Investigation of viral biomarkers in clinical trials of novel antiviral strategies

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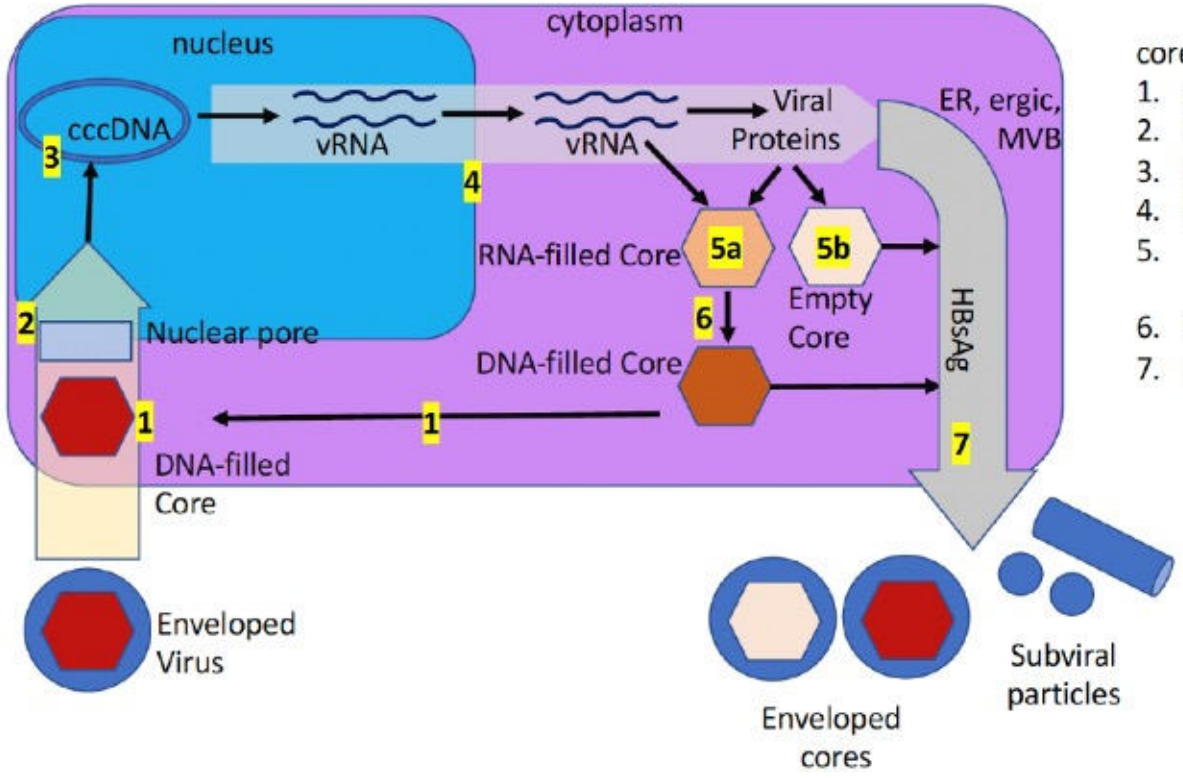
The biology of HBV RNAs and core proteins



core protein and core mediated activity:

