Why do we need novel biomarkers for the clinical development of HBV cure therapies?

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Disclosure

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HBV treatment endpoints

Current

- Undetectable serum HBV DNA by PCR (LLOQ 10-20 IU/mL)
- Normal ALT (variable ULN)
- HBeAg seroconversion (in HBeAg+)
- HBsAg loss

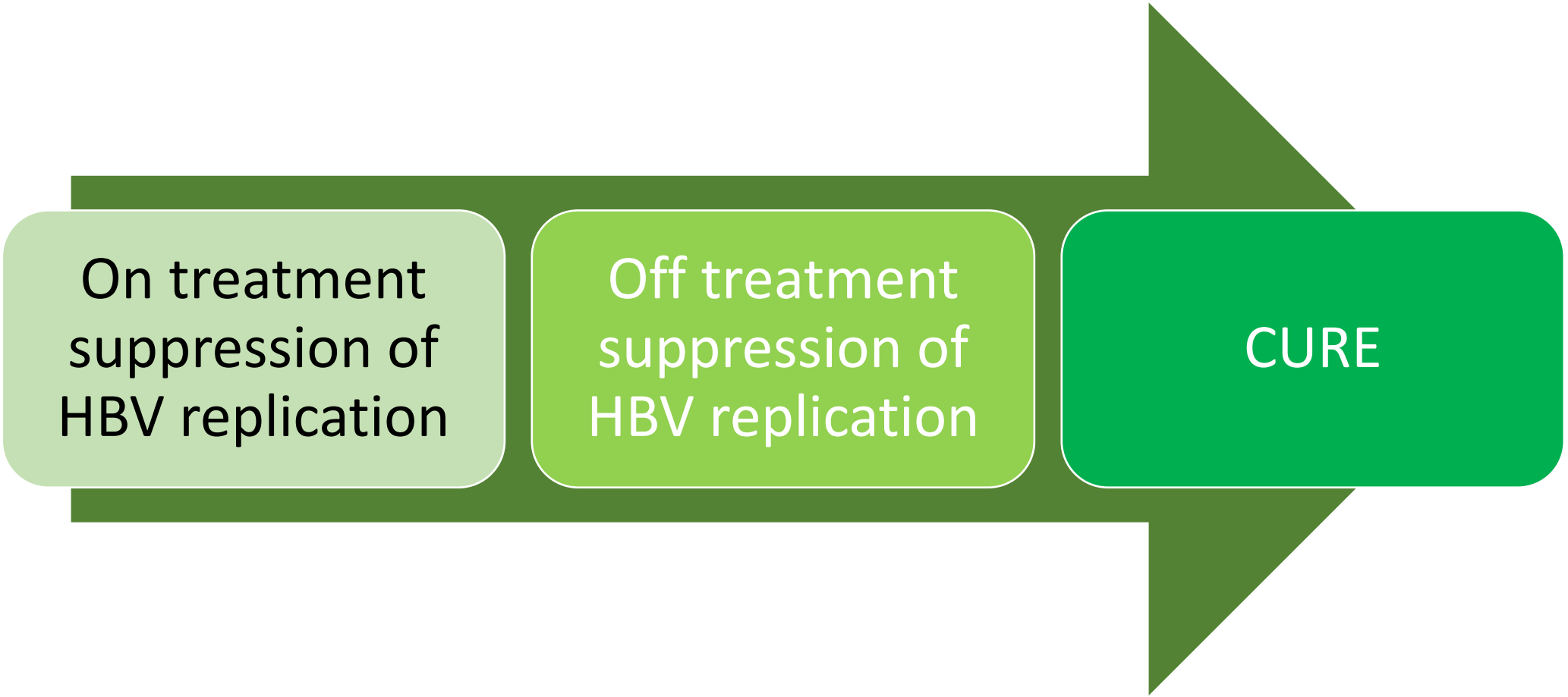
Historical

- Improvement in liver histology

Goal

- Decrease clinical outcomes: cirrhosis, decompensation, HCC, liver-related death

Evolution of HBV treatment goals



Definitions of HBV cure

	Complete/ Sterilizing cure	Idealistic functional cure	Realistic functional cure	Partial "cure"
Clinical scenario	**Never infected**	**Recovery after acute HBV**	**Chronic HBV with HBsAg loss**	**Inactive carrier off treatment**
HBsAg	Negative	Negative	Negative	Positive
Anti-HBs	Negative	Positive	Positive/negative	Negative
Serum HBV DNA	Not detected	Not detected	Not detected	Low level or not detected
HBeAg	Negative	Negative	Negative	Negative
Hepatic cccDNA, transcription	Not detected Not active	Detected Not active	Detected Not active	Detected Low level
Integrated HBV DNA	Not detected	Detected?	Detected	Detected
Liver disease	None	None	Inactive, fibrosis regress over time	Inactive
Risk of HCC	Not increased	Not increased	Declines with time	Risk lower vs. active hepatitis

How is HBV cure defined (AASLD-EASL 2016, 2019)?

- Sterilizing cure
  - Elimination of cccDNA and integrated HBV DNA (iDNA)
- Functional cure[1]
  - Sustained undetectable HBsAg, HBeAg, and HBV DNA after a finite course of therapy
  - Intended to reflect silencing of cccDNA transcription (assumed that cccDNA was sole or predominant source of HBV proteins and HBV DNA)

[1]Lok A et al., Hepatology 2017; 66: 1296 and Cornberg M et al., J Hepatol 2020; 72: 539

How is HBV cure defined (AASLD-EASL 2022)?

- Recommended endpoint is functional cure defined as HBsAg loss (<LLD 0.05 IU/ml) and HBV DNA below the limit of quantification (<LLQ 10 IU/ml) at 24 weeks off-therapy.
- An intermediate endpoint on the pathway to functional cure is partial cure, defined as HBsAg <100 IU/ml and HBV DNA <LLQ (<10 IU/ml) at 24 weeks off-treatment. Further data needed to establish that HBsAg decline to <100 IU/ml is a clinically meaningful threshold for use as part of composite clinical trial endpoint.
- qHBsAg, HBeAg/anti-HBe, and qHBV DNA should be assessed with every regimen at baseline, on-treatment, end-of-treatment, 24-weeks off-treatment and long-term off treatment.
- HBV genotype should be obtained at baseline on all viremic individuals.
- Other markers: qHBeAg (HBeAg+ patients only), qAnti-HBc, qHBV RNA, qHBcrAg, ultrasensitive qHBsAg, HBsAg isoforms should be explored.

Ghany M, Hepatology 2023 (in press)

How can HBV cure be accomplished?

- NA
- Entry inhibitors
- CAMs
- siRNAs/ASOs

Replication inhibition

- siRNA/ASO
- NAP/STOP
- Gene editing
- Epigenetic modifications

↓Viral antigen production / release

- TLR7/8 agonists
- Anti-PD1/anti-PDL1
- B/T cell vaccines
- IFN
- Pre-S/S antibodies

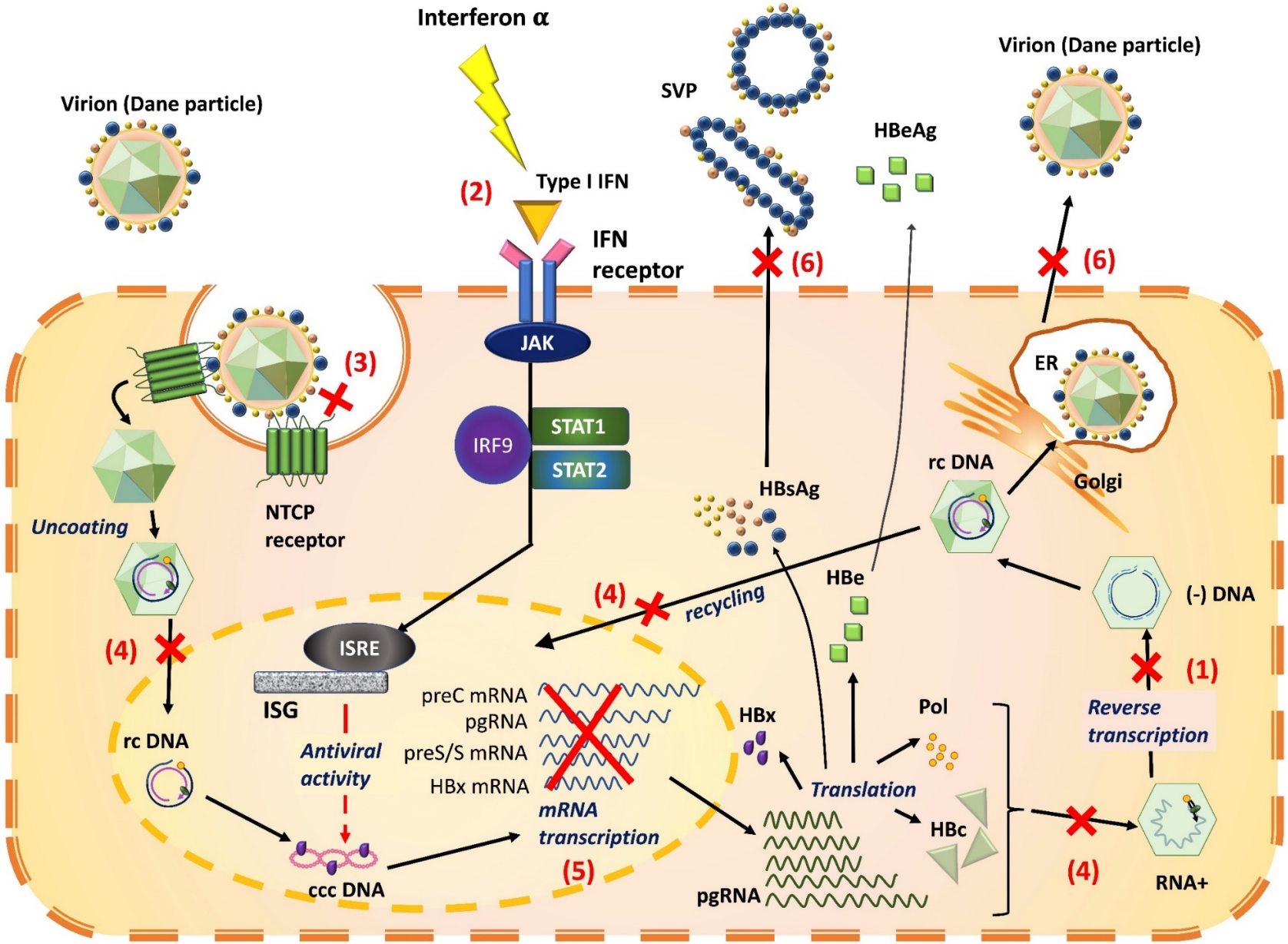
Enhance or restore innate/adaptive immune responses to HBV

Functional cure

Sustained HBsAg loss and undetectable HBV DNA after finite course of treatment

Antiviral strategies

1. Nucleos(t)ide analogues (NA)
2. Interferon (IFN)
3. Entry inhibitors
4. Capsid assembly modulators (CAM)
5. Post-transcription inhibition (short interfering RNA [siRNA], antisense oligonucleotide [ASO])
6. Secretion inhibition (nucleic acid polymer [NAP])

