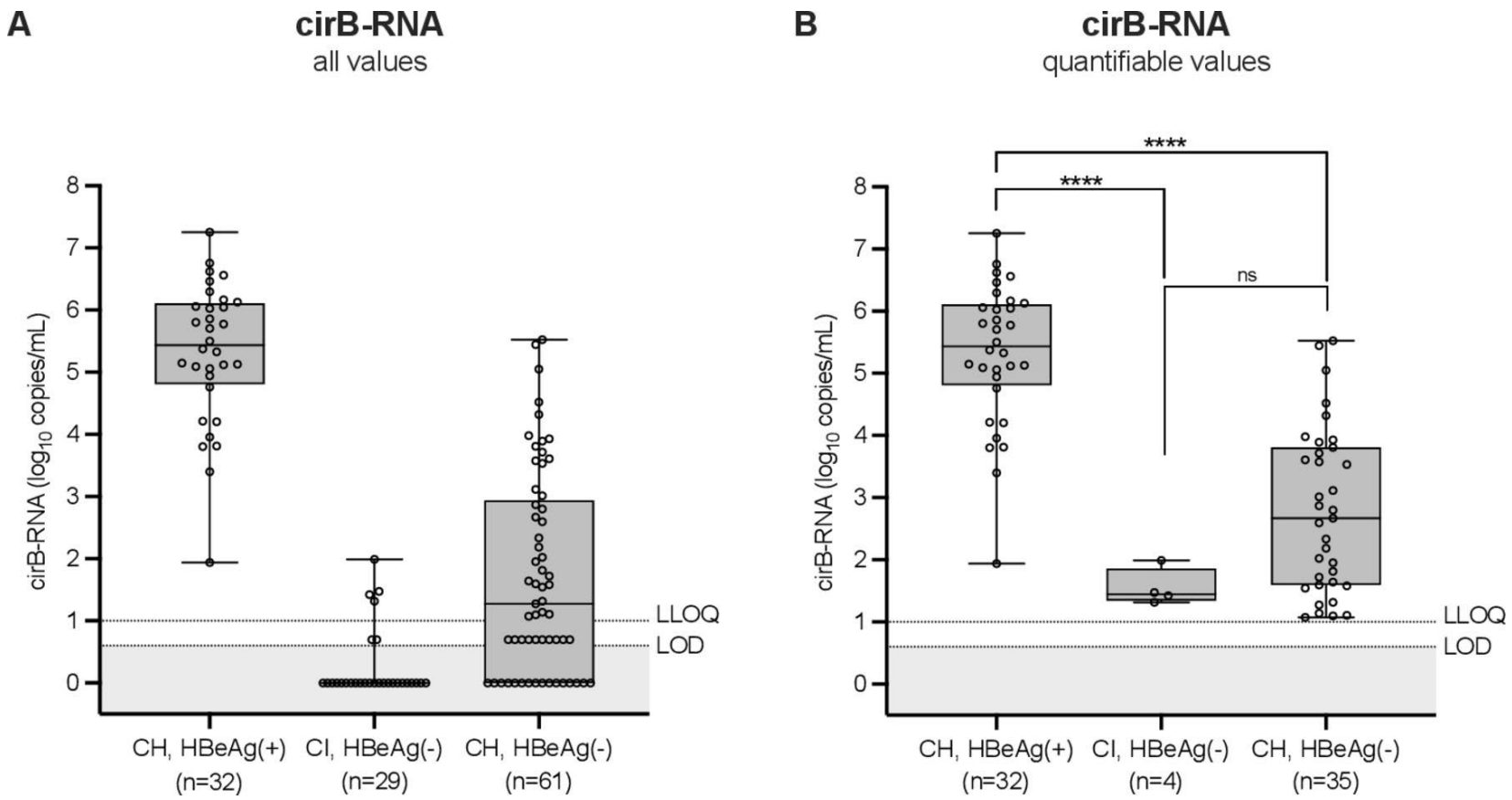


Preferential detection of HBV RNAs derived from cccDNA

Testoni et al. Gut 2023

cirB-RNA distribution according to disease status (untreated patients)

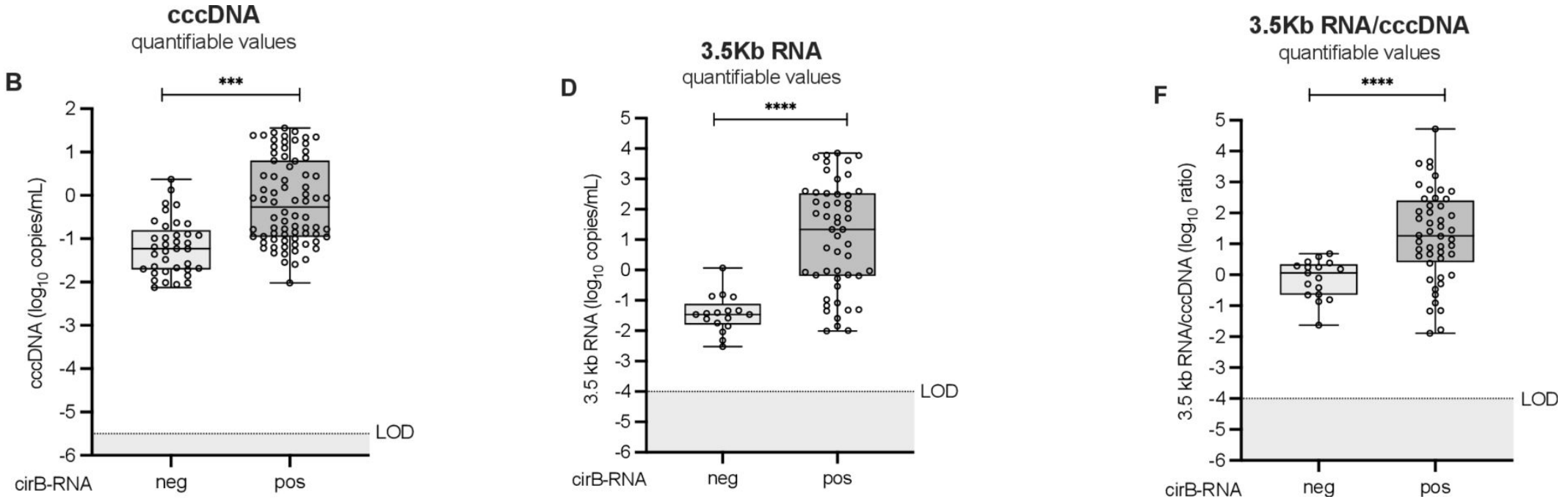
122 patients untreated patients with core liver biopsy