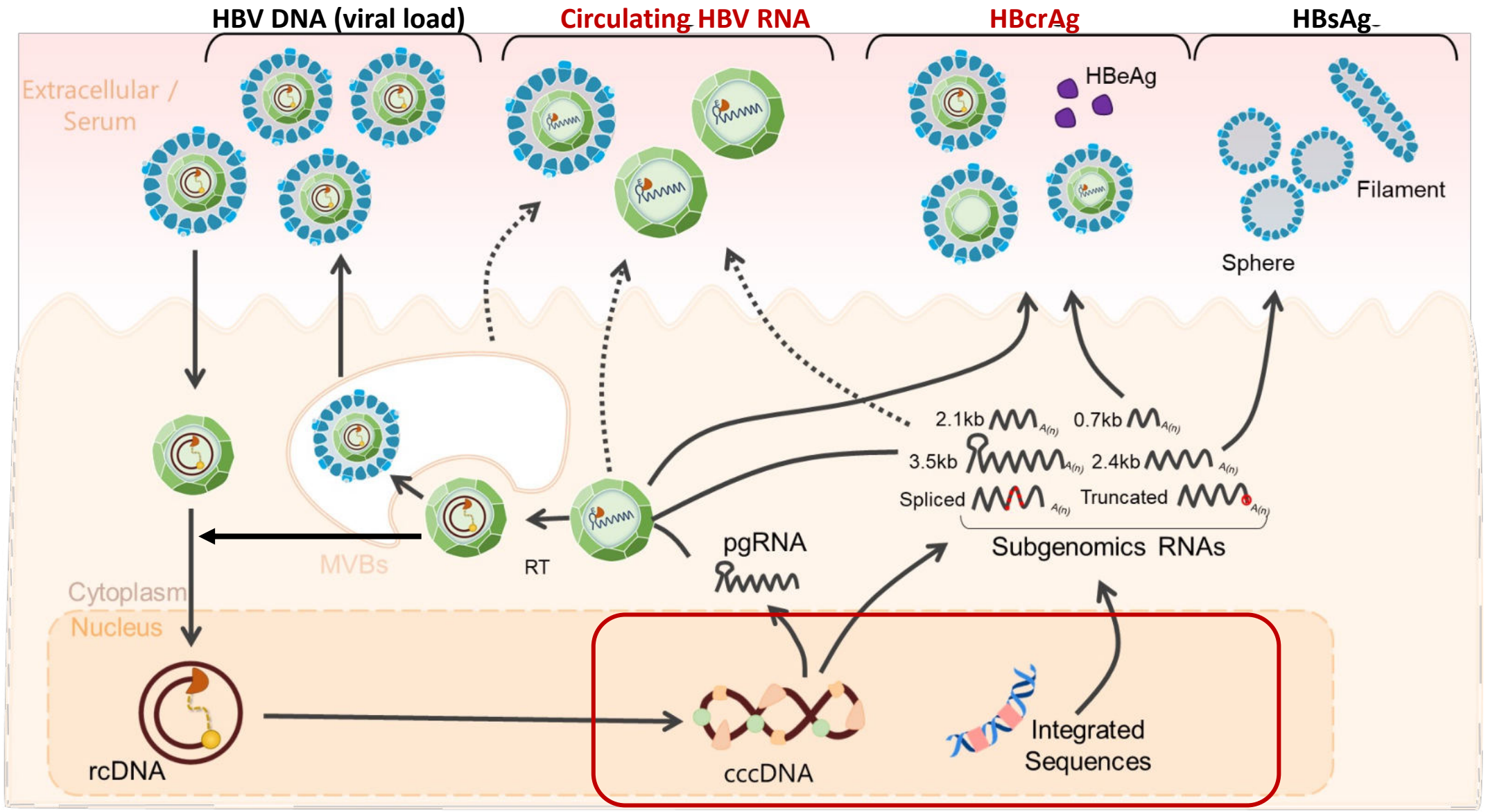
1. Transport to nucleus
2. Core dissociation, uncoating DNA
3. cccDNA
4. RNA export
5. a. nucleation by RNA+polymerase
   b. Spontaneous nucleation
6. Reverse transcription
7. Binding surface antigen

Nomenclature of HBV Core Protein Targeting Antivirals

Fabien Zoulim,* Adam Zlotnick,* Stephanie Buchholz, Eric Donaldson, John Fry, Anuj Gaggar, Jianming Hu, Michael Kann, Oliver Lenz, Kai Lin, Nagraj Mani, Michael Nassal, William Delaney, Su Wang, Gabriel Westman, Veronica Miller, and Harry Janssen

nature reviews gastroenterology & hepatology

Non invasive biomarkers of HBV infection



Testoni et al, Semin Liver Dis. 2017; Kramvis et al, Nat Rev Gastoenterol Hepatol 2022