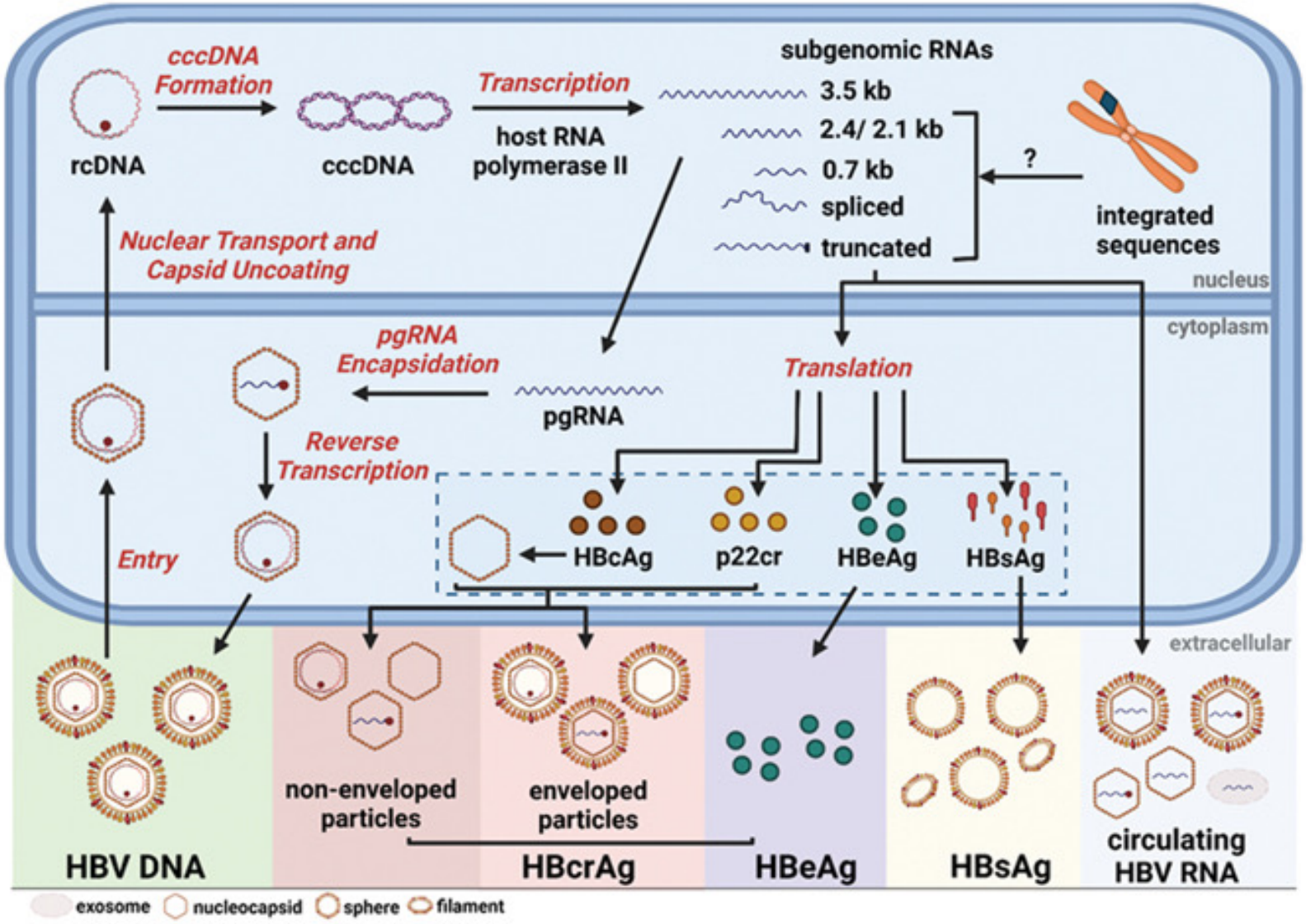


Jeng WJ and Lok AS

Why do we need novel biomarkers for the clinical development of HBV cure therapies?

- To confirm target engagement
- To assess virological response during treatment
- To predict likelihood of achieving cure
- To ascertain cure

HBV Replication Cycle and Circulating HBV Markers



Tu T, Semin Liver Dis 2022; 42: 327

Confirm target engagement

- Preferably blood markers but may also involve liver markers, commercial or research assays
- HBsAg production or release: HBsAg
- cccDNA transcription: HBV RNA, HBcrAg (HBcAg?)
- HBV DNA replication: HBV DNA
- Virus entry: HBV DNA, HBsAg, bile acid
- Integrated HBV DNA transcription: HBsAg (iHBsAg?)
- cccDNA elimination: hepatic cccDNA, hepatic pgRNA
- Integrated HBV DNA elimination: hepatic host-virus junction sequences?
- Restoration of immune response: hepatic/serum immune markers?

Assess virological response during treatment

- Assays
  - Blood-based, serial assessment
  - Standardized, sensitive, specific, quantitative
  - Perform equally well across genotypes and common variants
  - Readily available, quick turnaround of results
- Markers
  - HBV DNA, HBeAg (in HBeAg+), HBsAg - essential
  - HBV RNA, HBcrAg

Predict likelihood of achieving cure after treatment is stopped

- HBsAg:
  - Follow kinetics of HBsAg decline
- HBV DNA
- pgRNA, HBcrAg: as surrogates for cccDNA transcription, importance may vary depending on mechanism of action of therapy

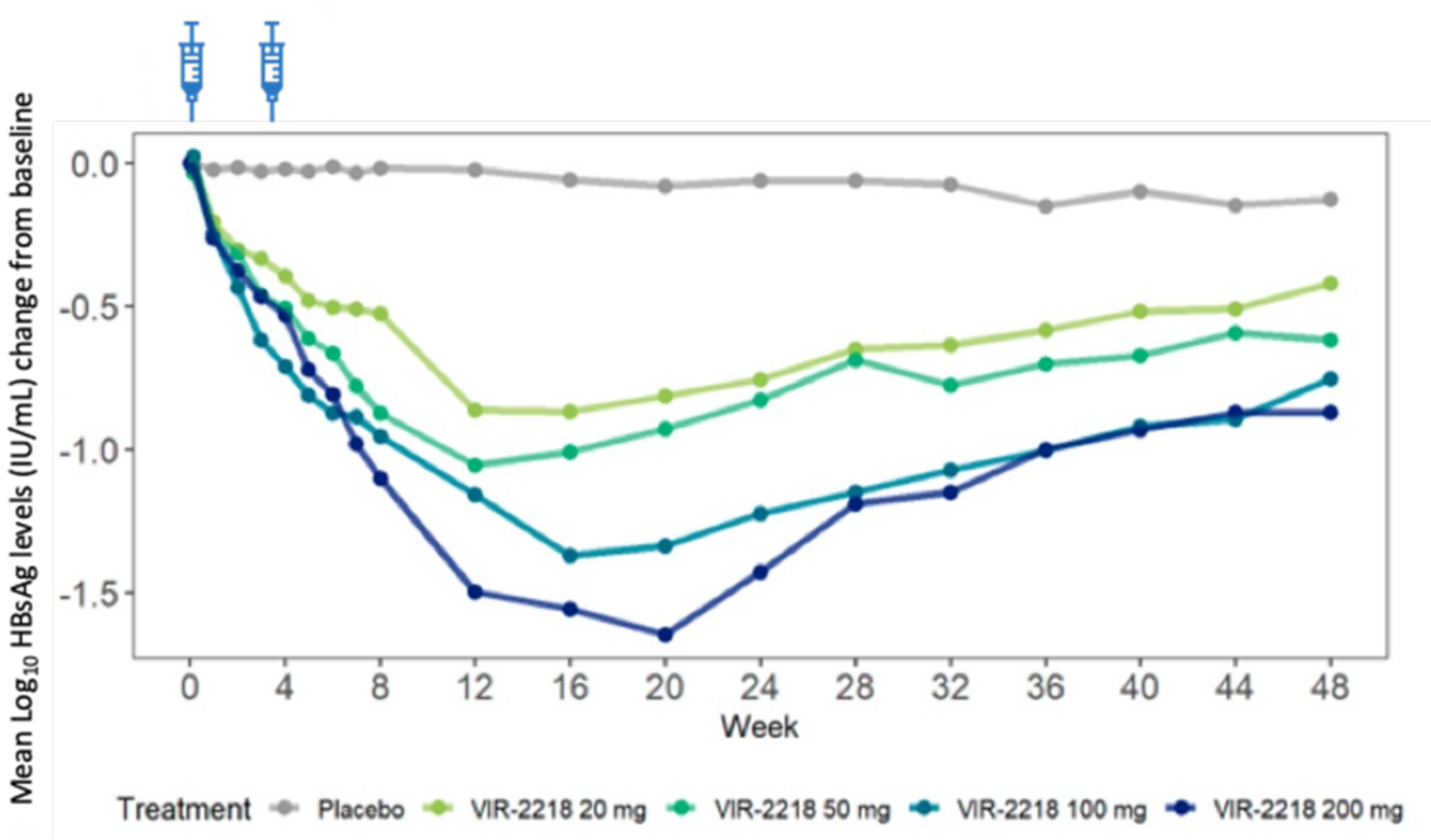
Ascertain HBV cure

- Sustained undetectable HBsAg and unquantifiable HBV DNA
  - Reliable assays widely available, need long-term follow-up
- Sustained suppression of cccDNA transcription
  - Necessary?
- Elimination of cccDNA and integrated HBV DNA
  - Ultimate goal, would eliminate risk of HBV reactivation
  - Liver-based, research assays – clinical utility?
  - cccDNA: research assays available, need standardization
  - Integrated HBV DNA: research assays reported, need validation and standardization

Serum quantitative HBsAg level

- Marker used to
  - Confirm target engagement
  - Select patients with high likelihood of response
  - Assess response during treatment
  - Predict response: kinetics during treatment, level at end of treatment
  - Determine success of curative therapy
- Limitations of current assays
  - Inadequate sensitivity
  - Cannot detect HBsAg bound to anti-HBs in immune complex
  - Cannot differentiate source from ccc or integrated HBV DNA – HBsAg may remain positive despite complete suppression of cccDNA transcription

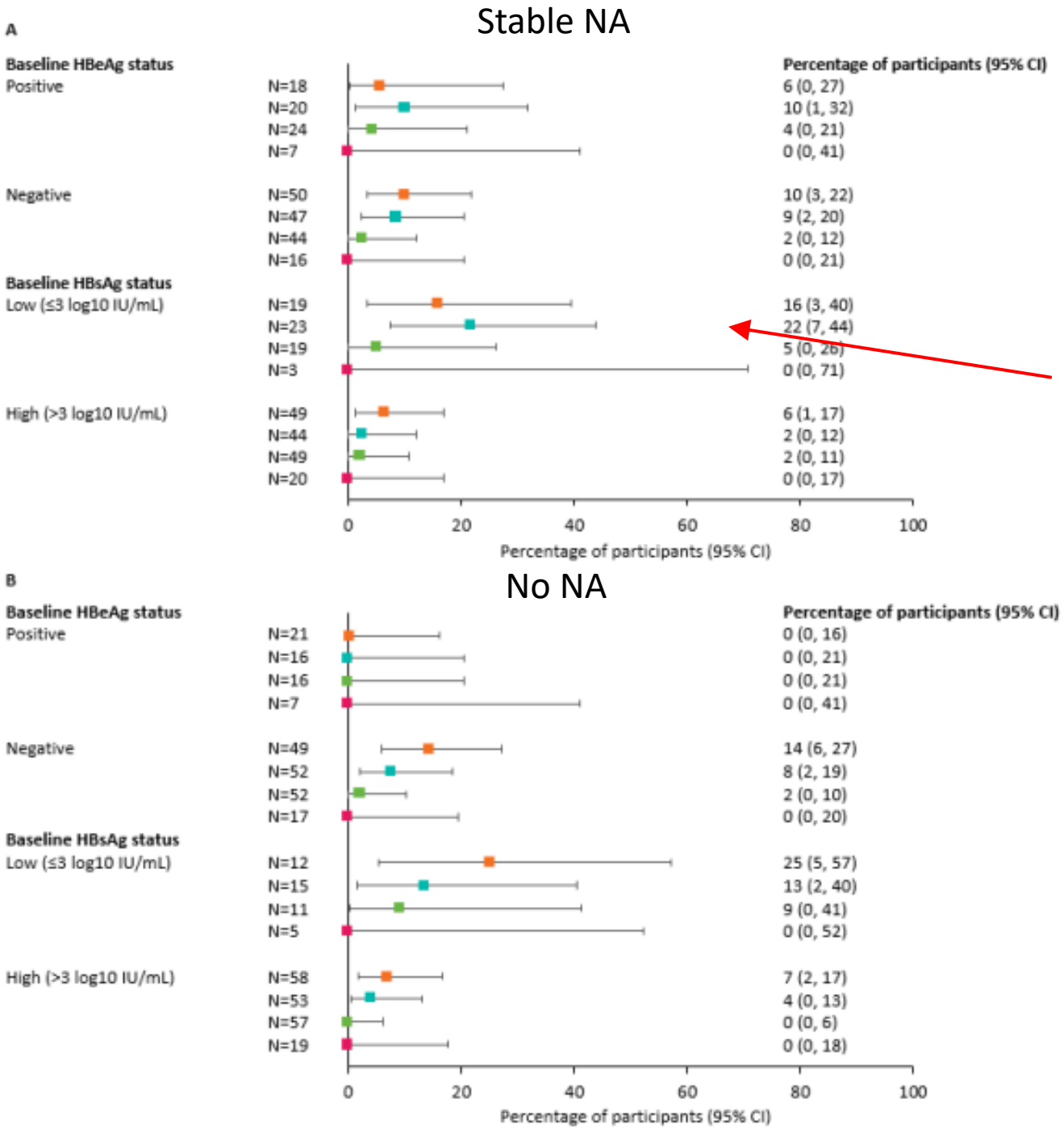
siRNA therapy VIR-2218 phase 2 study
Dose-dependent effect on qHBsAg