Barbara Testoni et al. Gut doi:10.1136/gutjnl-2023-330644

GUT

Intrahepatic viral markers in patients with detectable versus undetectable cirB-RNA (all untreated patients)



Barbara Testoni et al. Gut doi:10.1136/gutjnl-2023-330644

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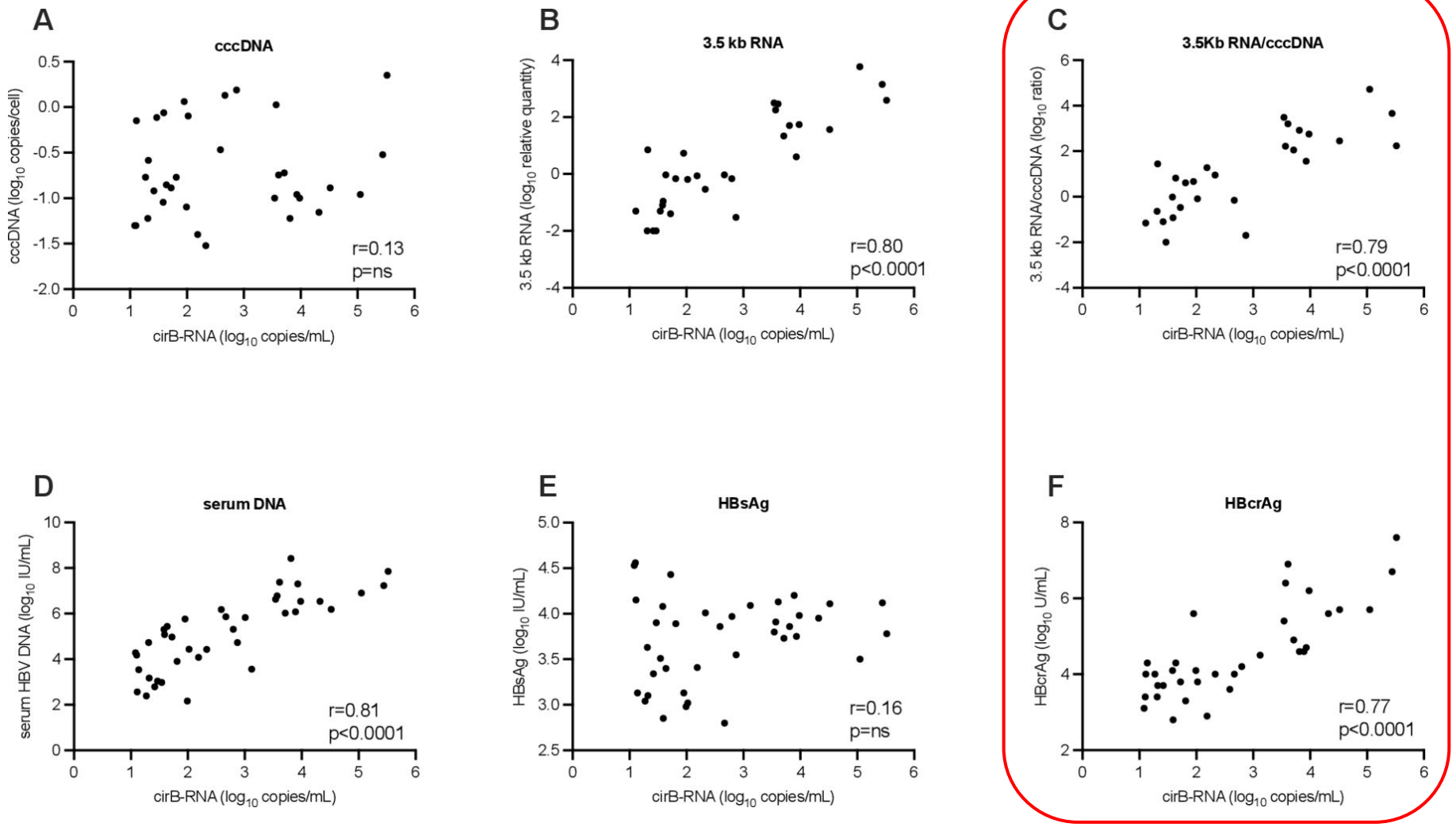
Correlations between cirB-RNA and intrahepatic and serum viral markers (HBeAg(–) untreated patients)