The HBV drug pipeline and the potential for combination therapy to cure HBV

Replication inhibition ± **Antigen reduction** ± **Immune stimulation**

hNTCP

Entry inhibitors: bulevirtide

HBV polymerase

NUC: ETV, TDF, TAF, novel NUCs and RNAseH inhibitors

Nucleocapsids

CAM: ABI-H0731, JNJ-56136379, RO7049389

Transcription

FXR agonist: EYP001

Viral RNAs

siRNA: JNJ-3989 VIR-2218

ASO: GSK3228836

LNA: RO7062931

RNA destabilizers

HBsAg release

NAPs: REP 2139, REP 2165

STOPS

Invigorate immune responses

Innate immunity

TLR7: GS9620, RO6864018, RO7020531, JNJ6479464

TLR8: GS9688

Immune check points

Anti-PD1: nivolumab

Anti-PDL1

PDL1 LNA

Oral PDL1 sm

Stimulate HBV specific B/T cells

Therapeutic Vaccines

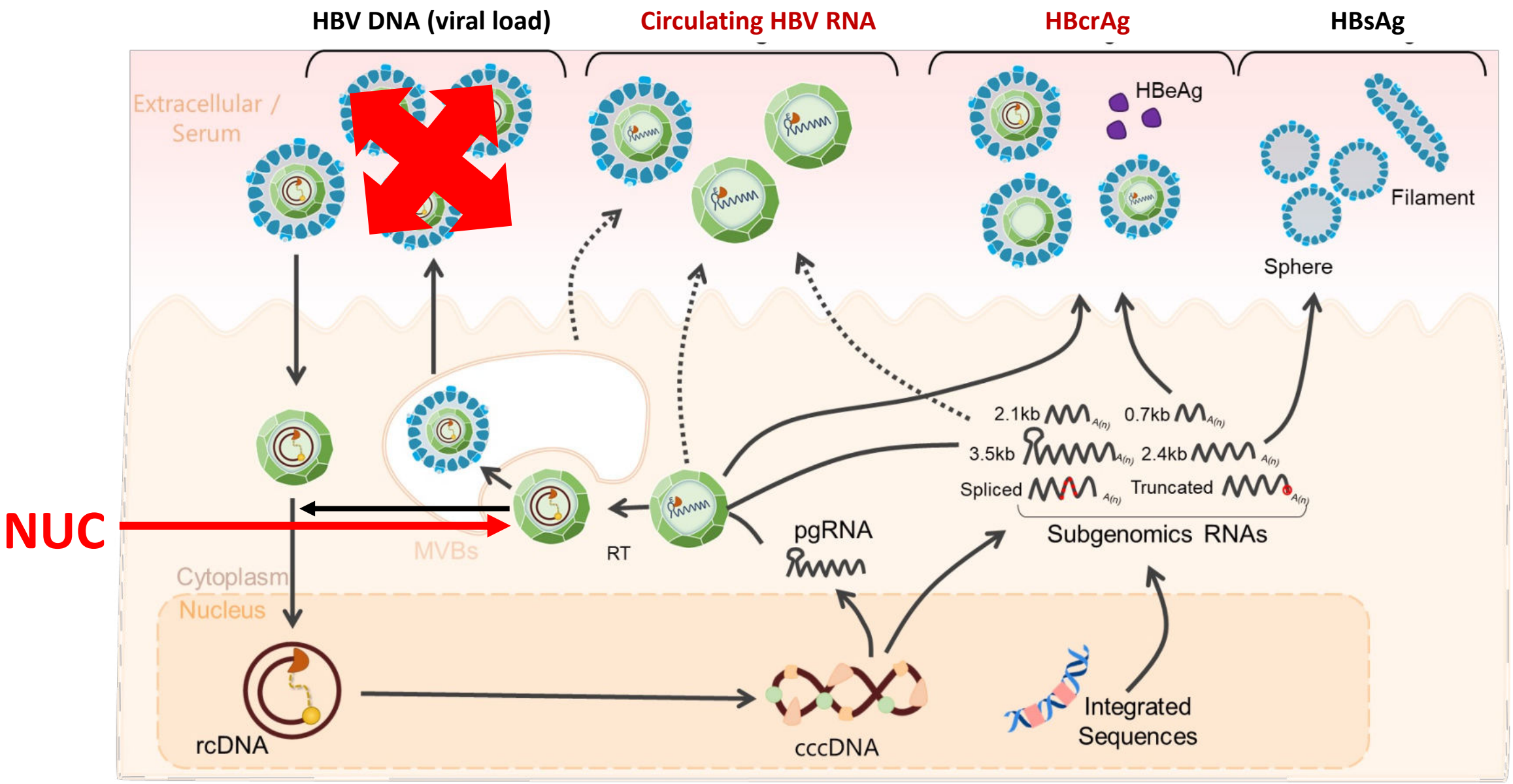
GS2829, GS6779
VTP-300
GSK3528869A

Exogenous HBs Mab

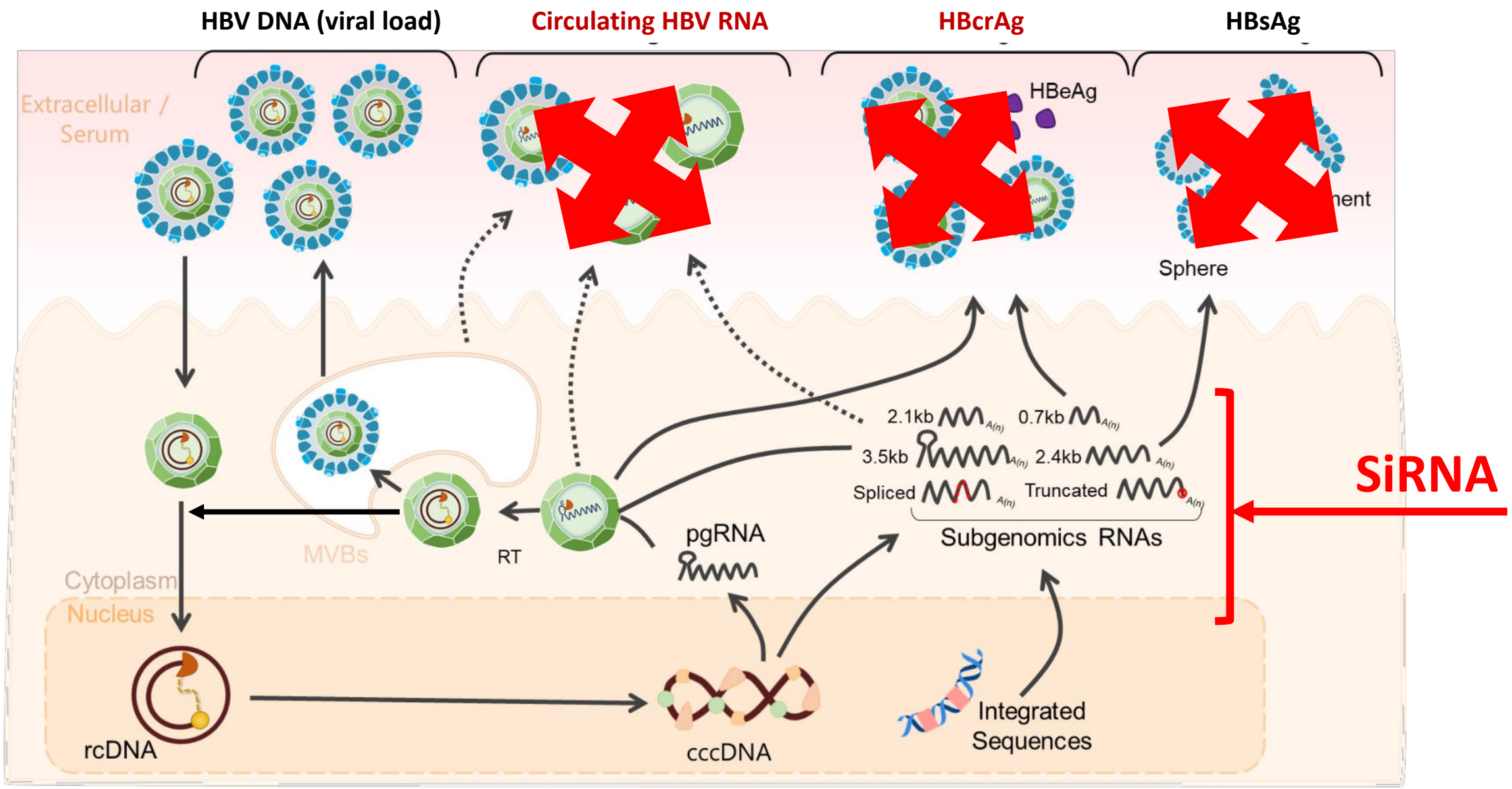
VIR-3434

Fanning et al. Nat Rev Drug Discov. 2019; Revill et al, Lancet Gastroenterol Hepatol 2019; Roca Suarez et al, Liver International 2021; Lim et al Nat Rev Gastroenterol Hepatol. 2023