- siRNA and antisense oligonucleotide therapies target multiple HBV RNAs - decrease production of virions and viral proteins
- Dose response effect on HBsAg kinetics
- Slow rebound after treatment

Gane E, ILC 2020

Bepirovirsen (ASO) B-CLEAR Trial: Proportion Achieving Primary Outcome in Participants (A) receiving NA versus (B) no NA



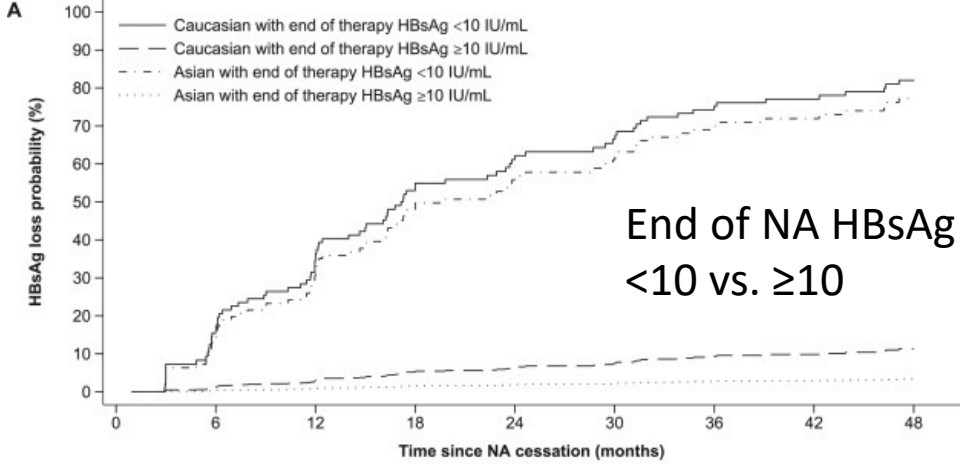
Higher rates of response in participants with low baseline HBsAg ≤3 log10 IU/mL

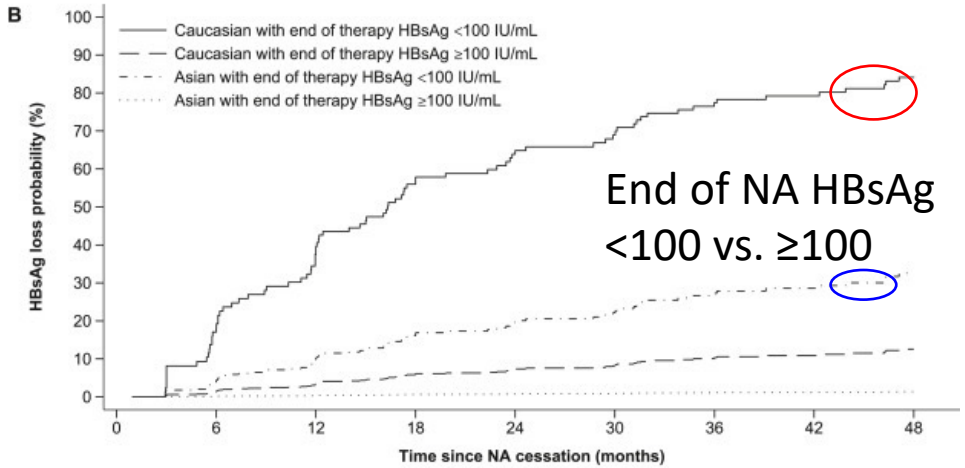
Response in Group 1 (300 mg x 24 weeks) higher in HBeAg- vs. HBeAg+ participants, particularly those not on NA

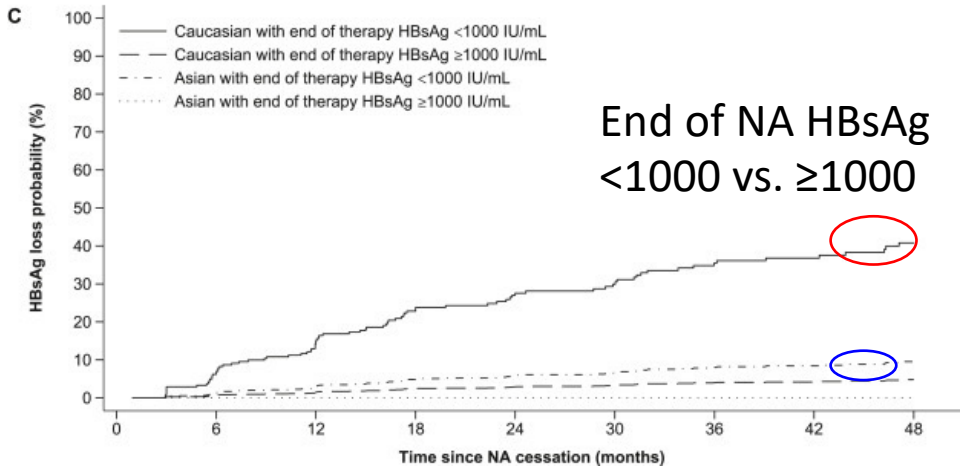
- On stable NA: 10% vs. 6%
- No NA: 14% vs. 0%

0% response across all 4 treatment groups among HBeAg+ not on NA

Yuen MF, 2022 NEJM 2022; 387: 1957





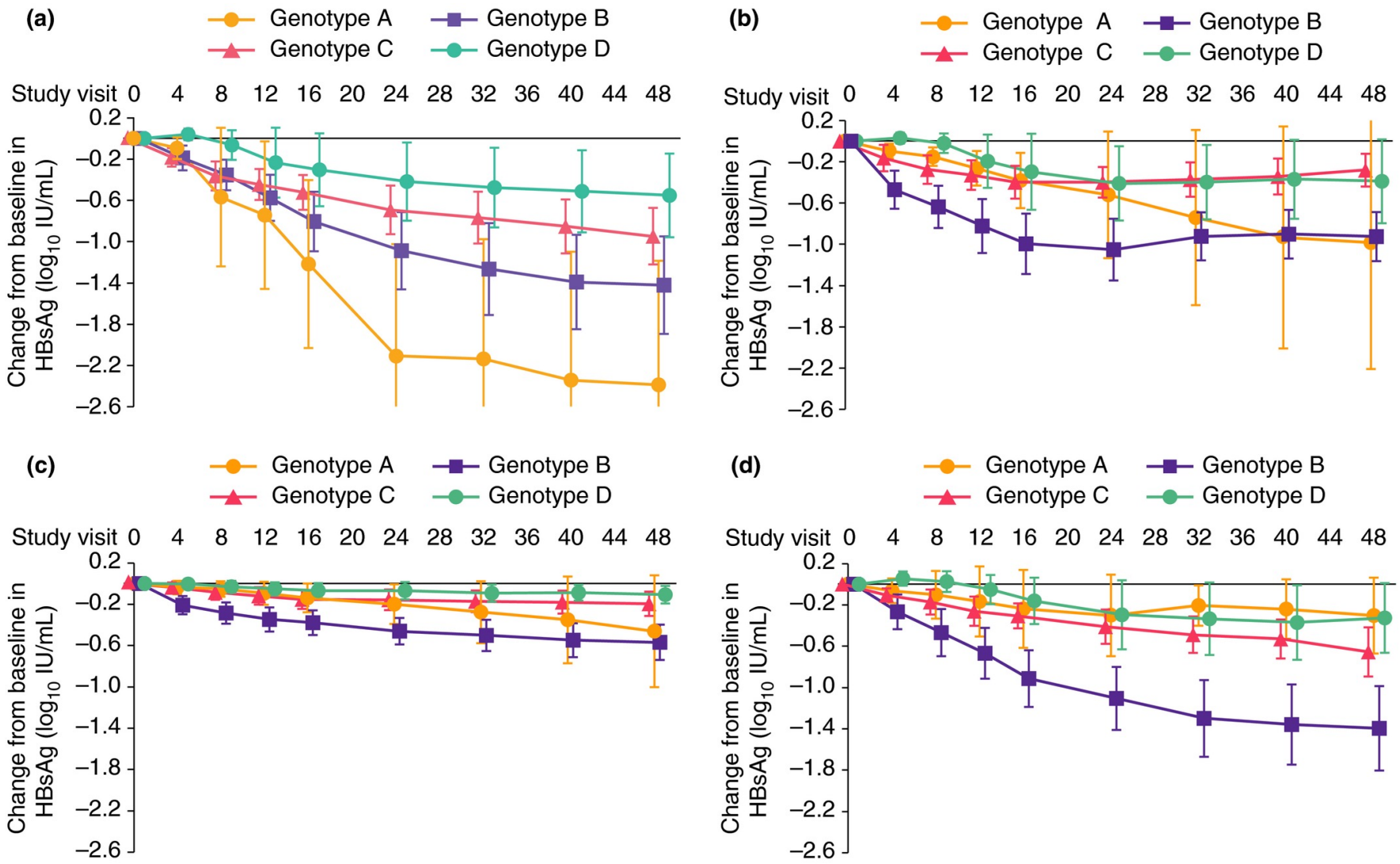


End of Treatment HBsAg Predicts HBsAg Loss after NA Withdrawal

- RETRACT-B – retrospective study of 1552 adult CHB patients, HBeAg- at time NA was stopped
- End of treatment HBsAg as predictor of HBsAg loss after NA withdrawal modulated by race
- Rates of HBsAg loss at FU year 4 according to end of NA HBsAg (IU/mL)
  - <10 – high in both Asians and Caucasians
  - <100 – significantly higher in Caucasians than Asians
  - <1000 - ~30% in Caucasians and <10% in Asians