Liver

Serum



Testoni et al. Gut doi:10.1136/gutjnl-2023-330644

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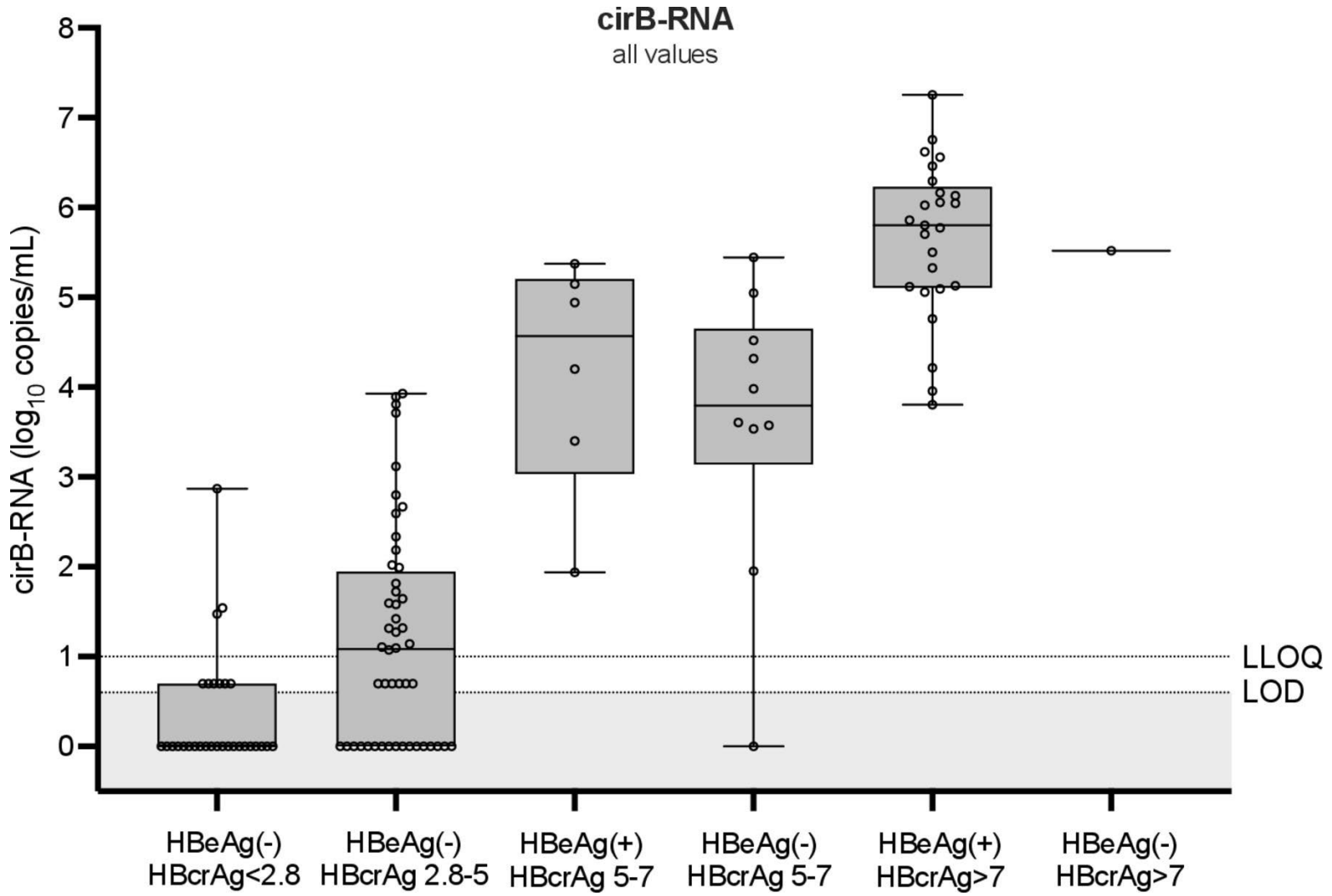
cirB-RNA distribution according to HBcrAg levels (untreated patients)



Testoni et al. Gut doi:10.1136/gutjnl-2023-330644

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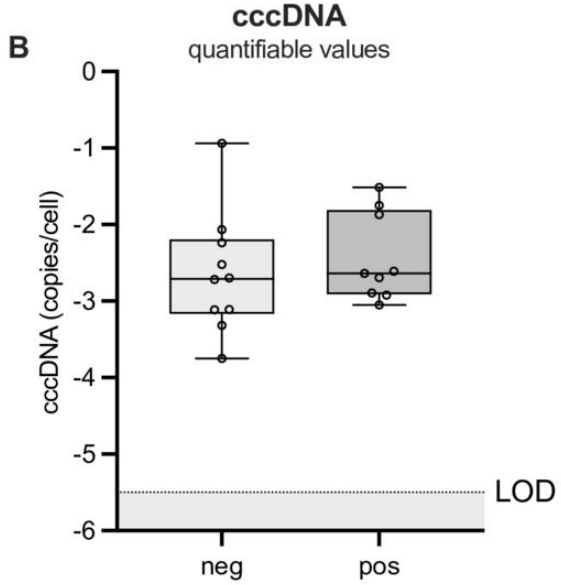
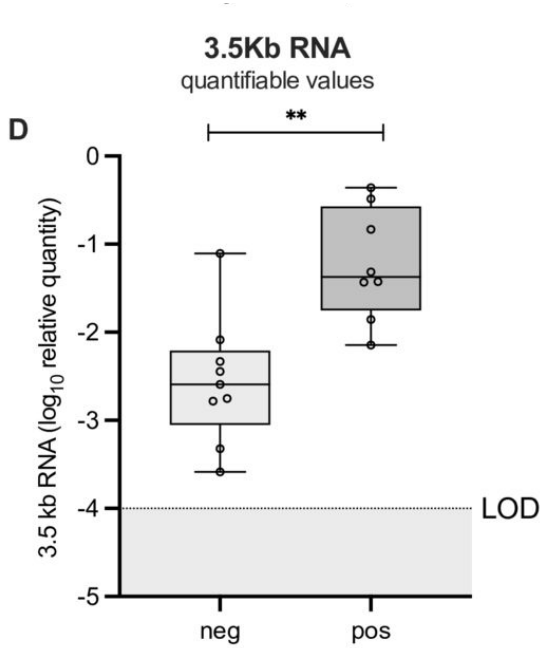
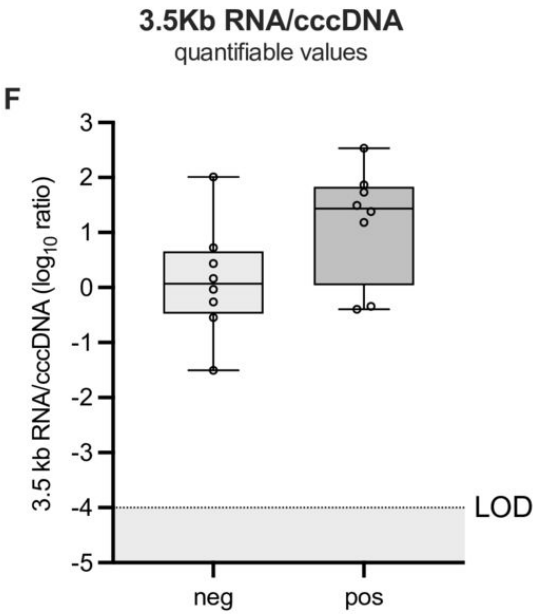
cirB-RNA and intrahepatic viral markers in NUC-treated patients

