RHU « CirB-RNA »
ANR-17-RHUS-0003

International Workshop on Viral Biomarkers

Prediction of outcome after cessation of nucleos(t)ide analog therapy

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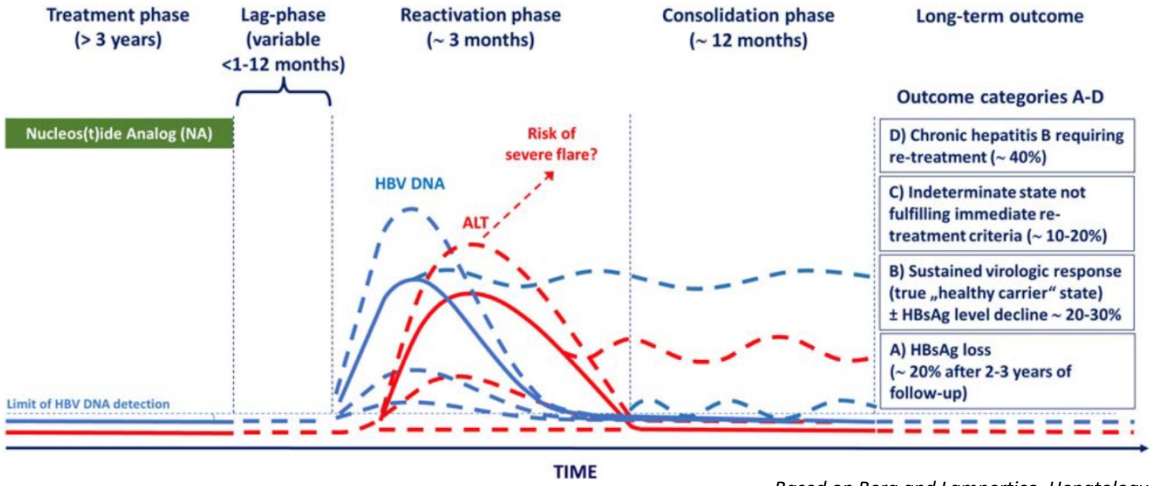
What are the **aims** of stopping NUCs?

Pre-treatment HBeAg **positive** → **Sustained virologic response**

Pre-treatment HBeAg **negative** → **HBsAg loss (Functional cure)**



Based on Berg and Lampertico. Hepatology (2018)

Virologic relapse: HBV-DNA > 2000 IU/ml
Clinical relapse: VR & ALT> 2 ULN

Clinical **guidelines** differ in NUC discontinuation recommendations

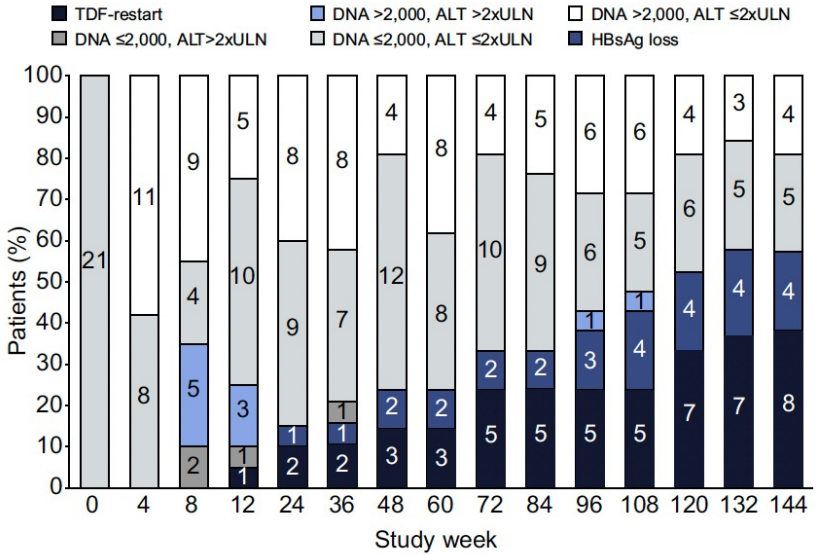
Guideline	HBeAg+	HBeAg-
EASL (2017)	HBeAg seroconversion & at least **6-12 months** of consolidation therapy following virological suppression	At least **3 years** of consolidation therapy following virological suppression
AASLD (2018)	HBeAg seroconversion & **at least 12 months** of consolidation therapy following virological suppression	**Indefinite** treatment unless compelling rationale
APASL (2015)	HBeAg seroconversion & **at least 1-3 years** of consolidation therapy following virological suppression	At least **2 years of treatment & 1 year of consolidation therapy** following virological suppression (tested 3 times, 6 months apart)

Sarin S, Kumar M, Lau G, Abbas Z, Chan HLY, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int 2016;10:1–98.
Lampertico P, Agarwal K, Berg T, Buti M, Janssen HL, Papatheodoridis G, et al. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol 2017;67:370–398.
Terrault NA, Lok AS, McMahon BJ, Chang K, Hwang JP, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology 2018;67:1560–1599.

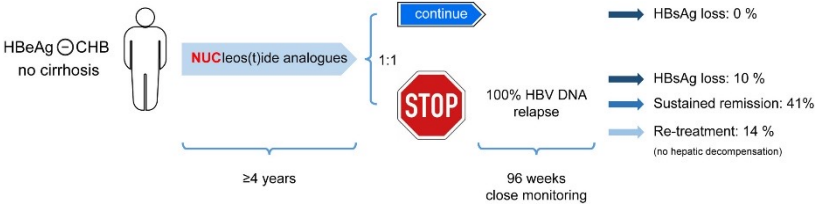
Why consider stopping NUCs in **HBeAg-neg**?

The rate of HBsAg loss can reach 10–20% during a relatively short follow-up period of 2–3 years whereas in the setting of continued NA therapy is only 1-2% per year.

Tseng et al. J Hep Reports 2023



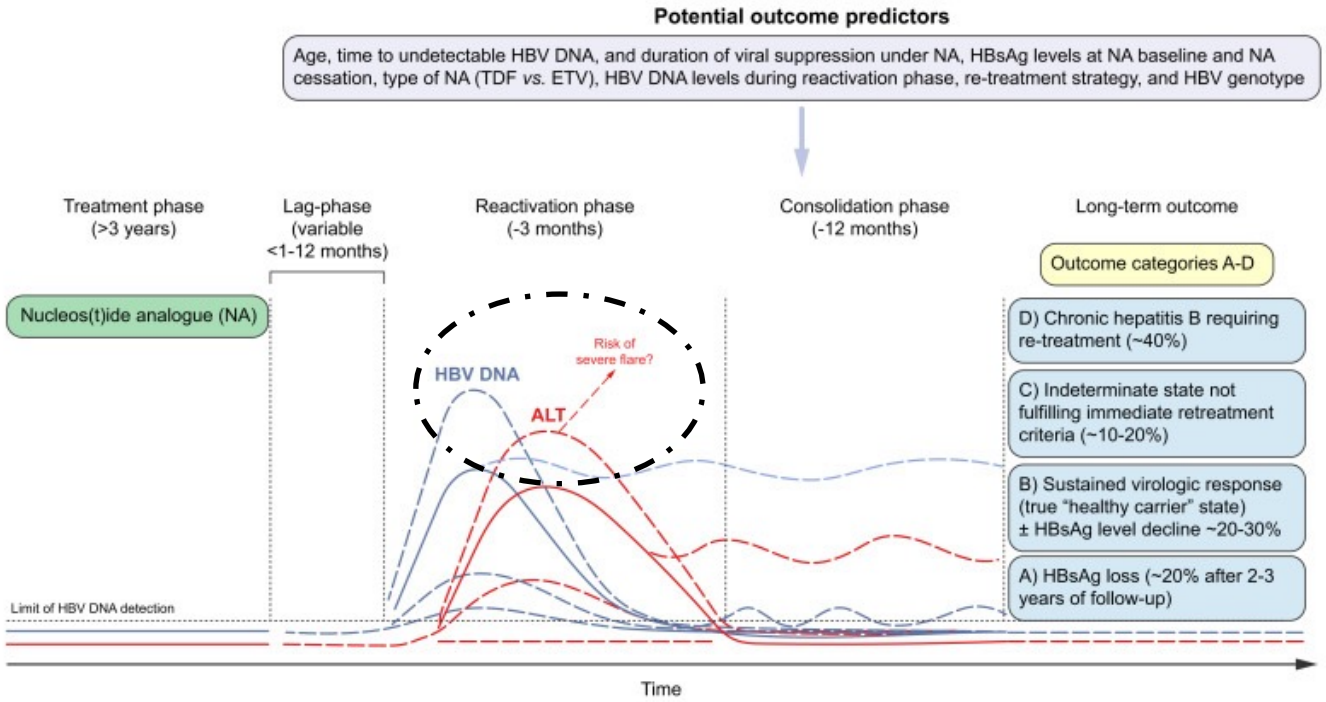
Berg et al, Journal of Hepatology 2017 vol. 67 j 918–924