Hirode G, Gastroenterol 2022; 162: 757

Kinetics of HBsAg Decline Vary by Treatment and HBV Genotype



a: TDF + PegIFN x 48 wks

b: TDF + PegIFN x 16 wks then TDF x 32 wks

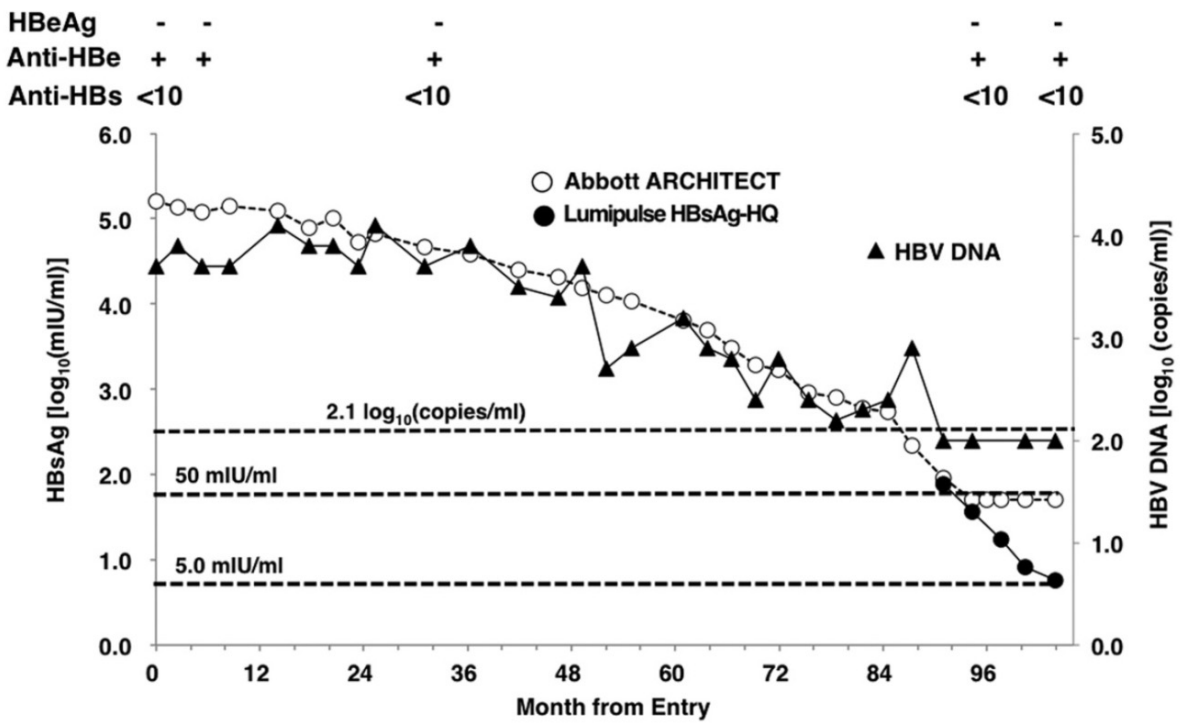
c: TDF alone x 120 wks

d: PegIFN alone x 48 wks

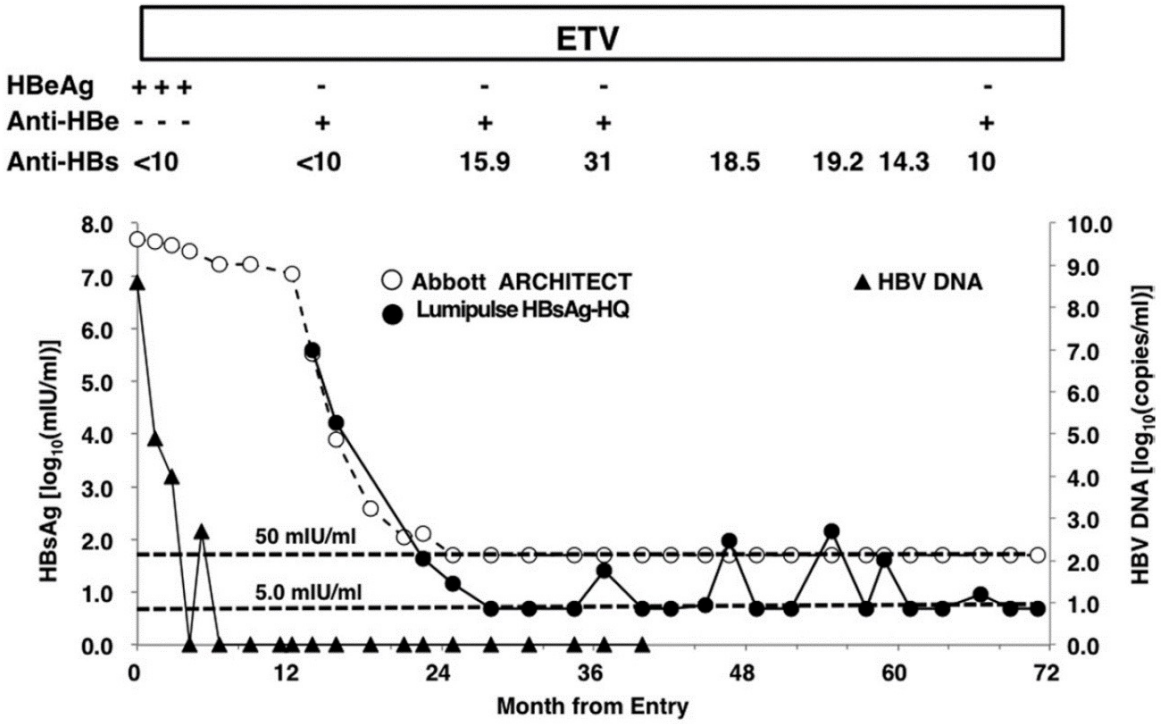
Marcellin P, Aliment Pharmacol Ther 2016; 44: 957

HBsAg Can be Detected in Some Patients with Spontaneous or Treatment-associated HBsAg Loss Using More Sensitive Assays

Spontaneous HBsAg clearance



Treatment-associated HBsAg clearance



Limit of detection: Abbott ARCHITECT 0.05 IU/mL, Lumipulse HBsAg-HQ 0.005 IU/mL

Shinkai N, J Clin Microbiol 2013; 51: 3484

Will a More Sensitive HBsAg Assay Be Needed?

To identify potential sustained responders who can stop treatment

Simulated HBsAg profiles following 300 mg QW dosing for 24 weeks

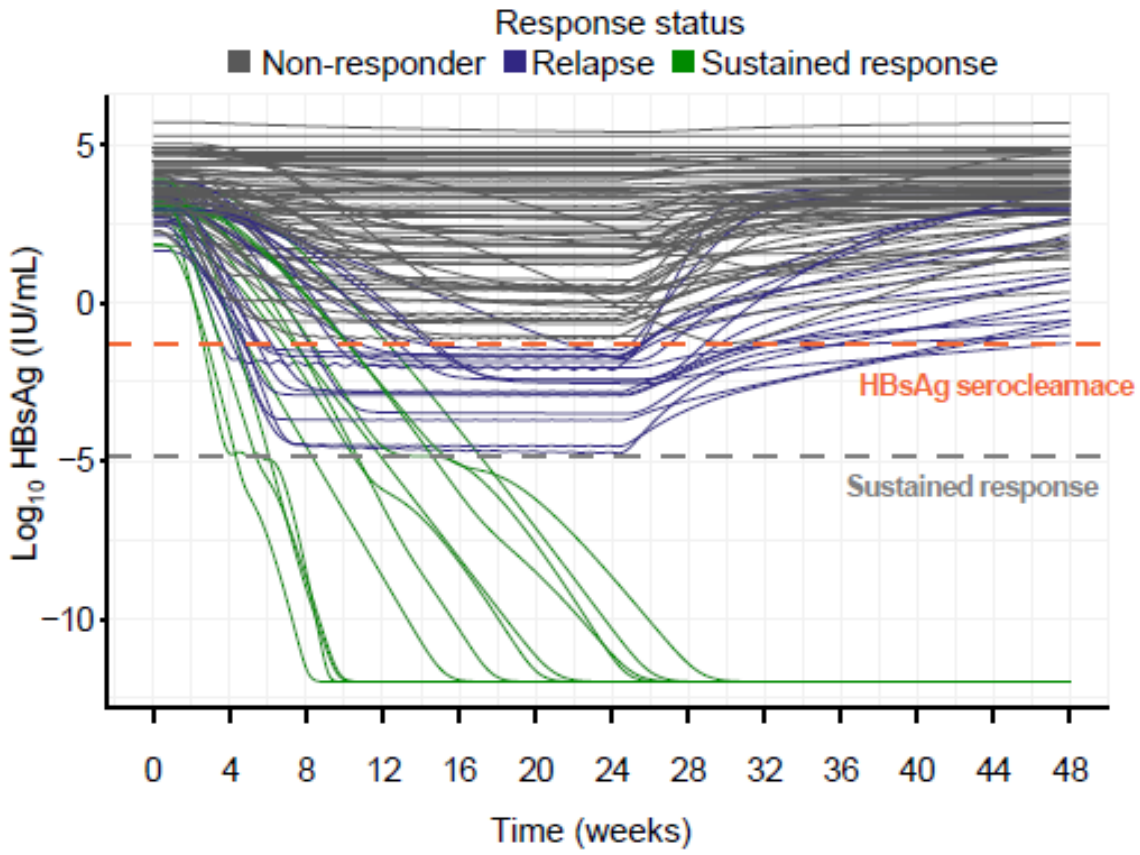
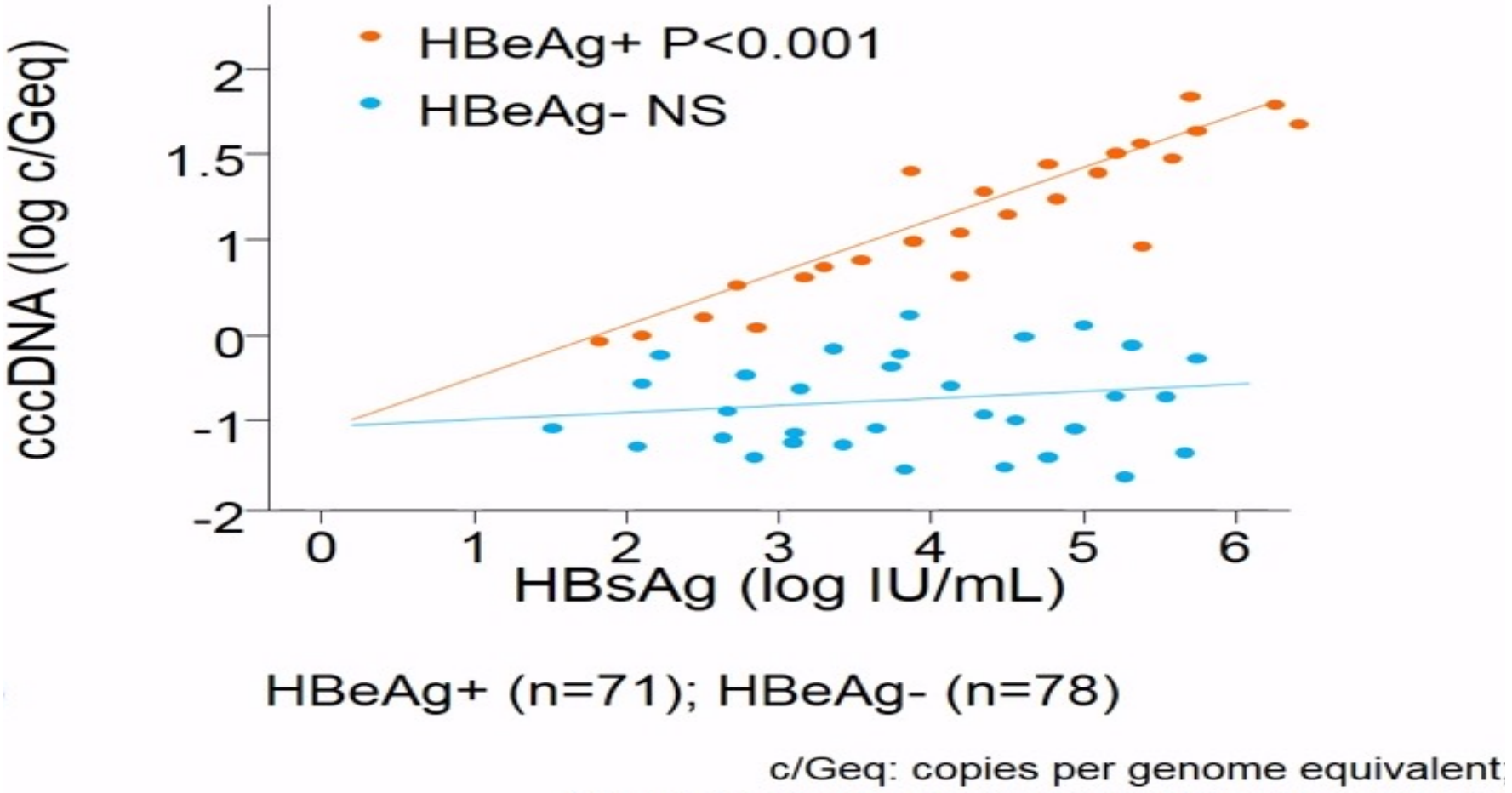