25 NUC treated patients with available liver samples







Testoni et al. Gut doi:10.1136/gutjnl-2023-330644

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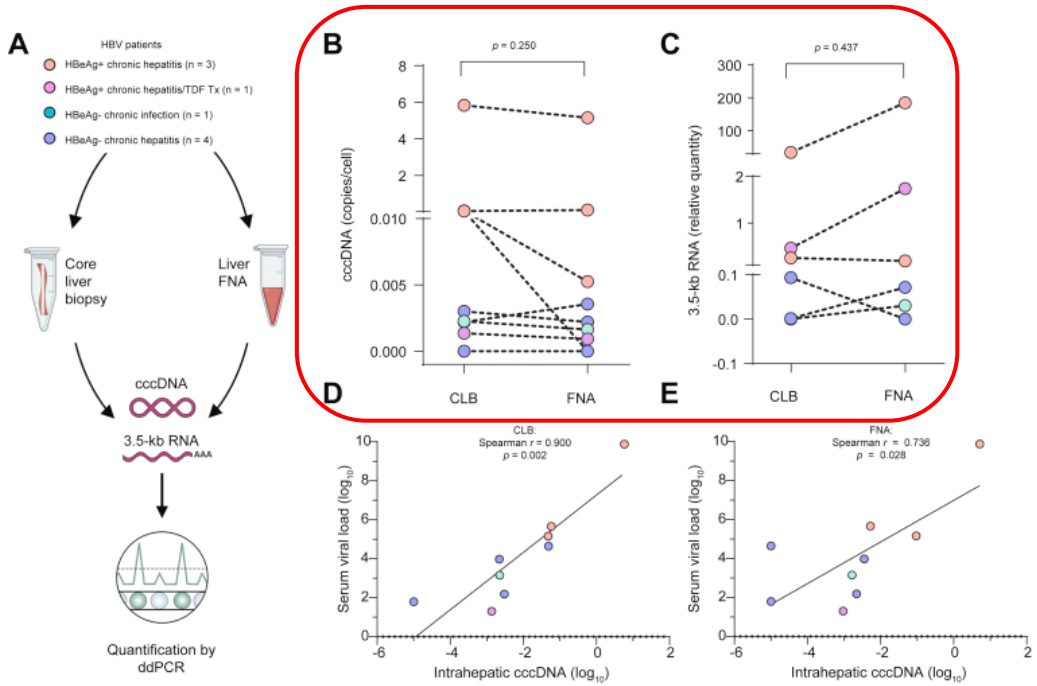
Evaluation of the HBV liver reservoir with fine needle aspirates