Van Bömmel et al. J Hepatol. 2023 May;78(5):926-936

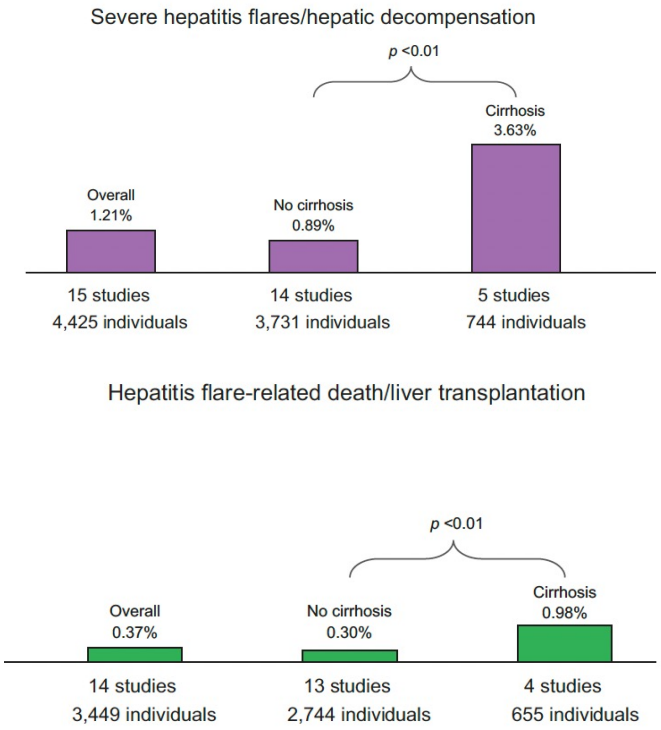
Challenges when stopping NUCs

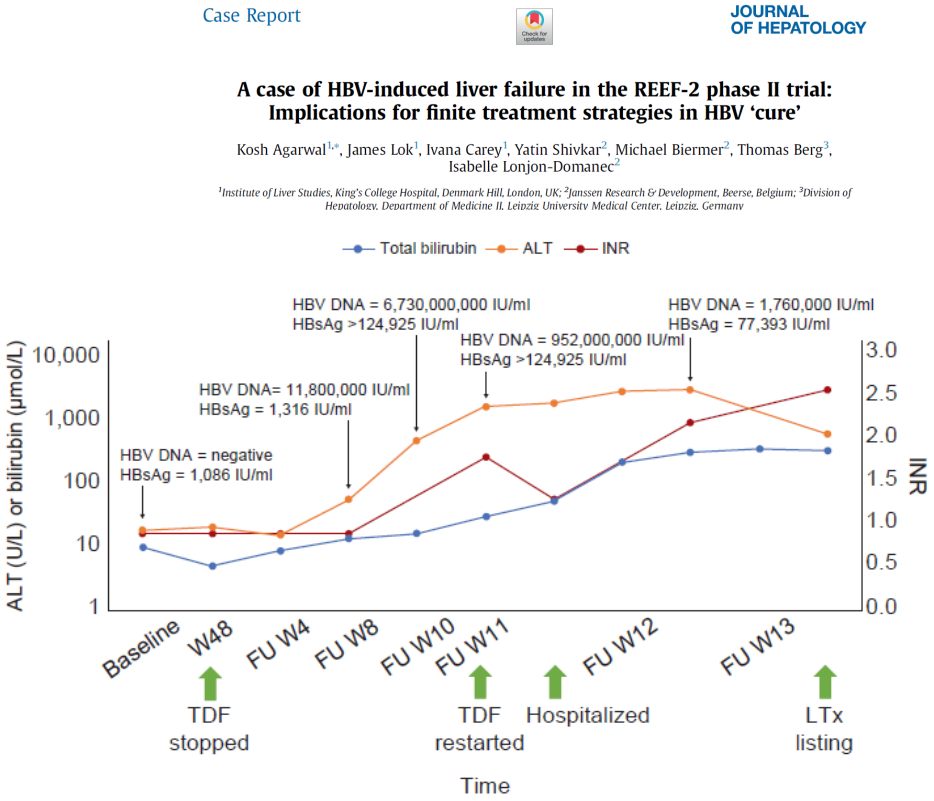
NUCs



Based on Berg and Lampertico. Hepatology (2018)

Risks when stopping NUCs





Tseng et al. JHEP Rep . 2022 Oct 28;5(1):100617.

To **consider** when evaluating the **role of HBV biomarkers** to guide stopping NUC therapy:

NUCs

Disease

- Pre-treatment HBeAg, HBV-DNA
- Genotype
- Ethnicity
- Treatment type / duration
- Stage of liver disease

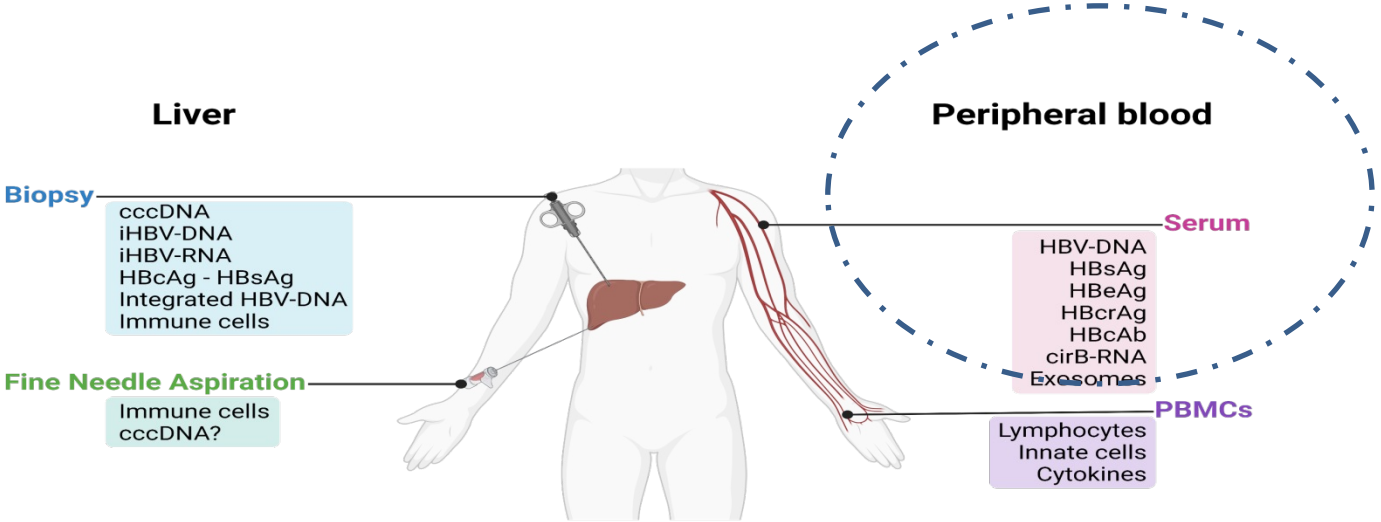
Biomarker

- Baseline or dynamic evaluation
- Alone or in combination
- Sensitivity
- Standardisation
- Reimbursement
- Commercial availability

Study design:
Endpoints
& definitions

- HBsAg loss
- Virologic / Clinical relapse
- Re-treatment criteria
- Time FU

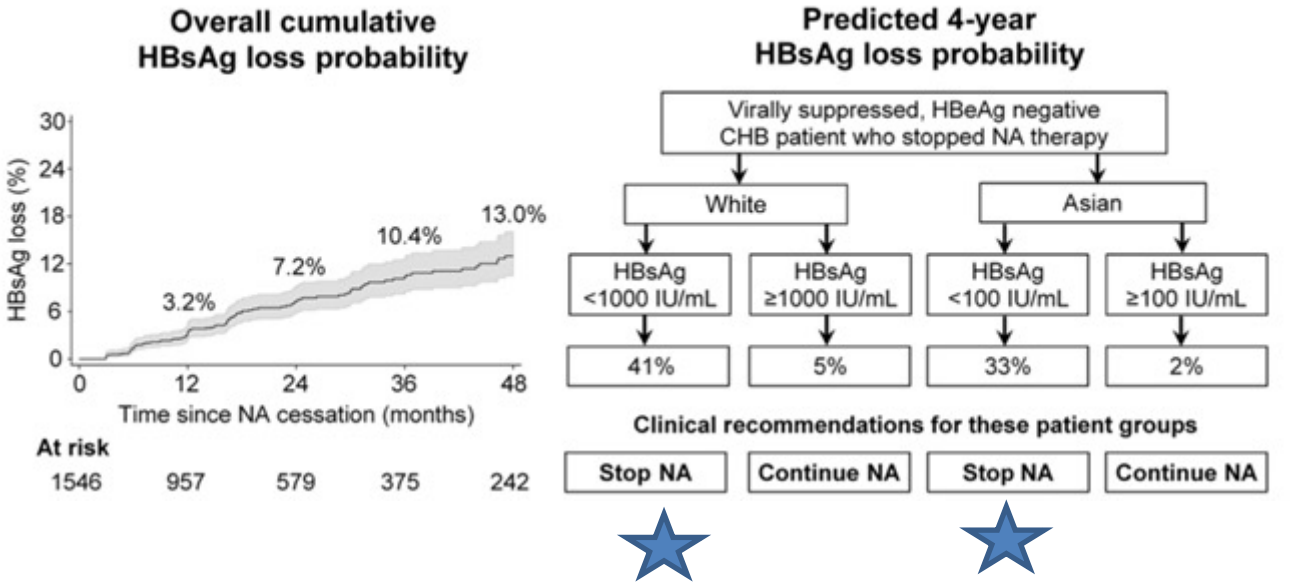
Potential **biomarkers** to predict outcome after NUC discontinuation



J Hep Reports 2022

qHBsAg levels: best **cut-off** to predict HBsAg loss?