Figure amended with persmission from Youssef A, et al. Poster presented at EASL 2022 (Poster No. SAT441). Chronic Hepatitis B Virus Infection: Developing Drugs for Treatment – Guidance for Industry. FDA CDER. April 2022.
FDA, Food and Drug Administration; HBsAg, hepatitis B surface antigen; QW, once a week.

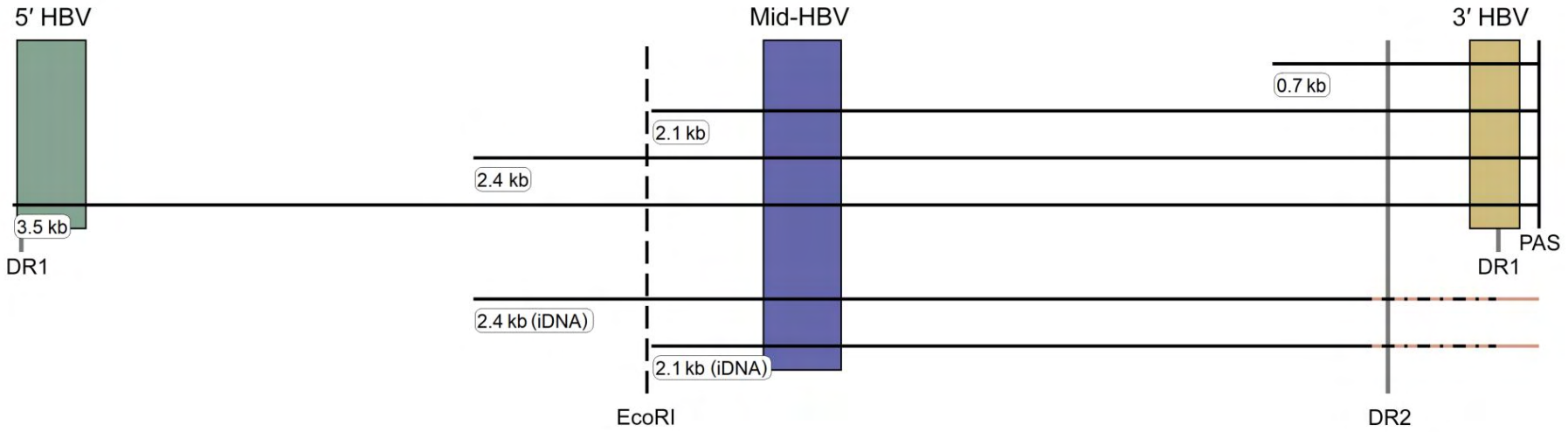
- FDA guidelines defines **HBsAg loss <0.05 IU/mL**
- A likelihood-based method was implemented to predict HBsAg values below the lower limit of detection (<0.05 IU/mL) to provide a complete HBsAg profile during on- and off-treatment periods
- Subjects who achieve HBsAg seroclearance but do not hit a lower threshold are predicted to eventually **relapse**.
- More sensitive assays may be needed:
  - → To help **validate** model predictions
  - → **Monitor** patients with precision

Serum HBsAg Level Correlates with Intrahepatic cccDNA in HBeAg+ but not HBeAg- Patients



Thompson A, Hepatology 2010; 51: 1933

Multiplex Digital Droplet PCR Assay for HBV S Transcripts from cccDNA vs. integrated HBV DNA



S transcripts from cccDNA

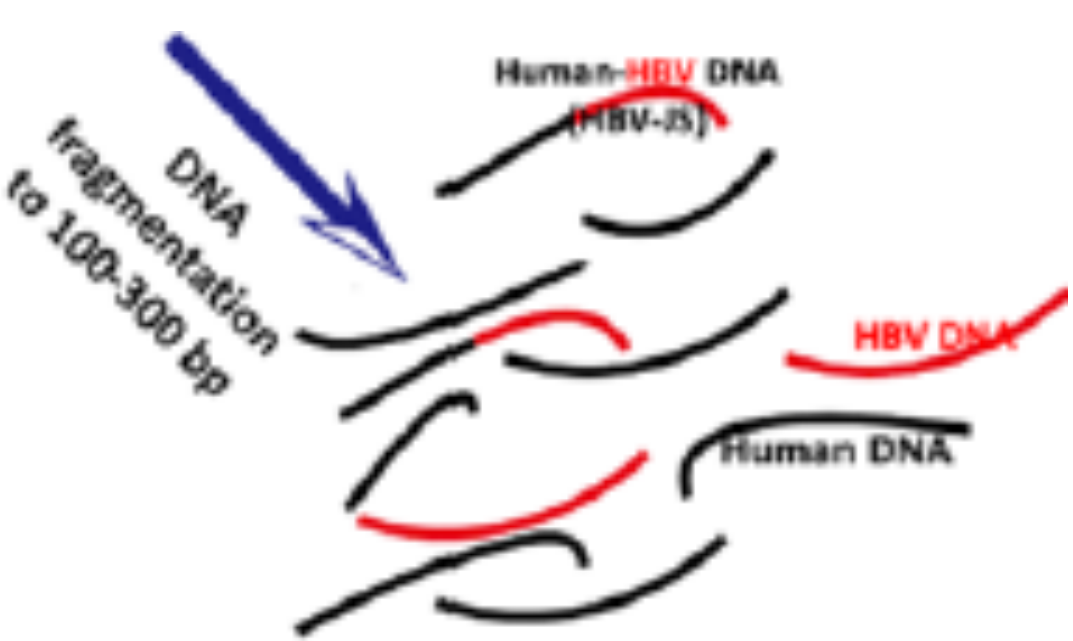
S transcripts from integrated HBV DNA
Lacks 3’ end

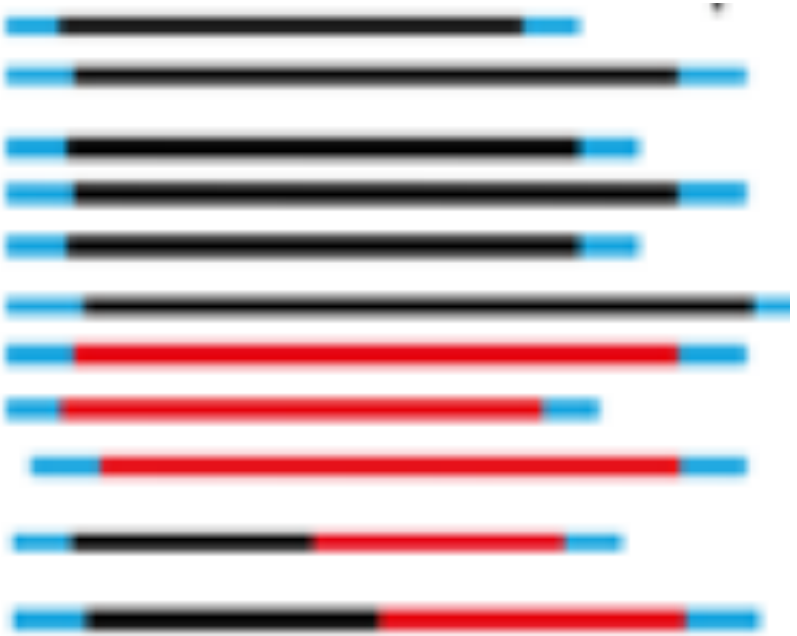
PCR target	S transcripts cccDNA	S transcripts integrated HBV DNA
Mid-HBV	Positive	Positive
3’ end	Positive	Negative

- Requires liver tissue
- Measures HBV S transcripts not HBsAg

Grudda T, J Clin Invest 2022; 132: e161818

Detection of Integrated HBV DNA Using Next Generation Sequencing (NGS) to Identify Virus-Host Junction Sequences





- Total DNA, HBV enrichment, NGS, identification of HBV-host junction sequences
- Potential to detect +/- quantify circulating integrated HBV DNA but not HBsAg
- Can be applied to liver tissue as well as urine samples
- Resource intense, unlikely to be clinically applicable

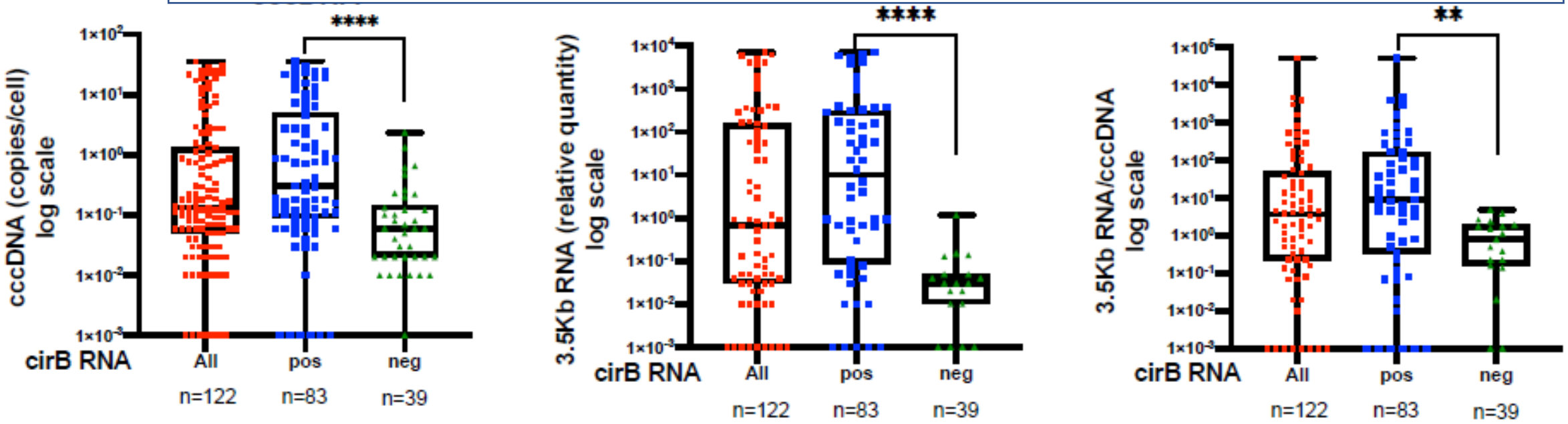
Shieh FS, PLOS One 2017; 12: e0182843; Lin SY, Hepatol Commun 2021; 5: 1649