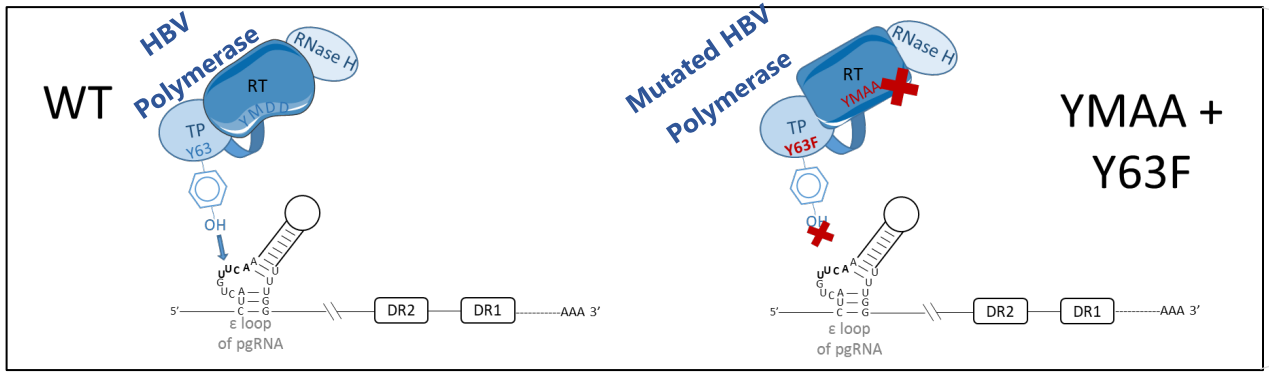
Age (years)	Sex	Ethnic origin	Genotype	HBsAg (log IU/ml)	HBeAg	HBeAb	HBV DNA (log IU/ml)	HBcrAg (log U/ml)	HBV RNA (log IU/ml)	ALT	Ishak fibrosis stage (/6)	EASL category
30	Male	Caucasian – European	D	4.88	Neg	Pos	2.19	3.7	N.D.	55	2	HBeAg– chronic hepatitis
30	Female	Asian – Bangladeshi	n.d.	4.34	Pos	Neg	9.88	9.1	8.8	100	3	HBeAg+ chronic hepatitis
33	Male	Asian – Bangladeshi	D	3.77	Neg	Pos	3.98	3	N.D.	34	1	HBeAg– chronic hepatitis
59	Male	Asian – Bangladeshi	n.d.	2.81	Neg	Pos	3.15	<2	d.n.q.	13	0	HBeAg– chronic infection
35	Male	Asian – Bangladeshi	C	1.41	Pos	Neg	5.16	6.6	3.5	65	3	HBeAg+ chronic hepatitis
35	Male	Asian – Pakistani	n.d.	3.89	Pos	Neg	1.30	5.3	2.8	54	2	HBeAg+ chronic hepatitis
42	Male	Afro Caribbean	n.d.	2.26	Neg	Pos	1.79	3	N.D.	35	1	HBeAg– chronic hepatitis
22	Male	Afro Caribbean	E	4.16	Pos	Neg	5.67	5.5	2.6	25	2	HBeAg+ chronic hepatitis
33	Male	Asian – Bangladeshi	A	4.42	Neg	Pos	4.64	4	1.8	19	1	HBeAg– chronic hepatitis

Testoni & Roca-Suarez et al, JHEP Rep . 2023 Jul 11;5(10):100841. doi: 10.1016/j.jhepr.2023.100841. eCollection 2023 Oct.

Generation of a stable cell line overexpressing and secreting HBV RNA particles

Potential advantages:

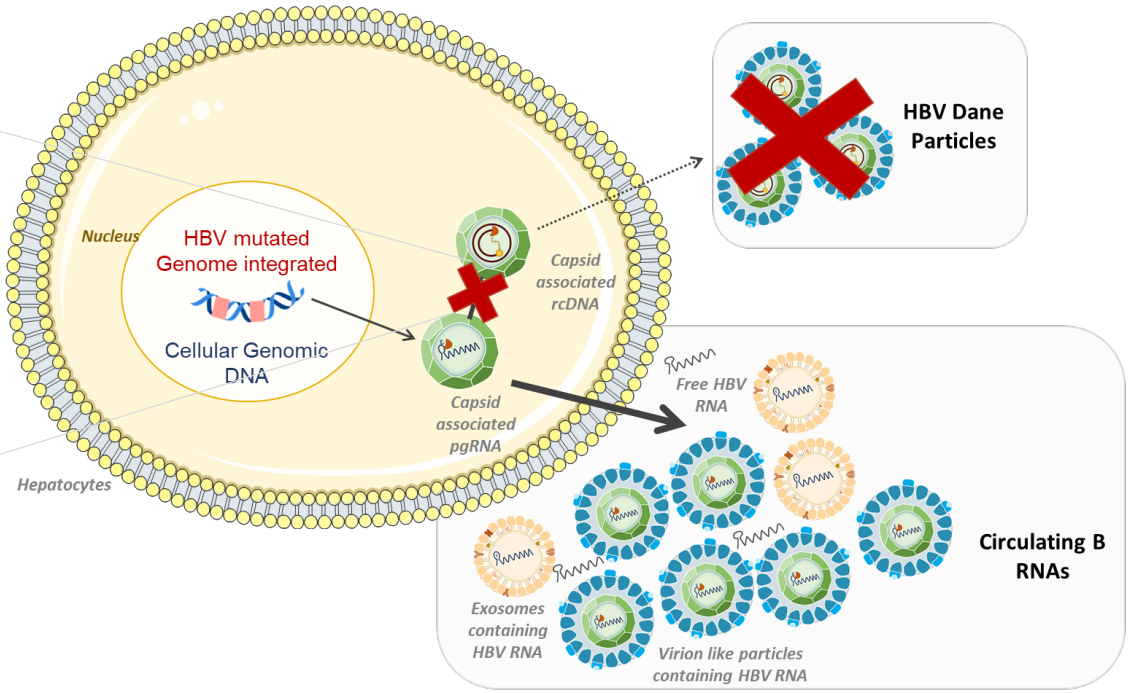
- Unlimited supply of HBV RNA
- Can be integrated in all the assay analytical steps (e.g., from samples extraction)
- Standardization across HBV RNA assays



- **HBV Polymerase interaction with ε loop of pgRNA allows the encapsidation of the pgRNA**

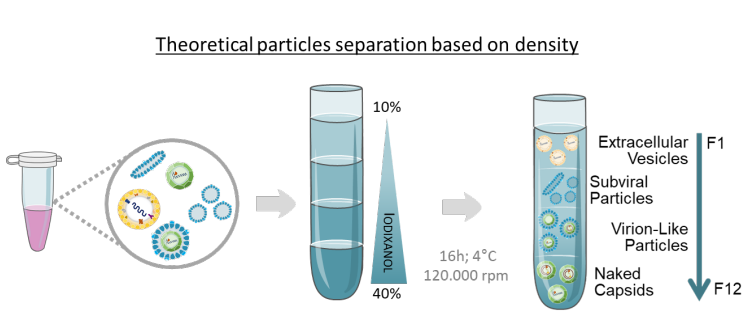
- **Mutated HBV Polymerase is unable to reverse transcribe the pgRNA into the viral rcDNA**