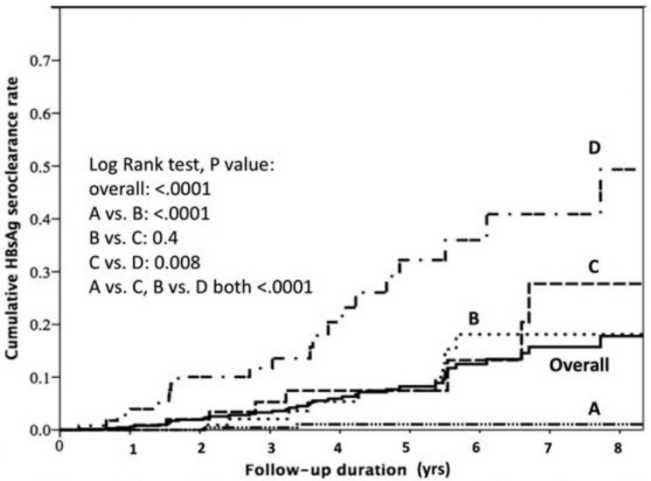
RETRACT-B Study: n=1552 patients HBeAg-negative, 87.6% Asian



Hirode et al. Gastroenterology 2022;162:757–771

qHBsAg levels: EOT, **kinetics and early re-treatment**

n=691 HBeAg- patients → 42 HBsAg loss
6-year cumulative incidence 13%



Patient at risk

Overall			691	595	472	351	237	150	101	61	31
A	CR+	reTx	269	262	226	179	126	77	47	29	13
B	CR+	reTx-	150	135	107	77	49	36	25	15	9
C	CR-	VR+	128	109	76	47	33	17	15	8	6
D	CR-	VR-	144	89	63	48	29	20	14	9	3

Cox regression analysis:

- lower EOT HBsAg level (<100 IU/mL)
- After NA stop: HBsAg reduction (>1log10), sustained response (VR-), and relapsers (CR+) not retreated

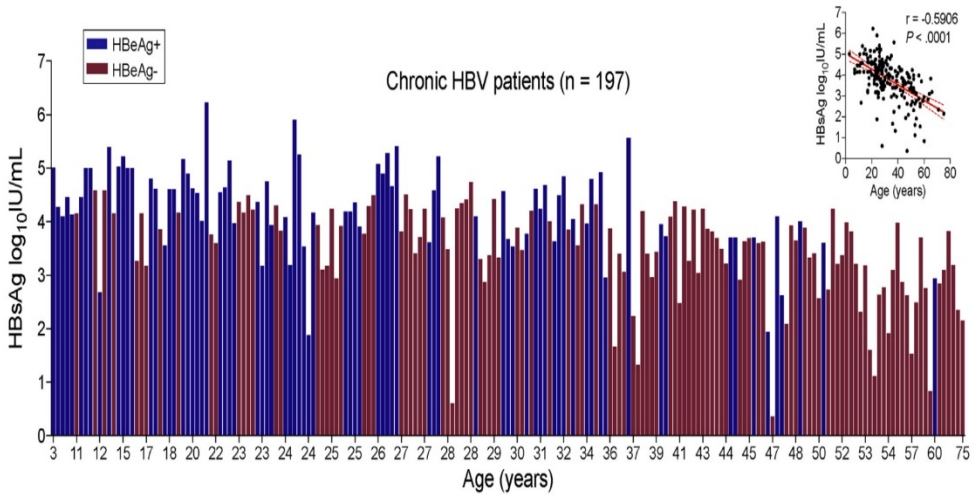
clinical relapse not treated → x7 HBsAg loss

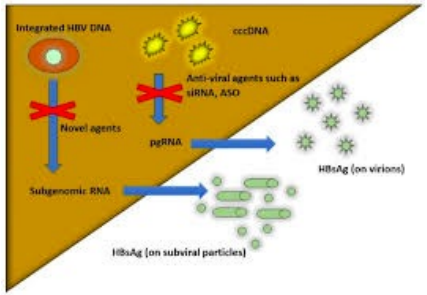
Jeng et al, Hepatology 2018

qHBsAg levels: challenges

Different qHBsAg according to HBV genotype

HBeAg-negative, age (duration of infection) and long-term NA therapy → lower **qHBsAg**



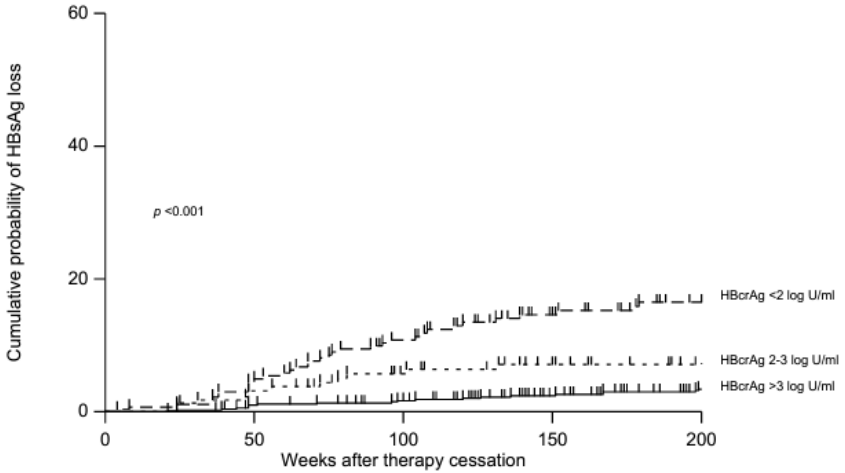


Role of HBsAg production from integrated HBV-DNA?

Le Bert et al. Gastroenterology 2020

HBcrAg in VR, CR and functional cure

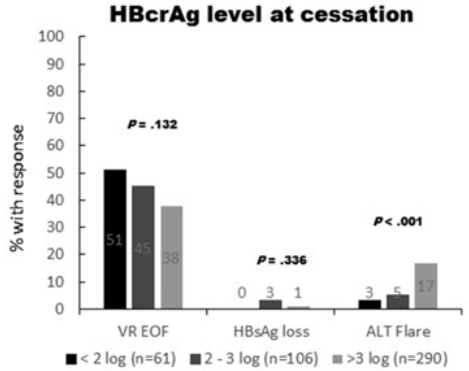
CREATE study, n= 1216, 291 (24%) HBeAg+



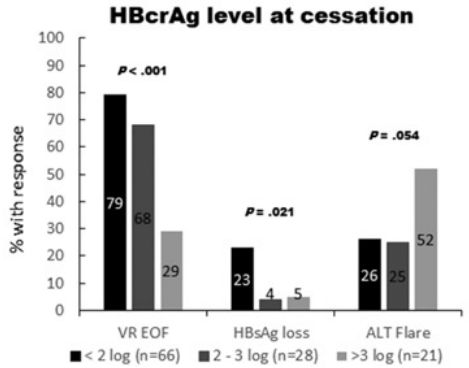
N° at risk*					
HBcrAg <2 log	272	214	167	143	123
HBcrAg 2-3 log	234	156	135	124	114
HBcrAg >3 log	710	619	540	507	477

Sonneveld et al. Journal of Hepatology 2022vol.76j1042–1050

Asian



Non-Asian



Sonneveld et al. Clinical Gastroent and Hepatol 2022

HBcrAg in VR, CR and functional cure

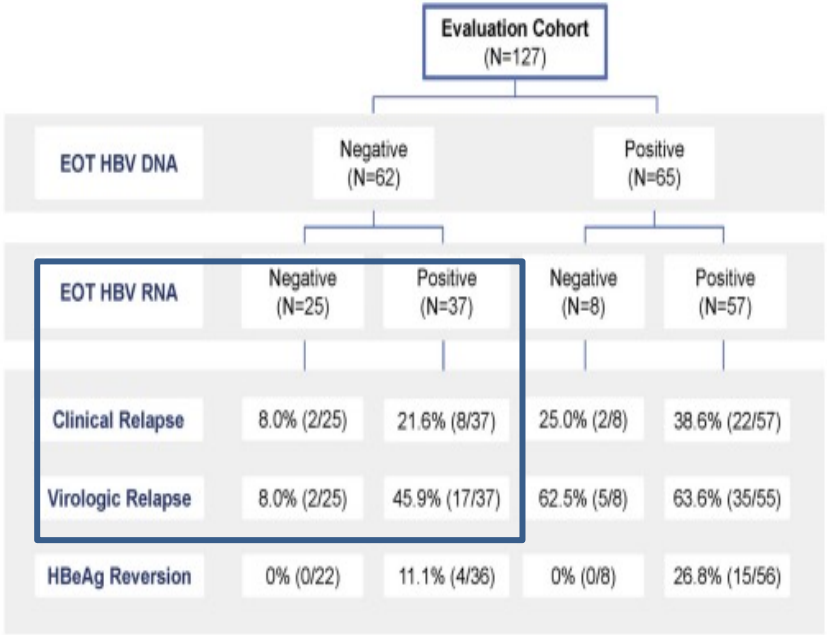
				> cut-off vs < cut-off		
Study	**Time-point**	**FU**	**Cut-off**	**VR**	**CR**	**HBsAg loss**
Kaewdech, et al. 2020	EOT	48 weeks	3 log U/mL	**74% vs 44%**	48% vs 8%	0% vs 6%
Huang et al. 2021	Baseline	5 years	4 log U/mL	**79% vs 56%**	**75% vs 41%**	**13% vs 29%**
Kuo et al. 2021	Baseline	3 years	4.7 log U/mL	**82% vs 55%**	**72% vs 39%**	**8% vs 43%**
Sonneveld et al. 2022	EOT	48 weeks	2-3 log U/mL	**65% vs 38%**	20% vs 15%	**2% vs 12%**

In **bold** if difference p<0.05

Zeng et al. JHEP Reports 2023 vol. 5 j 100720

HBV-RNA in VR, CR and functional cure

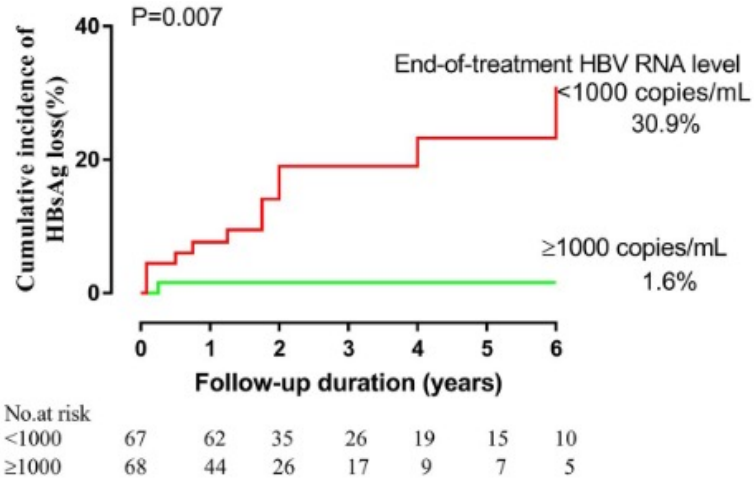
VR/CR in pre-treatment **HBeAg+ patients**



Fan et al, Clin Gas Hep 2020

HBsAg loss in pre-treatment **HBeAg+/- patients**

n=135, 70% HBeAg+, FU 6 yrs



Xia M, et al. Aliment Pharmacol Ther. 2021;54:709– 714.