Serum HBV RNA Levels

- Pregenomic RNA – most direct indicator of cccDNA transcriptional activity
  - Indicator of target engagement in capsid assembly modulator therapy
  - Predictor of cure after novel therapies: limited data
- Current assays not standardized, inadequate sensitivity, may be influenced by presence of HBV DNA, may include other RNA species such as HBV mRNA and spliced RNA in addition to pgRNA
- HBV RNA kinetics predicts HBeAg seroconversion during treatment with pegylated interferon or NA, data on prediction of HBsAg loss limited for current and new therapies
- Persistent detection of serum HBV RNA at end of NA therapy associated with higher likelihood of viral rebound after discontinuation of treatment, data on prediction of HBsAg loss limited for NA and new therapies

Detection of Circulating HBV RNA Correlates with Intrahepatic cccDNA Transcriptional Activity

cccDNA quantity | pgRNA quantity | cccDNA transcription

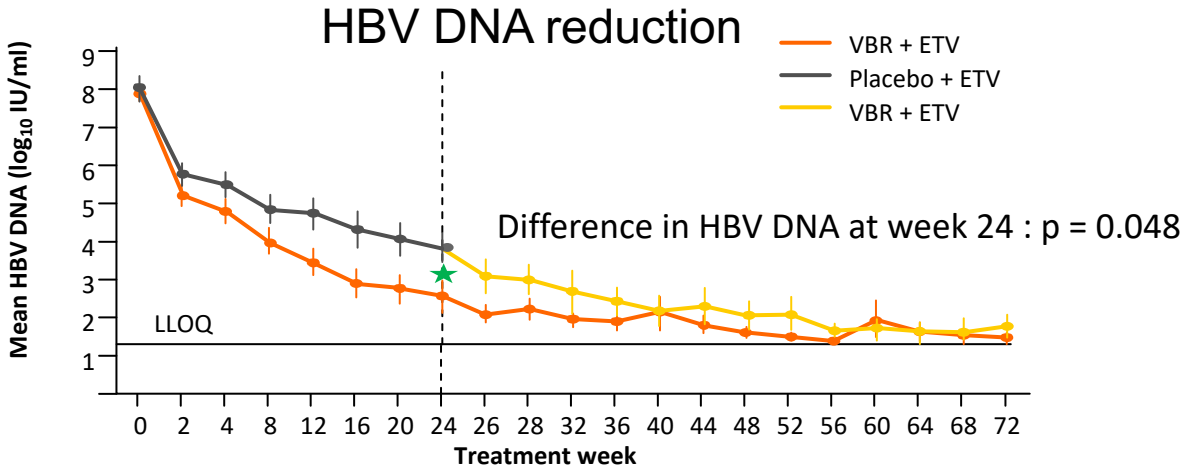


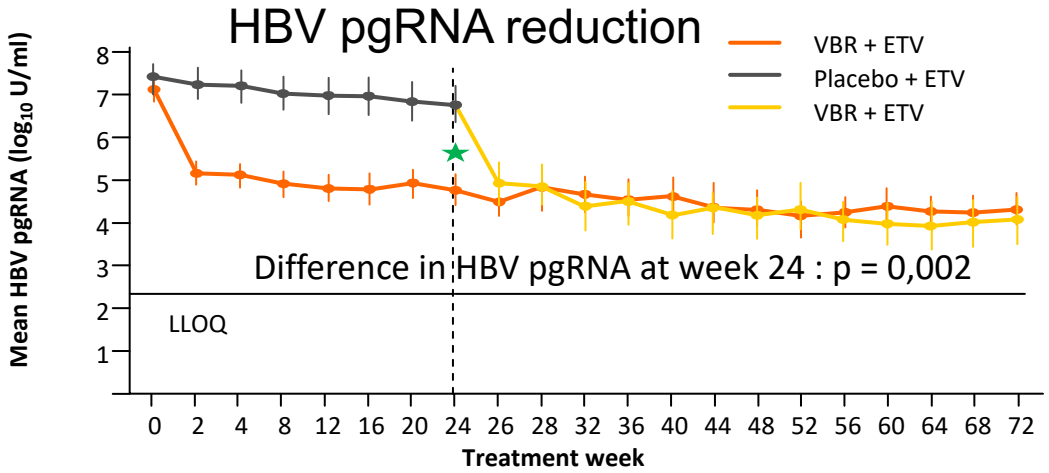
Testoni B, ILC 2021

Entecavir +/- Vebicorvir (CAM) in HBeAg+ CHB

Combination therapy more pronounced suppression of pgRNA than ETV alone

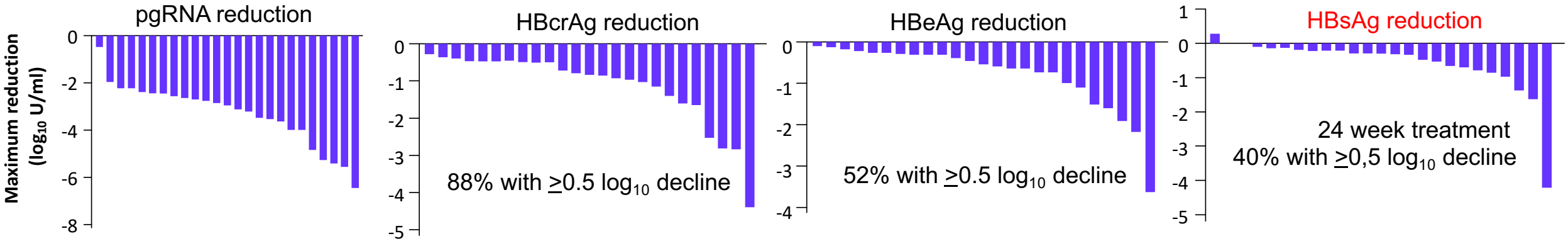
Treatment-naive patients





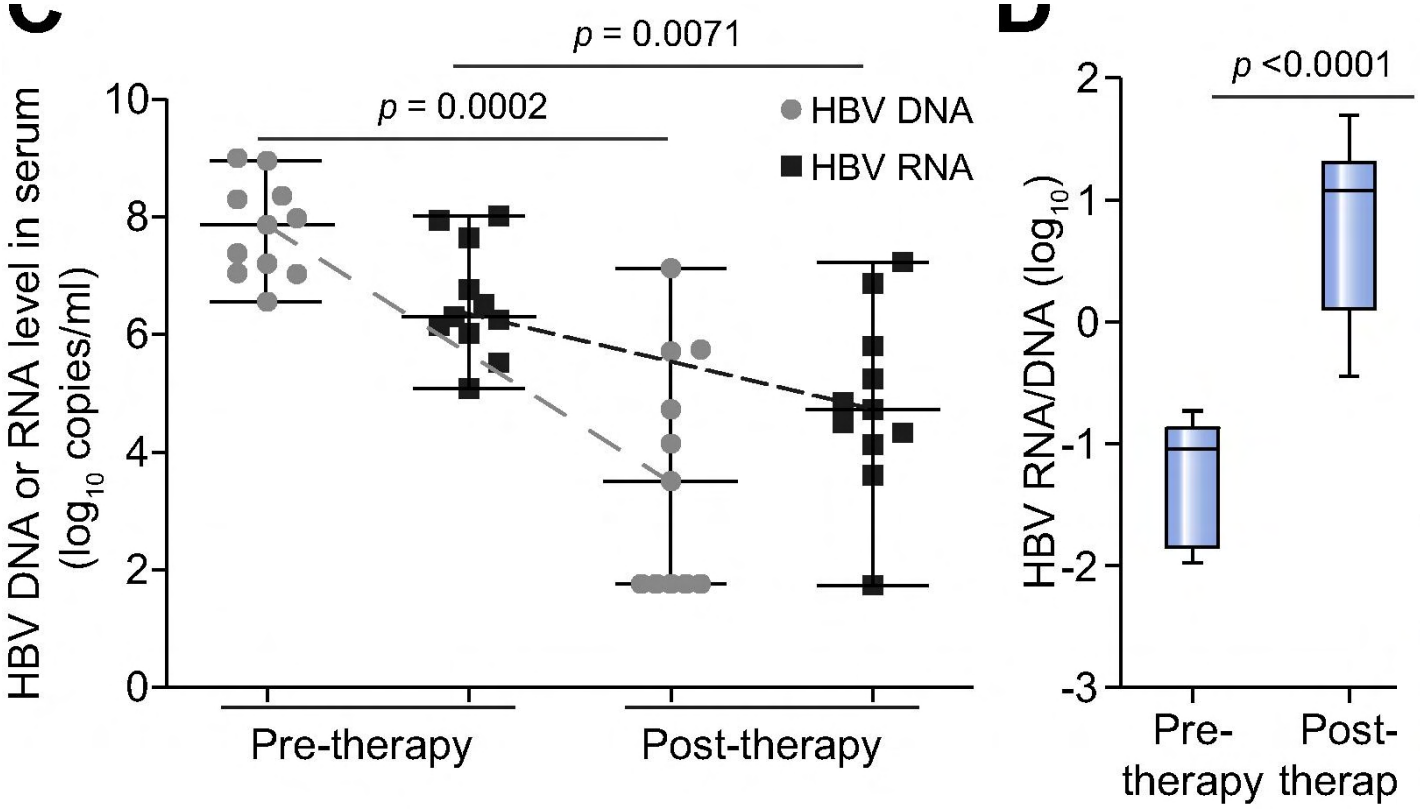
ABI-H0731 + ETV more marked decrease in HBV DNA and pg RNA than ETV alone

Change at week 24 from baseline for individual patients



Yuen MF et al. EASL 2020, Abs. LP30; Sulkowski M, J Hepatol 2022; 77: 1265

Serum HBV RNA at End of Nucleos(t)ide Analogue (NA) Treatment Predicts viral Rebound after Treatment