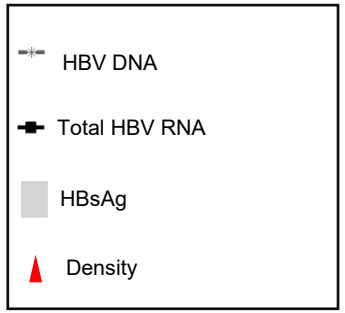
Genetically modified HBV-replicating cell lines

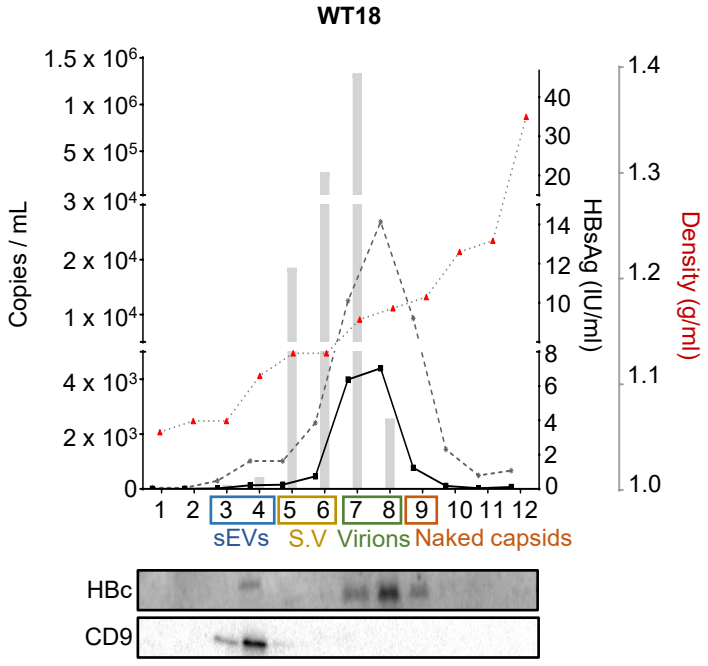


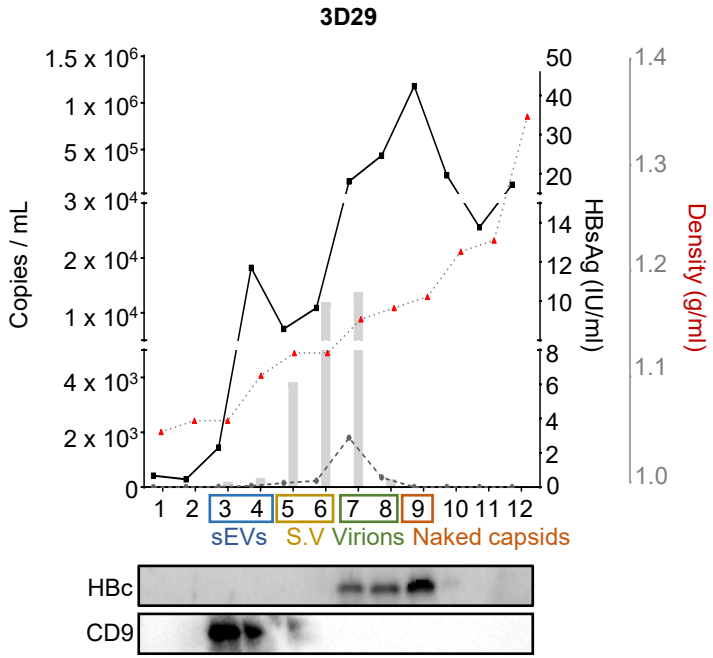
Paturel et al, in revision

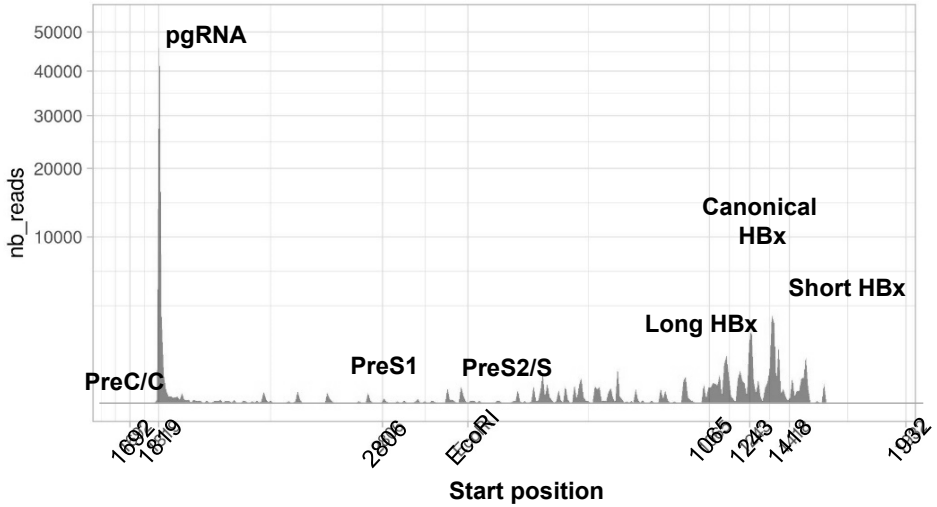
Phenotypic characterization of the selected clones