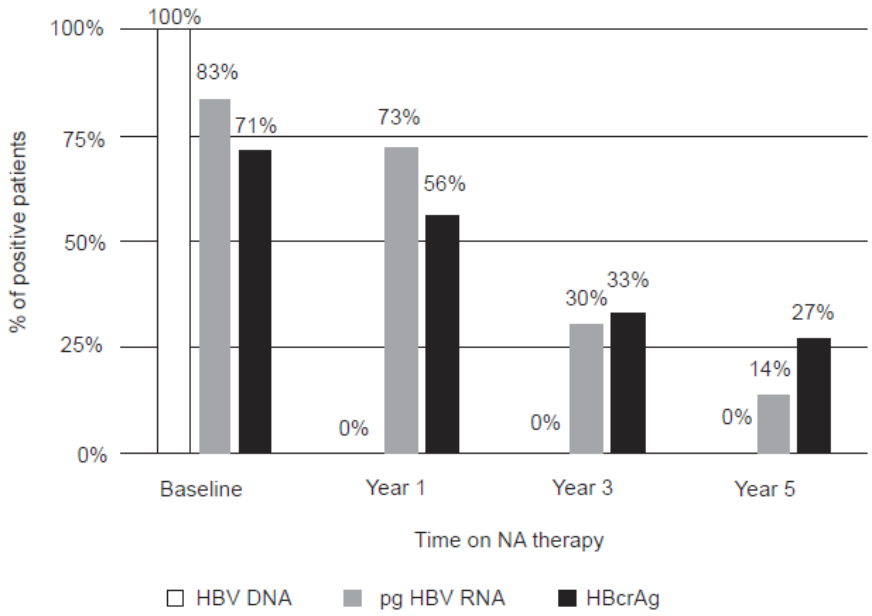
HBV-RNA in VR, CR and functional cure

				> cut-off vs < cut-off		
Study	**Time-point**	**FU**	**Cut-off**	**VR**	**CR**	**HBsAg loss**
Kaewdech, et al. 2020	EOT	48 weeks	2 log U/mL	72% vs 50%	**43% vs 21%**	0% vs 4%
Seto et al. 2020	EOT	48 weeks	1.65 log U/mL	**93% vs 36%**		
Xia et al. 2021	EOT	6 years	3-4.3 log U/mL		**100% vs 24%**	**2% vs 31%**
Papatheodoridi et al. 2022	EOT	48 weeks	LLD	**100% vs 68%**	**100% vs 28%**	0% vs 17%

In **bold** if difference p<0.05

Zeng et al. JHEP Reports 2023 vol. 5 j 100720

HBcrAg and HBV-RNA: challenges



Better correlation with cccDNA in HBeAg+

Sensitivity

Standardisation

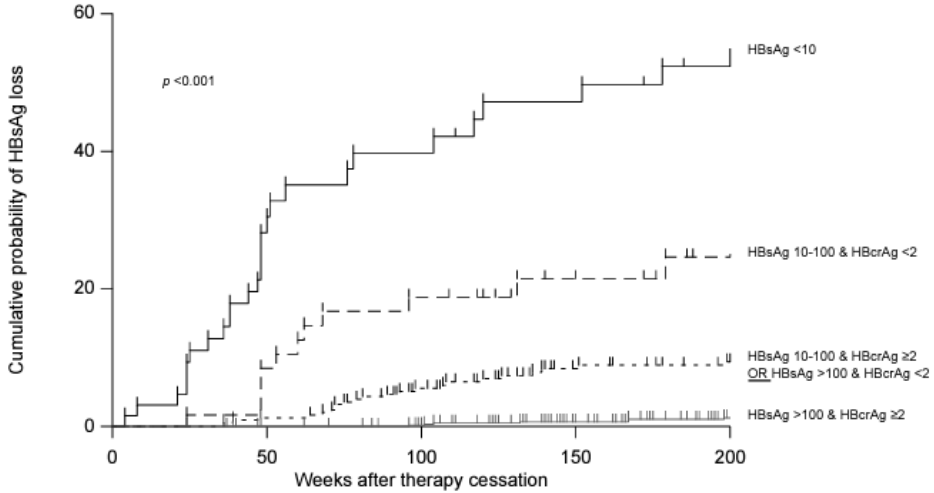
Reimbursement

While detectable levels of HBV-RNA and HBcrAg at EOT clearly predict an unfavourable outcome,the opposite–non-detectability of these markers–is not highly predictive of HBsAg loss

Carey et al. Hepatology . 2020 Jul;72(1):42-57.

Combination strategies: qHBsAg and HBcrAg

CREATE study n=1,216 patients
Non-Asian ethnicity was associated with the highest chance of HBsAg loss.
Among Asian patients, genotype C was associated with a higher chance of HBsAg loss.



N° at risk*					
HBsAg <10	64	31	25	21	16
HBsAg 10-100 & HBcrAg <2	59	45	36	28	20
HBsAg 10-100 & HBcrAg ≥2 OR HBsAg >100 & HBcrAg <2	322	260	209	185	174
HBsAg >100 & HBcrAg ≥2	771	653	572	540	504

Patients with HBsAg >100 IU/ml and detectable HBcrAg had a very low chance of HBsAg loss irrespective of HBV genotype

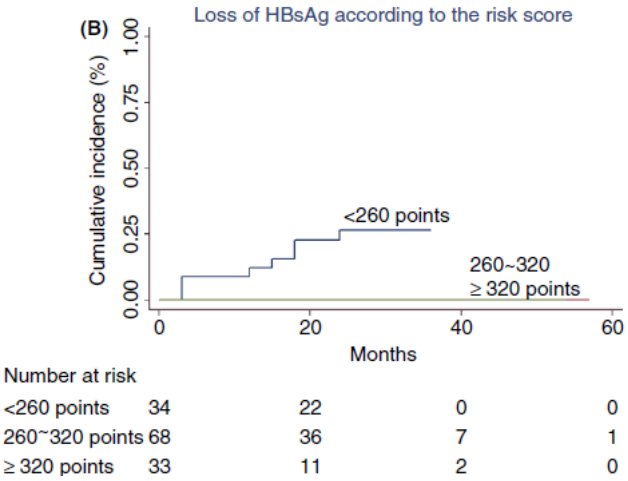
Sonneveld et al. Journal of Hepatology 2022 vol.76j1042–1050

SCALE-B score

35*HBsAg (log IU/mL) + 20*HBcrAg (log U/mL) + 2*age (year) + ALT (U/L) + 40 for tenofovir use

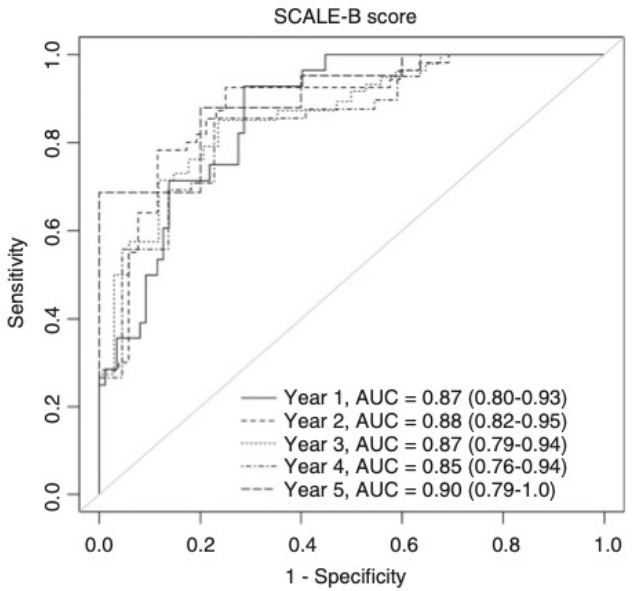
SCALE-B score cut-off for HBsAg loss

n=135 patients, FU 26 months



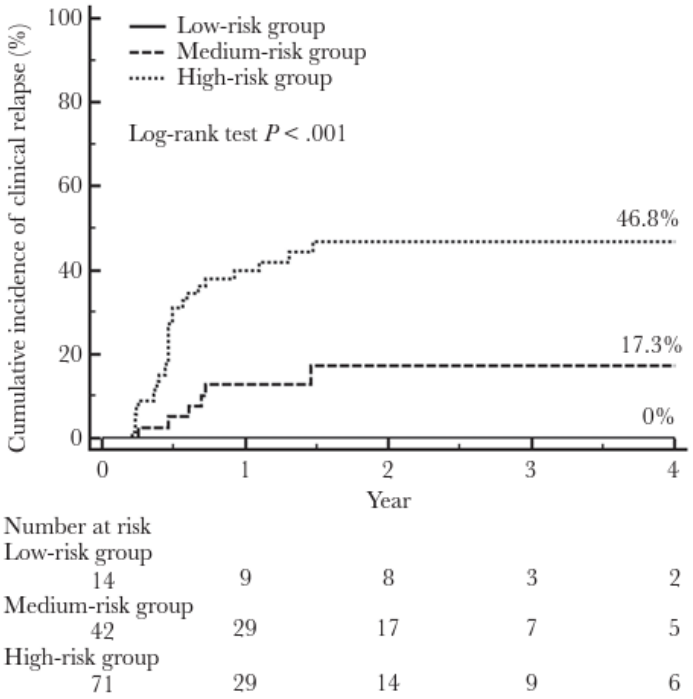
Hsu et al. Aliment Pharmacol Ther. 2019 Jan;49(1):107-115.