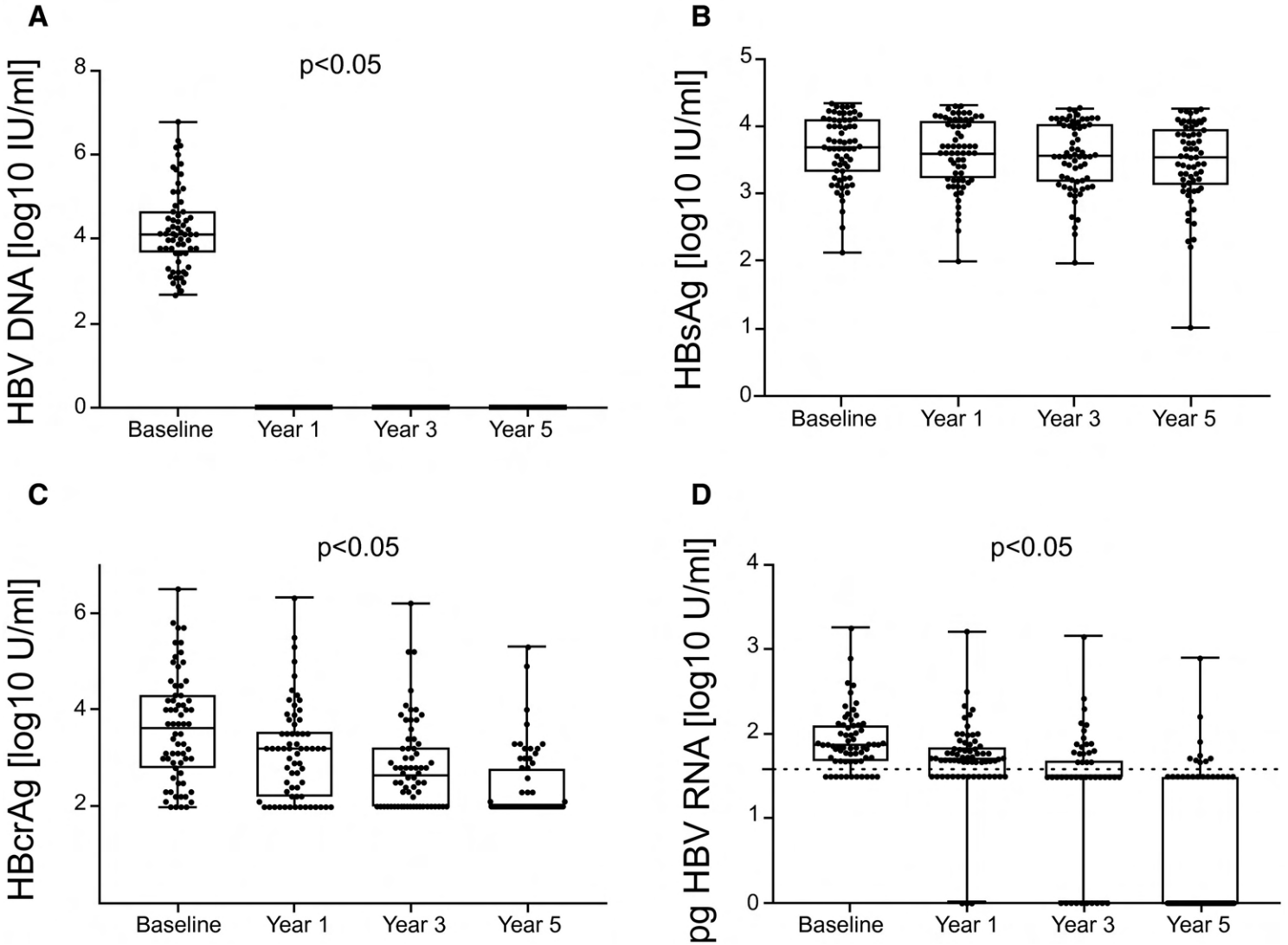


- NA results in greater reduction in serum HBV DNA than HBV RNA
- Viral rebound in 21/21 vs. 3/12 patients with and without detectable serum HBV RNA after cessation of NA therapy

HBV RNA	Viral rebound (n)	No viral rebound (n)	Total (n)	*p value
Positive	21	0	21	
Below the LoQ	3	9	12	0.001
Total (n)	24	9	33	

Wang J, J Hepatol 2016; 65: 700

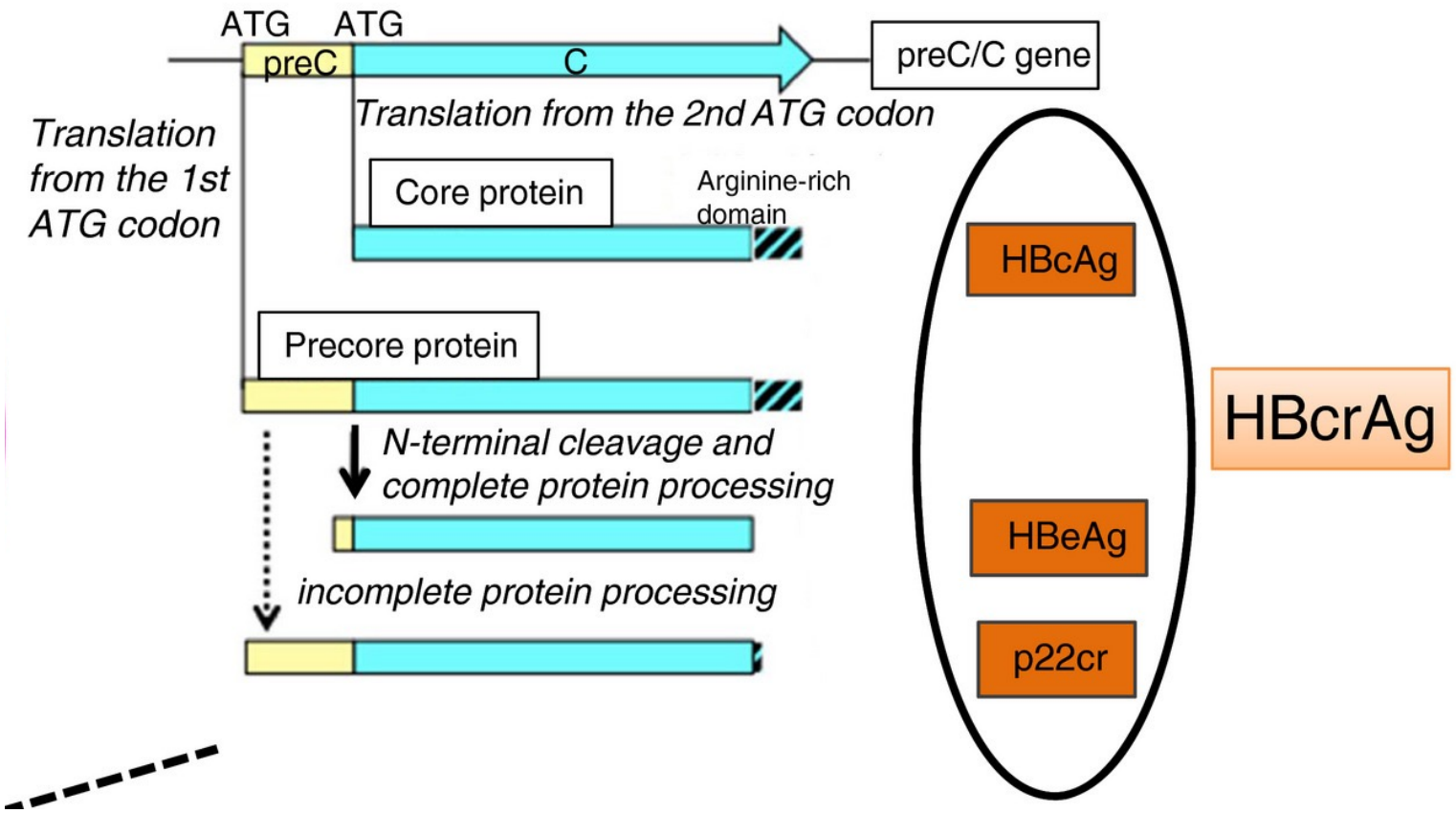
Kinetics of HBV markers in HBeAg- patients receiving nucleos(t)ide analogue (NA) therapy



- HBV RNA and HBcrAg remain detectable in patients receiving NA therapy despite undetectable HBV DNA, reflecting continued cccDNA transcription and translation
- HBV RNA and HBcrAg at end of NA therapy predict ALT flares after NA withdrawal
- Minimal change in qHBsAg after 5 years treatment

Carey I, Hepatology 2020; 72: 42

Hepatitis B Core Related Antigen (HBcrAg)

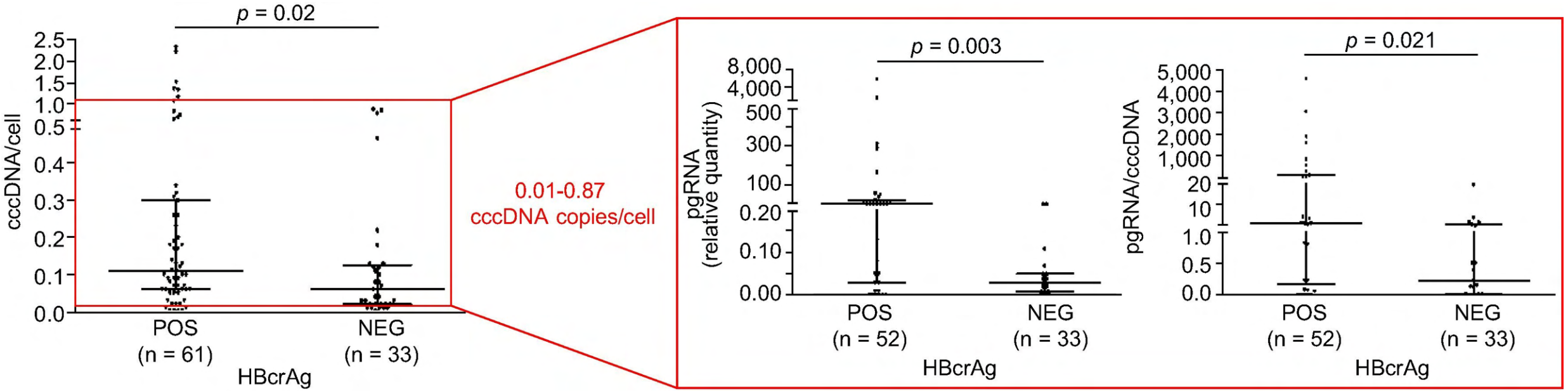


- Measures 3 antigens including HBeAg
- Marker of intrahepatic HBV cccDNA and its transcription and translation
- Sensitivity of current assay is suboptimal

Utility of HBcrAg Assays

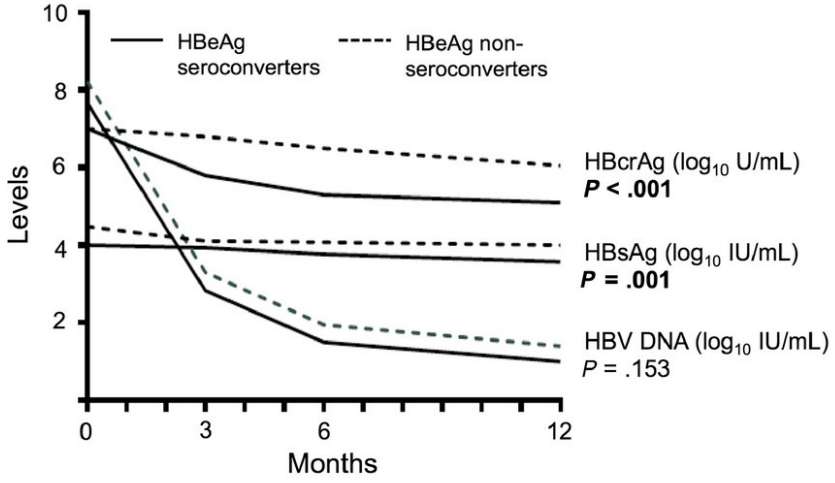
- Serum HBcrAg concentration correlates with
  - Intrahepatic cccDNA transcriptional activity
  - Quantitative HBsAg levels (modest correlation)
  - Serum HBV DNA levels (good correlation except for inactive carriers)
  - Serum ALT levels and histologic activity (modest correlation)
- During nucleos(t)ide analogue or Peg-IFN therapy, serum HBcrAg level
  - Better indicator of decrease in cccDNA than serum HBV DNA
  - Low baseline level and greater decline during treatment associated with higher likelihood of HBeAg clearance
  - Remains detectable after serum HBV DNA becomes undetectable, predictor of post-treatment viral relapse and to a lesser extent with HBsAg loss

Circulating Hepatitis B Core-related Antigen (HBcrAg) Correlates with Higher Intrahepatic cccDNA Quantity and Transcriptional Activity