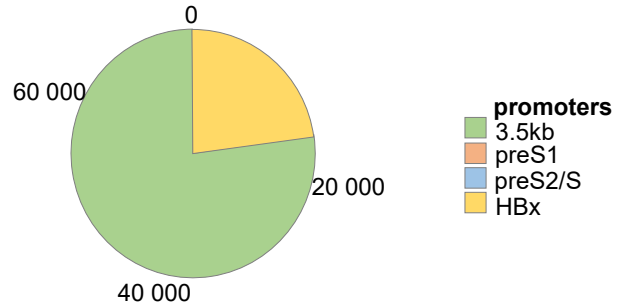












Huh7-3D29: Inversion of HBV RNA/DNA ratio and high HBV RNA productivity

Paturel et al, in revision

Perspectives

- HBV RNAs: a relevant biomarker to assess target engagement in early phase clinical studies (CAMs, SiRNAs, ASO)
- Currently considered as exploratory biomarker for endpoint assessment
- Role of HBV RNAs (and other viral biomarkers) in emerging therapies, i.e. CAMs, SiRNA, ASO containing regimens?
- Need for standardization of the different assays to allow comparison across studies
- Next questions to address the clinical utility of the biomarker:
    - Role in predicting functional cure?
    - Role in predicting viral rebound after stopping therapy?
    - Role in response guided therapy?
    - Patient stratification?
    - Role in the clinical monitoring of patients?

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  Samuel Didier,
  Roche Bruno
- **Montpellier Hospital:**
  Pageaux Georges-Philippe
- **Grenoble-Alpes Hospital:**
  Leroy Vincent
- **Nice Hospital:**
  Anty Rodolphe

Hepatitis viruses and pathobiology of chronic liver diseases

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Doohyun Kim
Hyoseon Tak
Caroline Scholtes

TCH
Françoise Berby
Isabelle Bordes

Epigenetics, microenvironment and liver cancer

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Alexia Paturel

Roche Team
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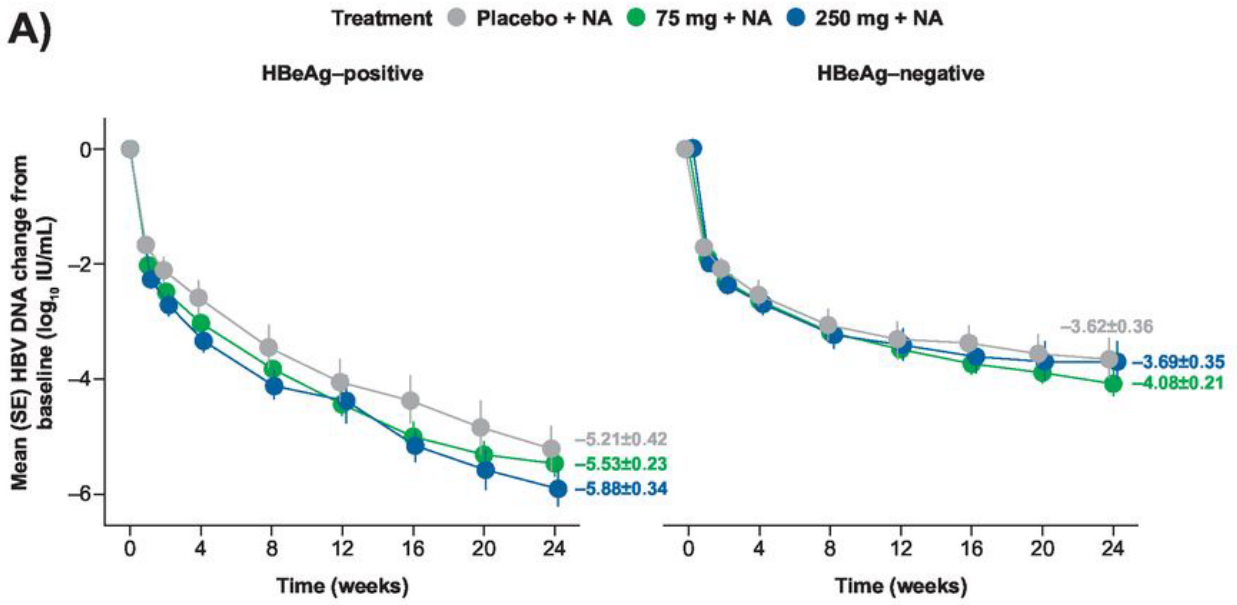
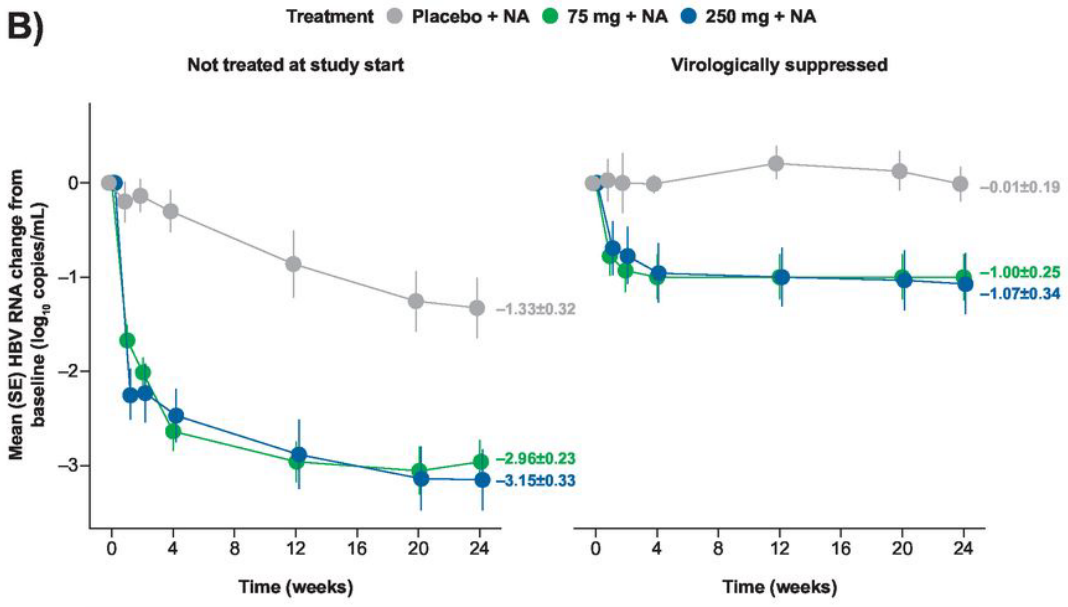
Aaron Hamilton