SCALE-B score cut-off for clinical relapse



Sonneveld et al. Journal of Hepatology 2022 vol.76j1042–1050

Combination strategies: HBcrAg and HBV-RNA



n=127 HBeAg+ achieving SC

High risk: Both positive

Medium risk: HBV-RNA negative & HBcrAg > 4 log10 U/mL
Or HBV-RNA positive & HBcrAg < 4 log10 U/mL

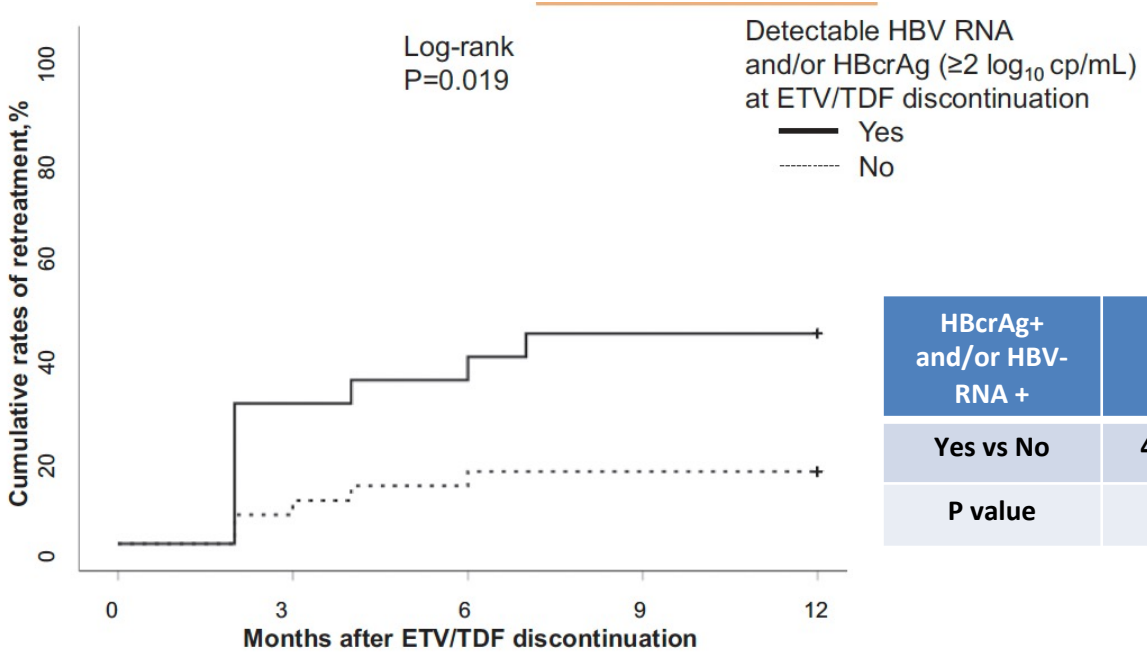
Low risk: Both negative

HBsAg loss was higher in the low-risk patients (16.1% [5/31] vs 1.3% [2/155], P = .002).

Fan et al. J Infect Dis. 2020 Jul 23;222(4):611-618.

Combination strategies: HBcrAg and HBV-RNA

n=57 HBeAg-negative

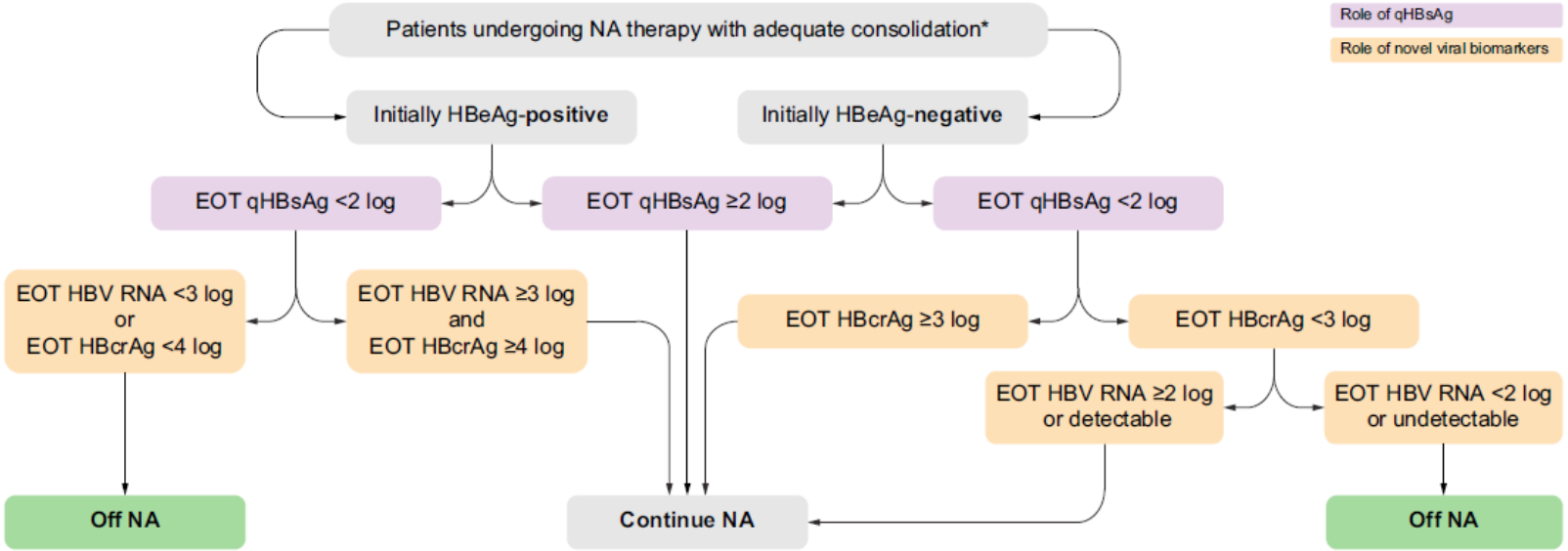


HBcrAg+ and/or HBV-RNA +	VR	CR	HBsAg loss
Yes vs No	**47% vs 18%**	59% vs 29%	**0% vs 46%**
P value	**0.04**	0.07	**0.009**

, Apatheodoridi et al. J Viral Hepatitis 2022

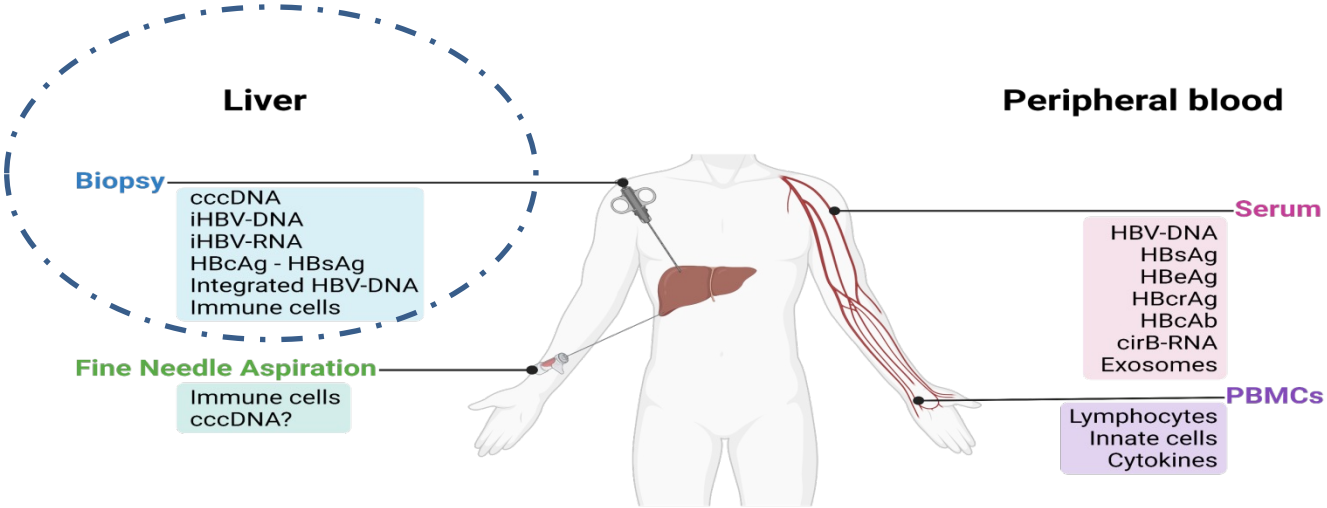
NUC discontinuation **algorithm** based on viral markers

Meta-analysis : 33 studies which provided data of 2986 patients undergoing treatment cessation



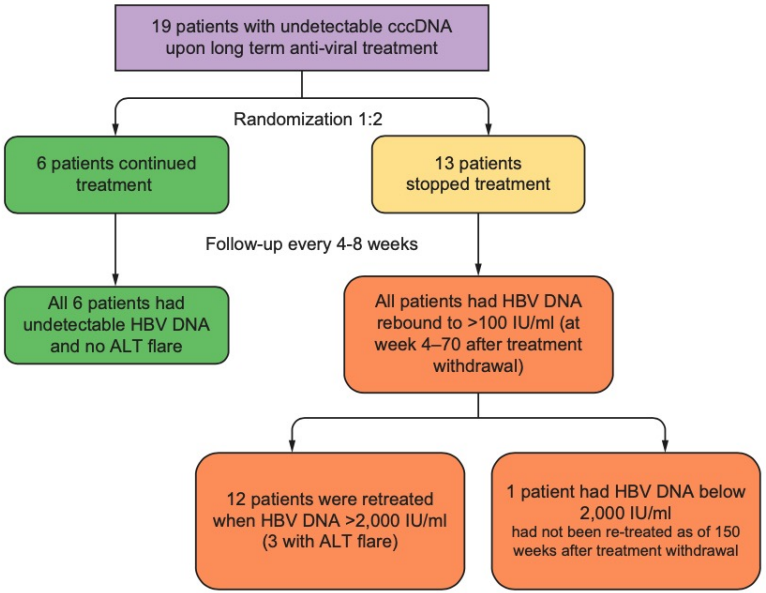
Zeng et al. JHEP Reports 2023 vol. 5 j 100720