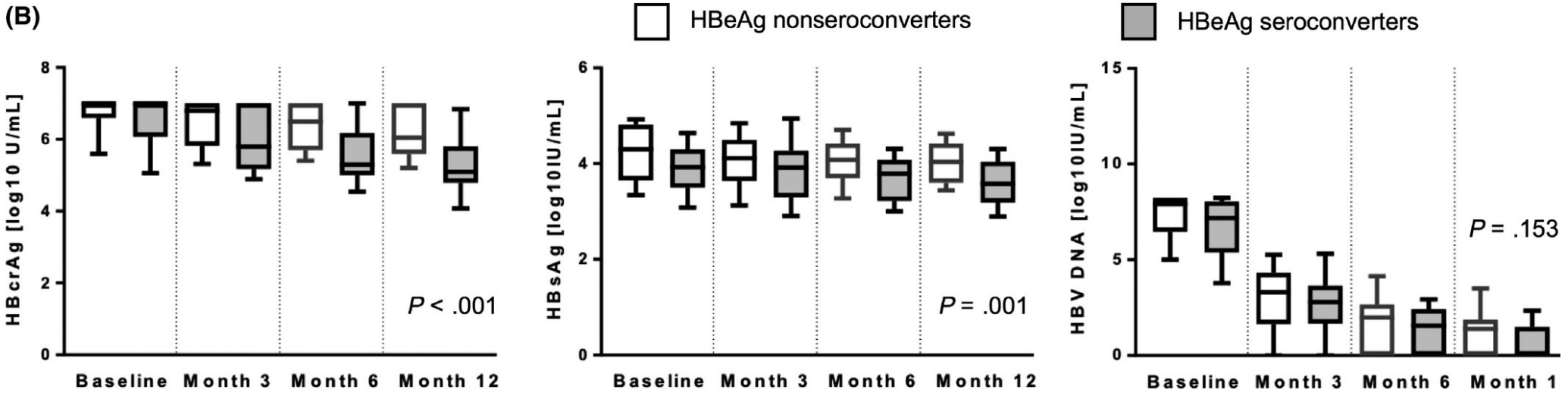


Testoni B, J Hepatol 2019; 70: 615

Baseline and Early Decline in HBcrAg Level Better Predictor of HBeAg Seroconversion during NA Therapy than HBsAg Level



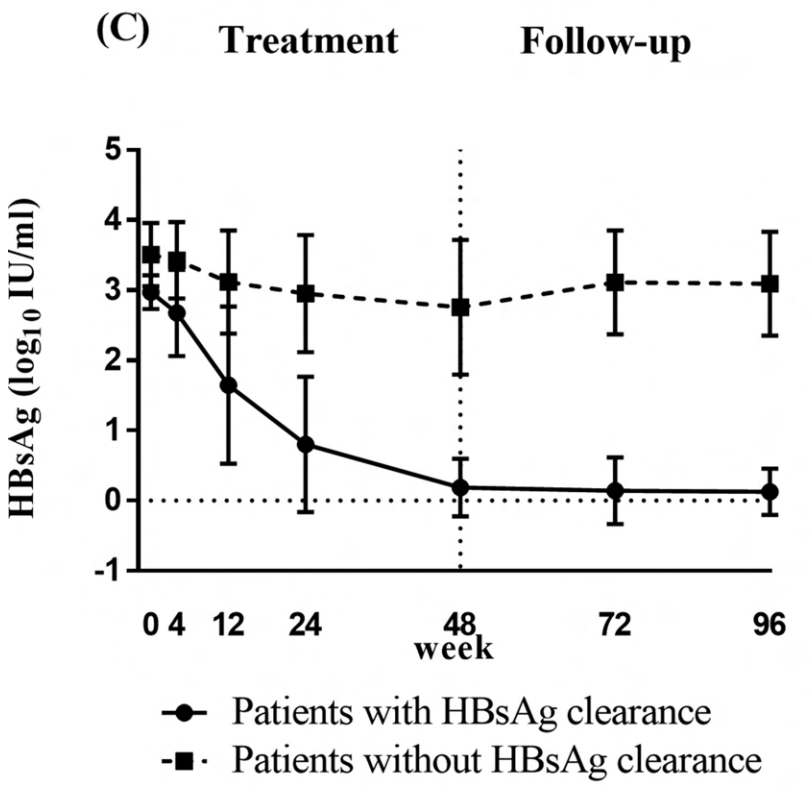
- 118 HBeAg+ patients received nucleos(t)ide analogue (NA), 43 HBeAg seroconversion
- HBcrAg better predictor of HBeAg seroconversion than HBsAg level, combination of both markers better than either alone



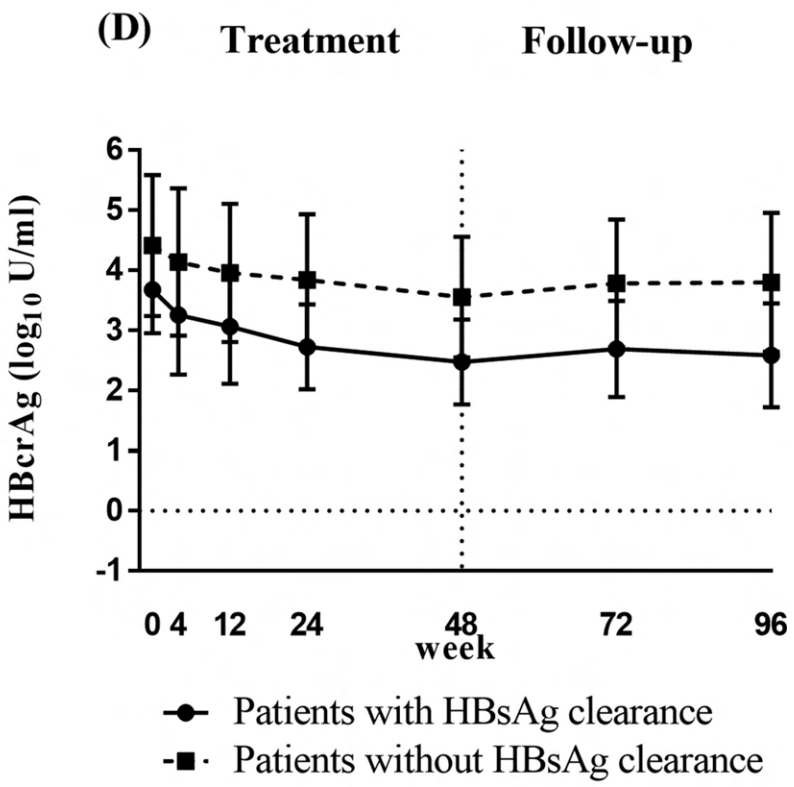
Wang B, J Viral Hepat 2018; 25: 886

HBsAg level better predictor of HBsAg loss than HBcrAg in HBeAg- patients receiving peginterferon +/- entecavir

qHBsAg



HBcrAg



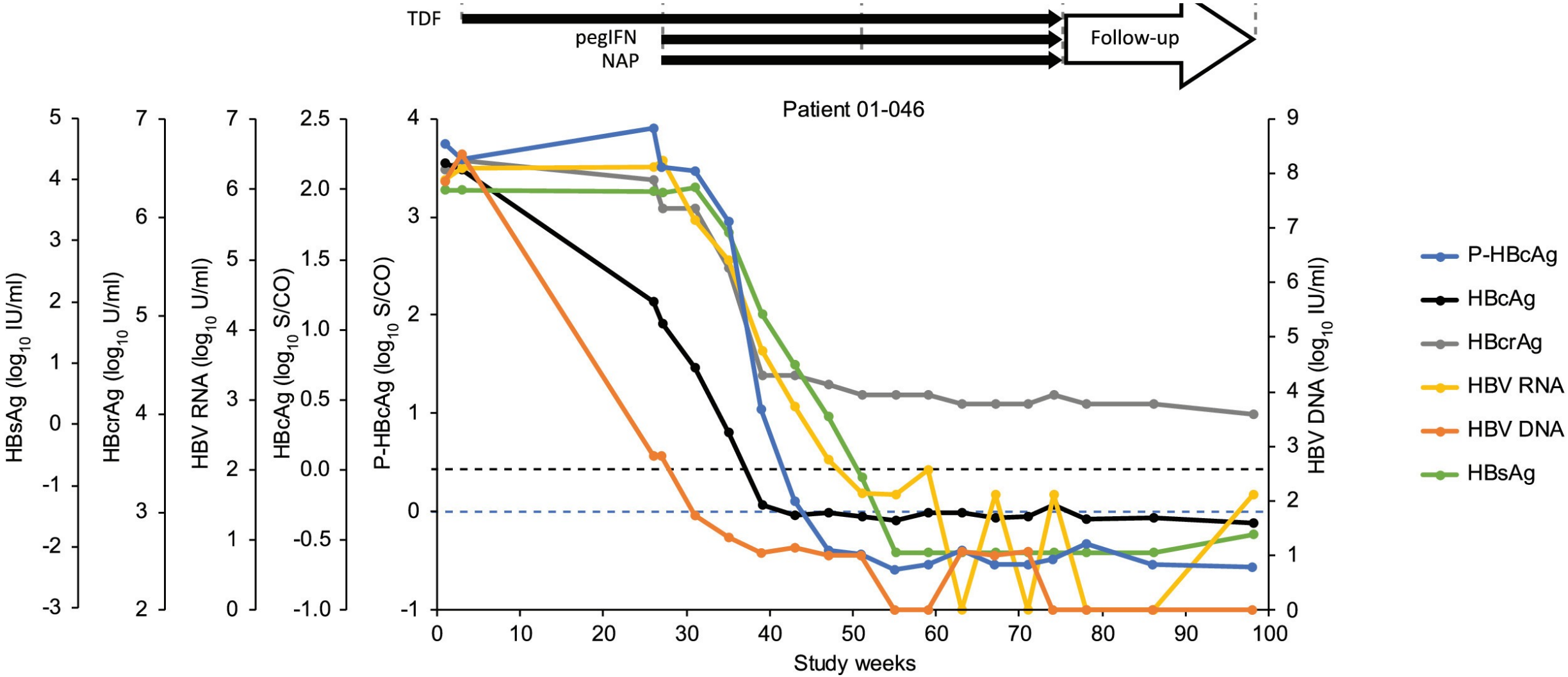
- 121 patients received 48 weeks peginterferon +/- entecavir
- 11 cleared HBsAg
- On treatment decline in HBsAg better predictor of HBsAg clearance than decline in HBcrAg (or HBV DNA)
- HBsAg decline at week 12 or 24 used as stop rule in peginterferon treatment

Chuaypen N, Clin Microbiol Infect 2018; 24: 306e7

Serum HBcAg level

- Circulating HBcAg
  - Reflects cccDNA transcriptional activity and secreted nucleocapsids
  - Not expressed from integrated HBV DNA
  - Expression from cccDNA not affected by precore/core promoter mutations
  - 2 forms: phosphorylated (C-terminal) in circulating virions with HBV DNA and non-phosphorylated in circulating empty nucleocapsids
- HBcAg assay
  - Chemiluminescent microparticle immunoassays using monoclonal antibodies to capture phosphorylated or non-phosphorylated C-terminus of HBcAg with no interference from anti-HBc
  - During antiviral treatment, HBcAg correlates with HBV DNA while P-HBcAg correlates more closely with HBV RNA and may remain detectable after HBcAg is undetectable.

HBV biomarker kinetics in a patient treated with TDF followed by addition of peginterferon and nucleic acid polymer (NAP)



Geissler R, J Clin Virol 2023 (in press)

Novel biomarkers for the clinical development of HBV cure therapies

- Purpose of biomarkers
  - Confirm target engagement
  - Assess response during treatment
  - Predict “cure”
  - Ascertain cure
- Available biomarkers
  - HBV DNA, HBsAg
  - HBV RNA, HBcrAg
- Additional assays / biomarkers needed
  - HBsAg assays
    - with improved sensitivity
    - can detect HBsAg bound to anti-HBs in immune complex
    - can differentiate source from integrated vs. ccc DNA
  - Standardized assays with improved sensitivity and specificity for cccDNA transcription activity
    - pgRNA, HBcrAg (or HBcAg?)