Mean change from baseline in HBV DNA and HBV RNA over 24 weeks of treatment (JADE trial)

(pooled placebo/JNJ-56136379+NA treatment arms)



Patients with HBV DNA <LLOQ at Week 24, n (%)	JNJ-56136379 75 mg + NA	JNJ-56136379 250 mg + NA	Placebo + NA
HBeAg–positive	0/12	4/11 (36)	1/8 (13)
HBeAg–negative	14/21 (67)	16/19 (84)	12/13 (92)



Patients with HBV RNA TND at Week 24, n (%)	JNJ-56136379 75 mg + NA		JNJ-56136379 250 mg + NA		Placebo + NA	
	NCT	VS	NCT	VS	NCT	VS
HBeAg–positive	3/12 (25)	9/9 (100)	4/11 (36)	10/10 (100)	0/8	1/5 (20)
HBeAg–negative	16/21 (76)	24/24 (100)	19/19 (100)	18/18 (100)	9/13 (69)	10/15 (67)

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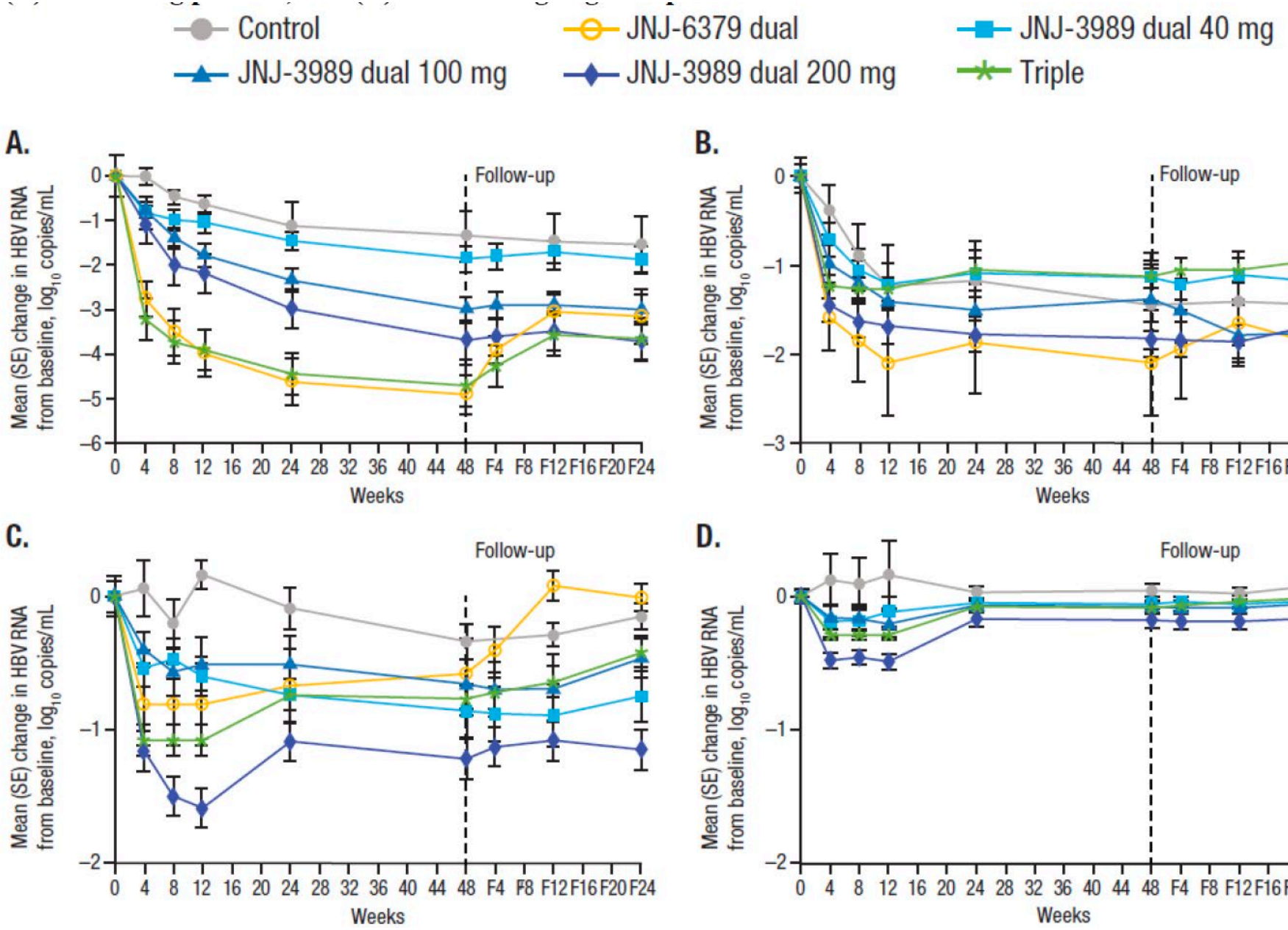
Harry L A Janssen et al. Gut 2023;72:1385-1398

Mean change in HBV RNA in the REEF-1 study



NCT HBeAg+

VS HBeAg+

NCT HBeAg-

VS HBeAg-