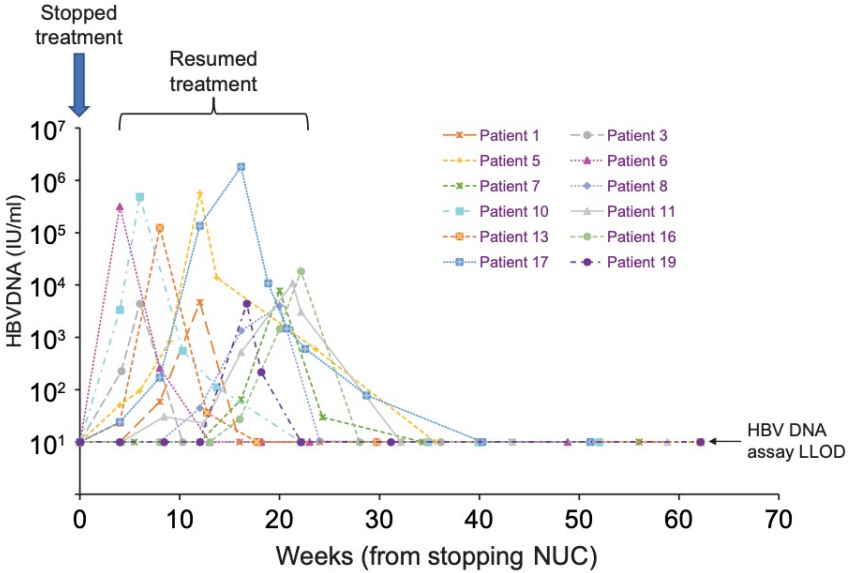
Potential **biomarkers** to predict outcome after NUC discontinuation



J Hep Reports 2022

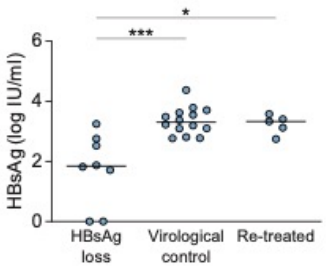
Intrahepatic viral markers: **cccDNA**

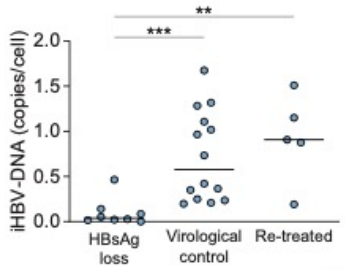


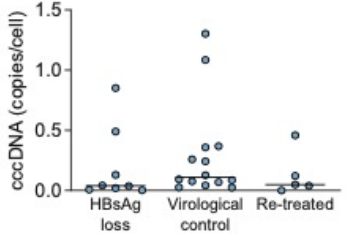


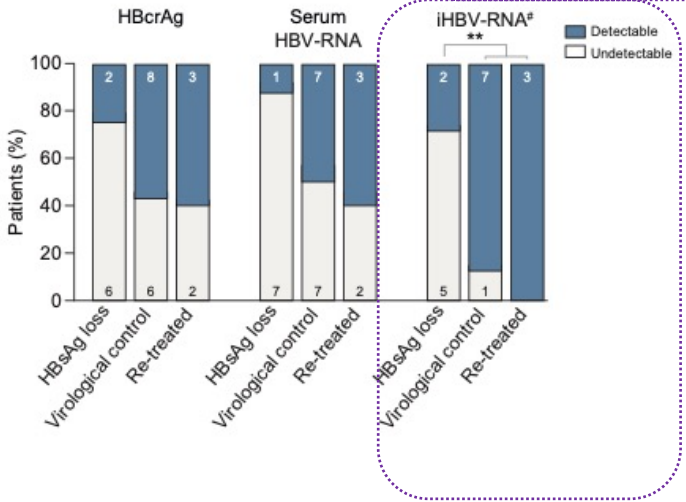
Lai et al, J Hep Reports 2020

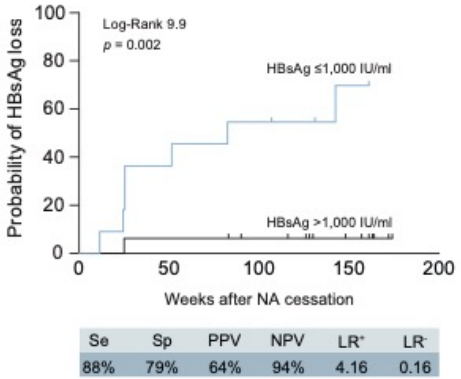
Intrahepatic viral markers: **cccDNA, iHBV-DNA and iHBV-RNA**











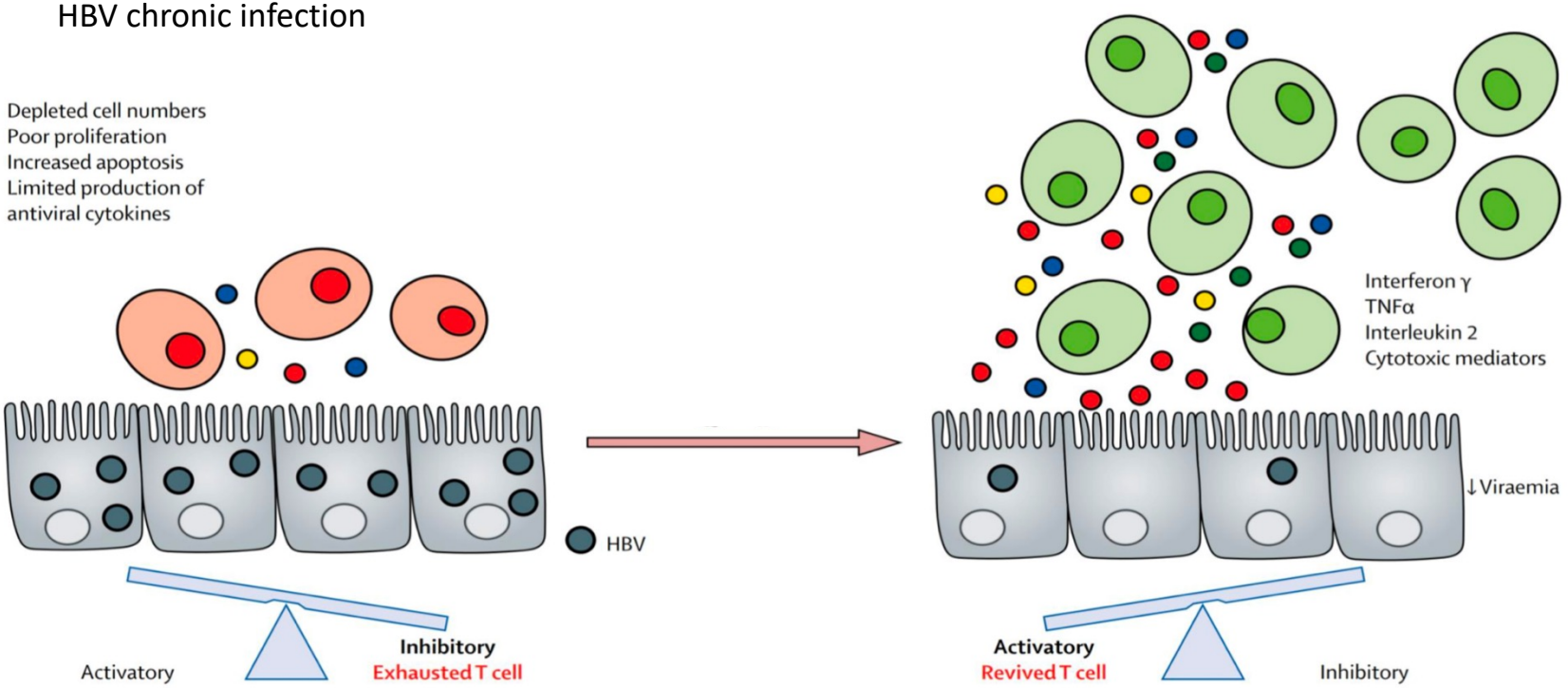
Se	Sp	PPV	NPV	LR+	LR-
88%	79%	64%	94%	4.16	0.16

García-López and Lens et al, J Hepatology 2021

Immune biomarkers

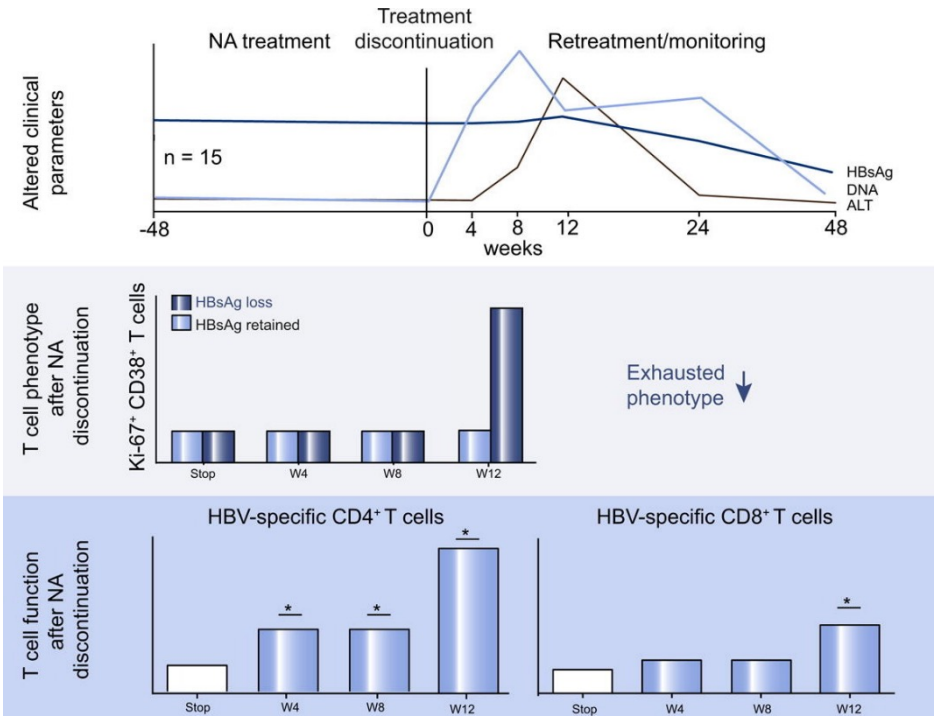
Acute-resolving HBV hepatitis

HBV chronic infection



Maini MK and Pallett LJ. Lancet Gastroenterol Hepatol 2018

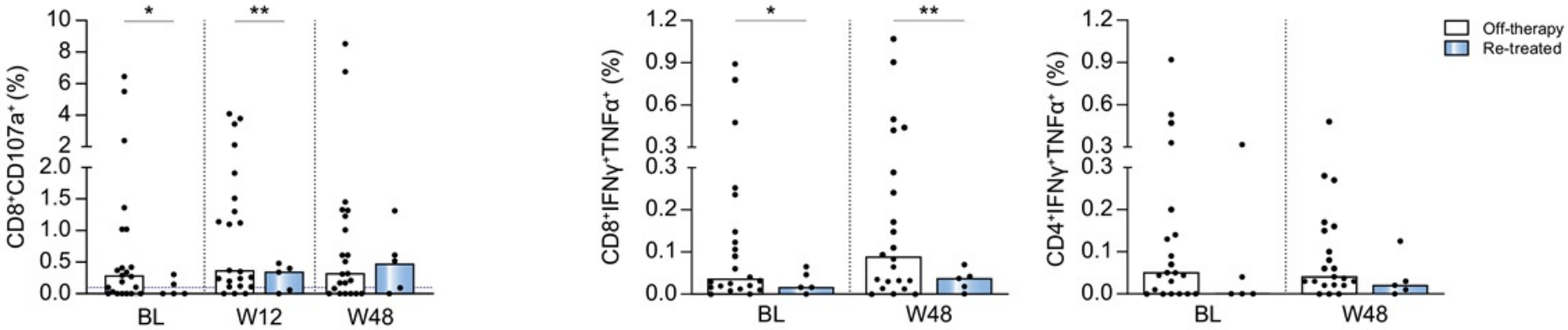
HBV-specific T cell responses



- ✓ T cells from patients with subsequent **HBsAg loss** showed a **less exhausted** phenotype.
- ✓ These T cells also expressed higher levels of activation and proliferation markers at week 12 after discontinuation of therapy

Rinker F et al, J Hepatol. 2018

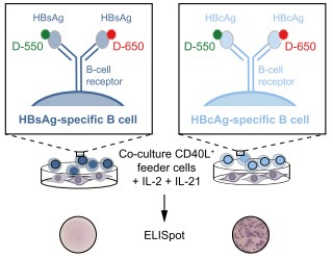
HBV-specific T cell responses



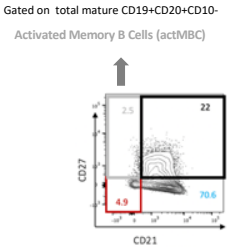
- ✓ Increased **frequency of functional HBV-specific CD8+ T cells** at baseline was associated with sustained viral control off treatment.
- ✓ The strength of HBV-specific T cell responses **did not correlate** with serum or intrahepatic virological markers

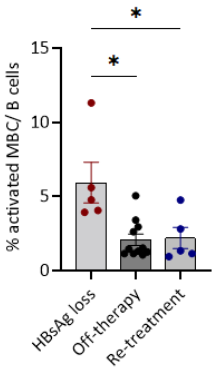
García-López M and Lens S et al, J Hepatol. 2021

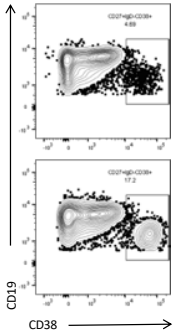
HBV-specific B cell responses

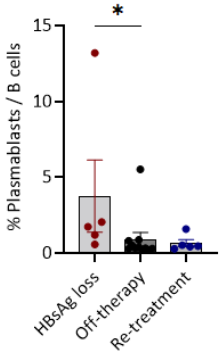


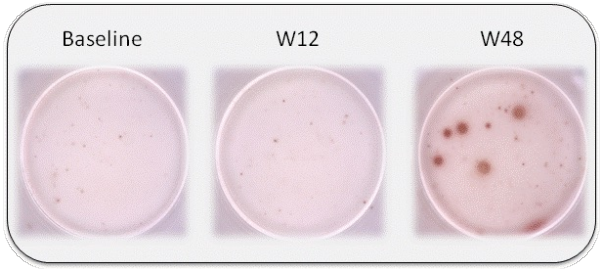
[1]Burton et al. JCI 2018
[2]Salimzadeh et al. JCI 2018
[3]Le Bert et al. J Hepatol 2019
[4] Vanwolhegem J Hepatol 2021









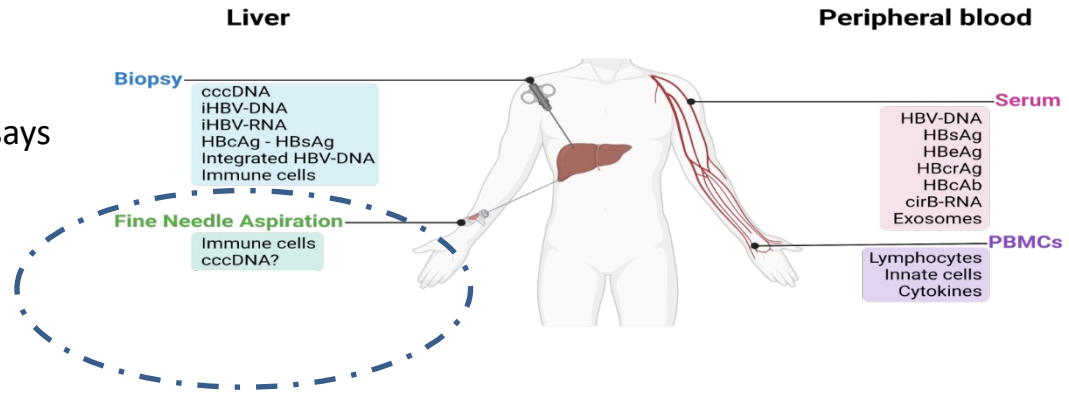


- ✓ Long-term NUC therapy and NUC discontinuation induce significant changes in **HBV-MBC frequency and phenotype**.
- ✓ HBsAg loss: ↑ activated global B cells (EOT and FU), ↑plasmablasts and recovery of B cell functionality (HBsAb production)

Lens S, et al. Manuscript in preparation

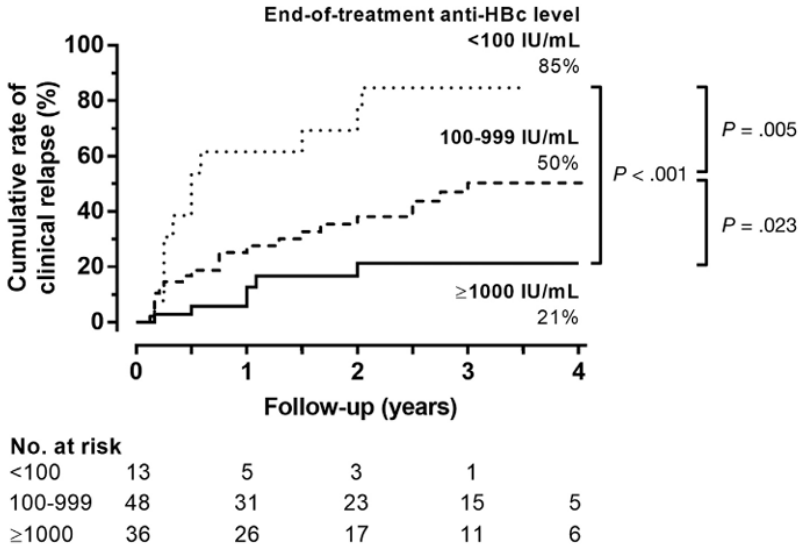
Immune biomarkers : challenges

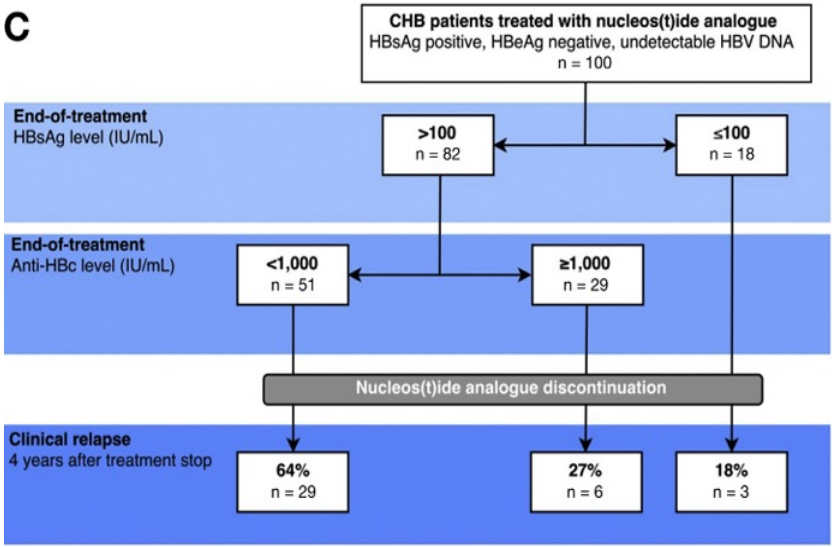
- Need of specialized laboratory
- Heterogeneity of results so far
- In vitro vs in vivo HBV-sp responses
- Lack of robust and reproducible assays
- Periphery = Liver?



Anti-HBc levels

n=100 patients (71% HBeAg+), FU 2 years, primary endpoint clinical relapse: HBV-DNA >2000 and ALT >2ULN





Chi et al. Clin Gastroenterol Hepatol . 2019 Jan;17(1):182-191.e1.

Anti-HBc levels

n=41 HBeAg-neg (control arm REEF-2 study)

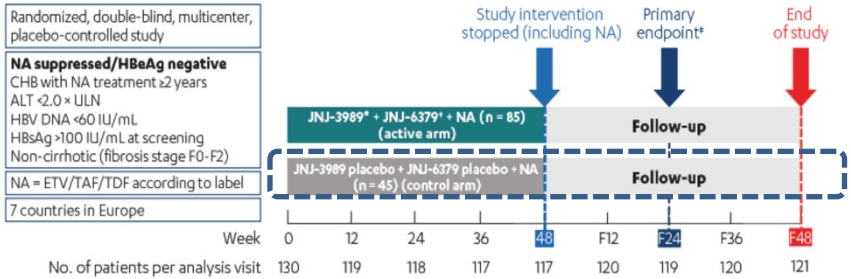


Table 2. Proportion of REEF-2 NA Control Arm Patients With Post-treatment Virologic Relapse and Biochemical Flare by EOT Virologic Parameters

EOT variables/type of NA	N	Virologic relapse (confirmed HBV DNA >2,000 IU/mL		Biochemical flare (ALT ≥3 × ULN)	
		Any virologic flare	Peak HBV DNA >100,000 IU/mL	Any ALT flare	Peak ALT ≥10 × ULN
Patients with EOT data who entered follow-up, n (%)	41	27 (65.9)	11 (26.8)	16 (39.0)	10 (24.4)
HBV RNA					
Detectable*	35	23 (65.7)	9 (25.7)	13 (37.1)	9 (25.7)
TND†	6	4 (66.7)	2 (33.3)	3 (50.0)	1 (16.7)
P value‡		1.0000	0.6514	0.6624	1.0000
HBcrAg					
Detectable*	21	17 (81.0)	**9 (42.9)**	10 (47.6)	**9 (42.9)**
TND†	20	10 (50.0)	**2 (10.0)**	6 (30.0)	**1 (5.0)**
P value‡		0.0516	**0.0325**	0.3408	**0.0089**
Anti-HBc IgG					
<300 IU/mL	23	16 (69.6)	**11 (47.8)**	12 (52.2)	**10 (43.5)**
≥300 IU/mL	18	11 (61.1)	**0**	4 (22.2)	**0**
P value‡		0.7417	**0.0008**	0.0626	**0.0021**
HBsAg					
<1,000 IU/mL§	13	9 (69.2)	6 (46.2)	8 (61.5)	**6 (46.2)**
≥1,000 IU/mL	28	18 (64.3)	5 (17.9)	8 (28.6)	**4 (14.3)**
P value‡		1.0000	0.0727	0.0835	**0.0485**

Van Bommel et al, EASL 2023

Take-home messages

- Serum HBV biomarkers are useful in predicting outcomes after **treatment discontinuation**.
- **Low quantitative HBsAg levels** are currently the most reliable predictive marker: <1,000 IU/ml in the Caucasian population and <100 IU/ml in the Asian population. The **dynamics** of qHBsAg may also be helpful.
- **Detectable** levels of **HBV RNA** and **HBcrAg** at EOT mostly predict an **unfavourable** outcome, BUT the opposite is not highly predictive of HBsAg loss.
- **Intrahepatic** viral markers do **not** seem to improve predictive capacity of serum markers.
- The effect of antiviral treatment on **HBV-specific immune** response may also contribute to define the population who would benefit the most from finite therapy but this requires broader and specialized evaluation.

Thank you for your attention!

PI15/00151
PI16/00111
PI18/00079
IFI18/00006

Viral Hepatitis Unit, Hospital Clínic Barcelona

Follow us on Twitter **@SaSi_Lens** and **@BCVirHep**

Clinical trials including NUCs withdrawal

What effect of new therapies would I expect in a HBeAg-neg CHB patient under long term NA therapy?

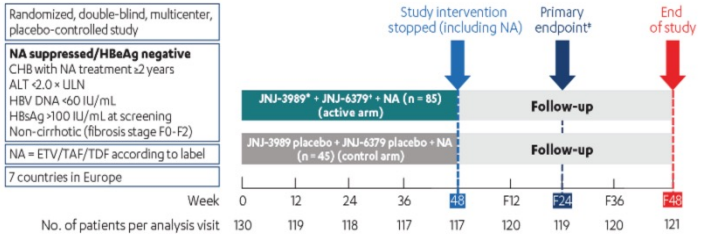
	HBsAg	HBcrAg	HBV-RNA
CAMS	↓	↓	↓
siRNA	↓↓	↓↓	↓↓
HBsAg secretion inhibitors	↓↓↓	↓	↓
Entry inhibitors	↓	↓	↓

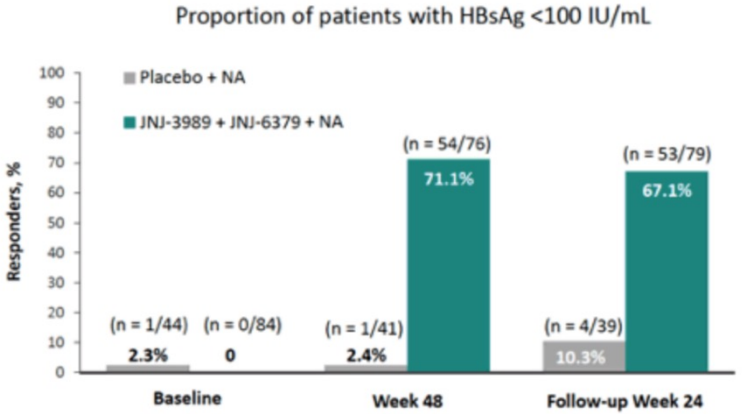
Personal

siRNA + CAM + NUC

REEF-2: Primary Endpoint and Proportion of Patients With HBsAg <100 IU/mL

No patients achieved the primary endpoint of HBsAg seroclearance* at Follow-up Week 24 without restarting NA treatment, in either treatment arm





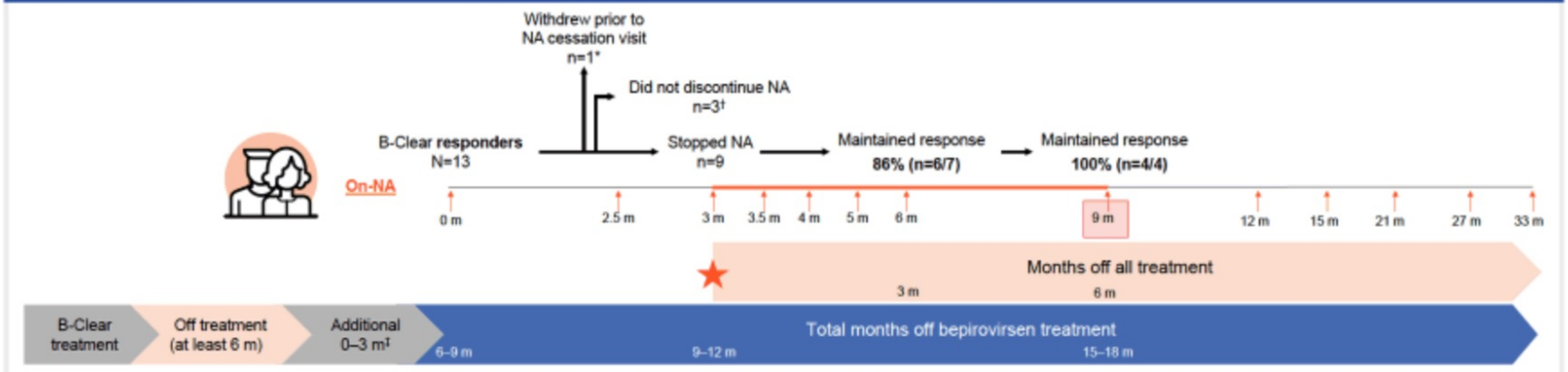
Agarwal K et al. EASL 2022

Bepirovirsen (ASO) + NUC

On-NA population:

- 69% (9/13) participants ceased NA as per protocol.
- Of the 7 participants who ceased NA and had ≥6 months of follow-up within B-Sure, 86% (6/7) maintained response 3 months post NA cessation (**Figure 3**).
- Of the 4 participants who ceased NA and had ≥9 months of follow-up within B-Sure, 100% (4/4) maintained response 6 months post NA cessation; no participants restarted NAs.

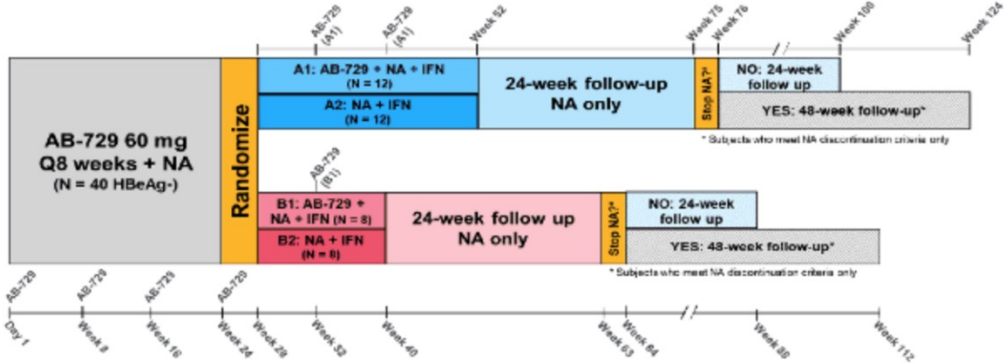
Figure 3. Responders from the On-NA population maintained durable treatment response off all treatment



Gadano A et al. EASL 2023

NUC discontinuation in clinical trials ongoing

AB-729-201 Study Design



NUC discontinuation if HBsAg <100

Yuen et al. EASL 2023

HEPATITIS B FOUNDATION

ANRS HB07 IP-Cure-B Proof of Concept (PoC) Clinical Trial. Educating the Liver Immune Environment Through TLR8 Stimulation Followed by NUC Discontinuation

Zoulim et al. H2020 IP-Cure-B project