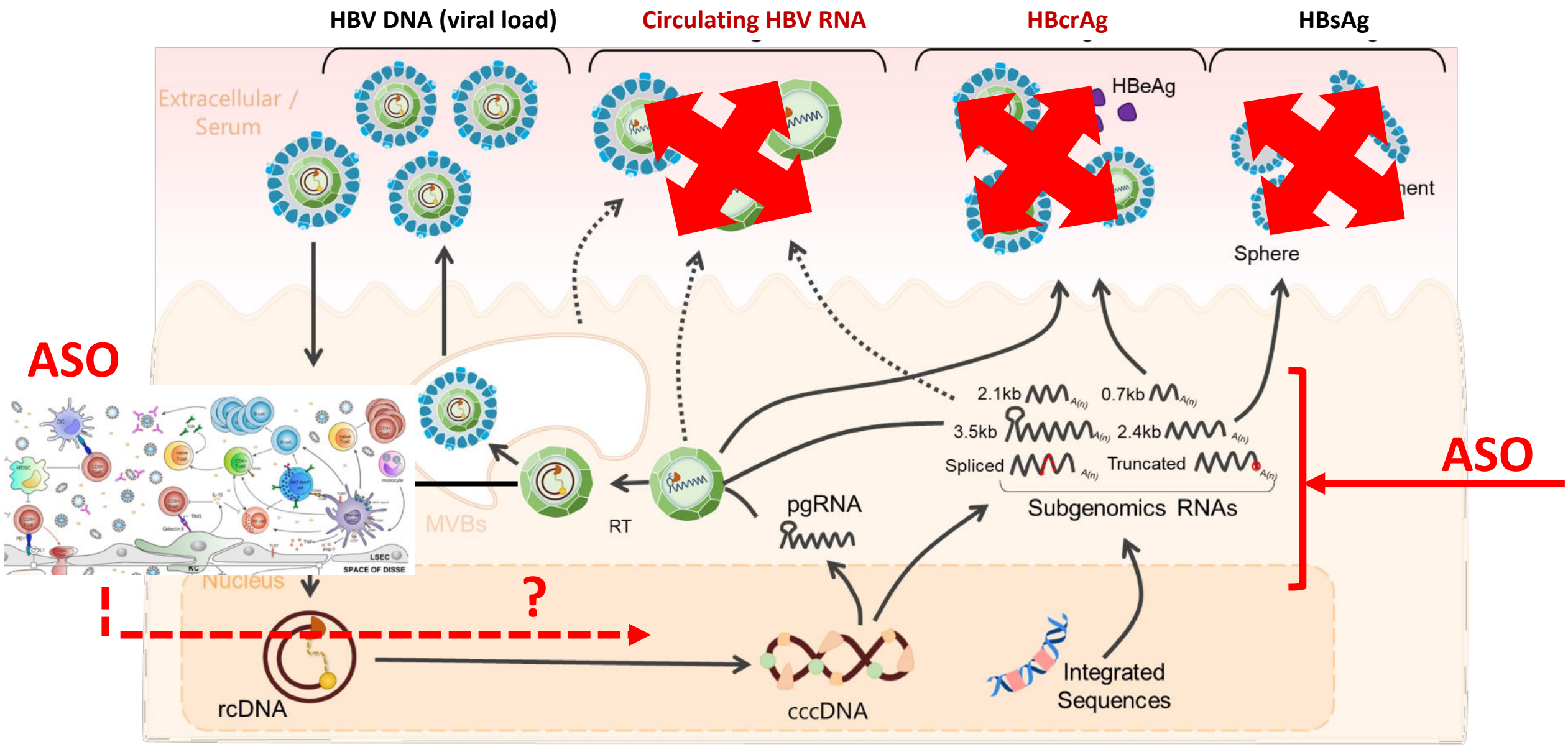
Effects of NUC – Target engagement



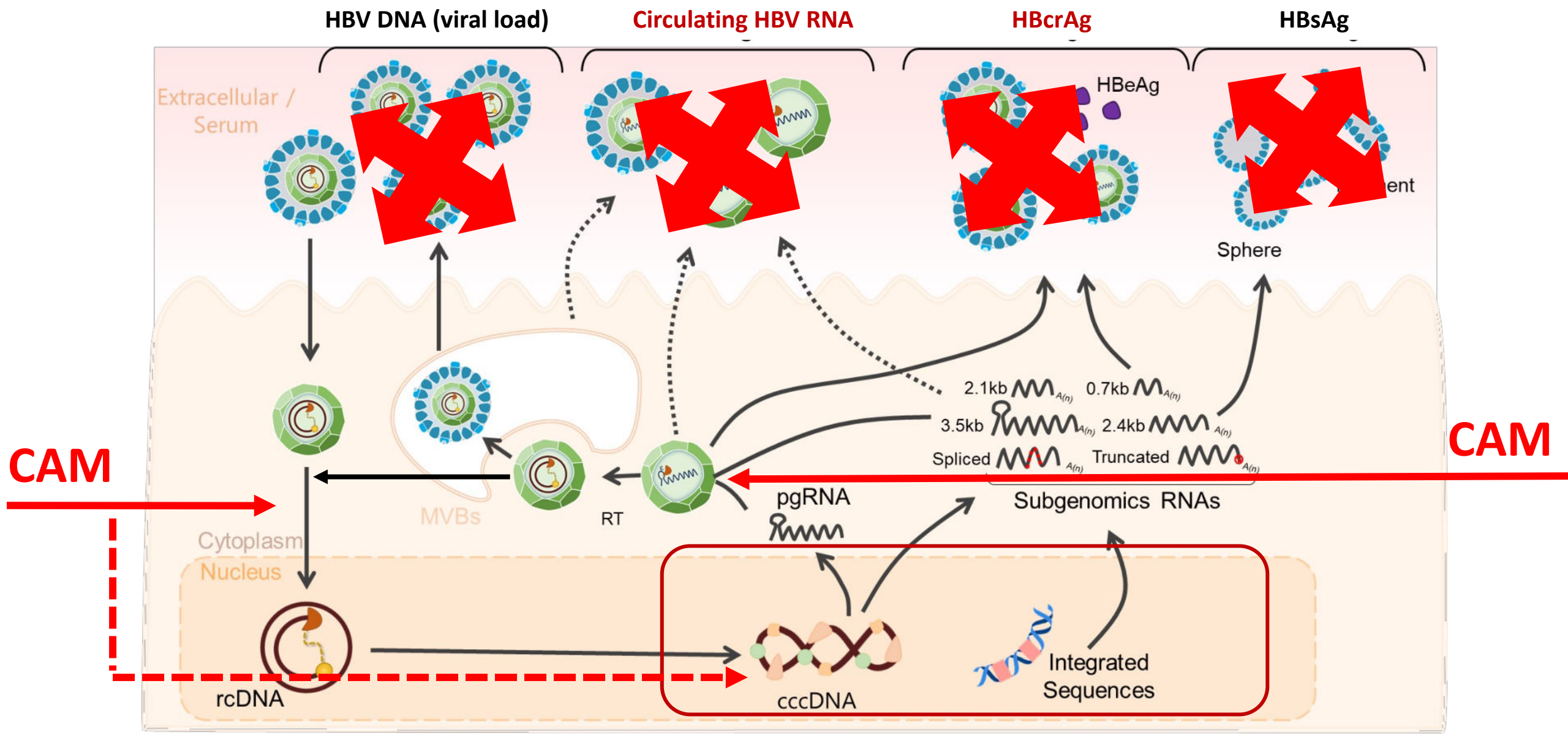
Effects of SiRNA – Target engagement



Effects of ASO – Target engagement



Effects of CAM – Target engagement



Effect of CAMs on viral biomarkers in clinical trials

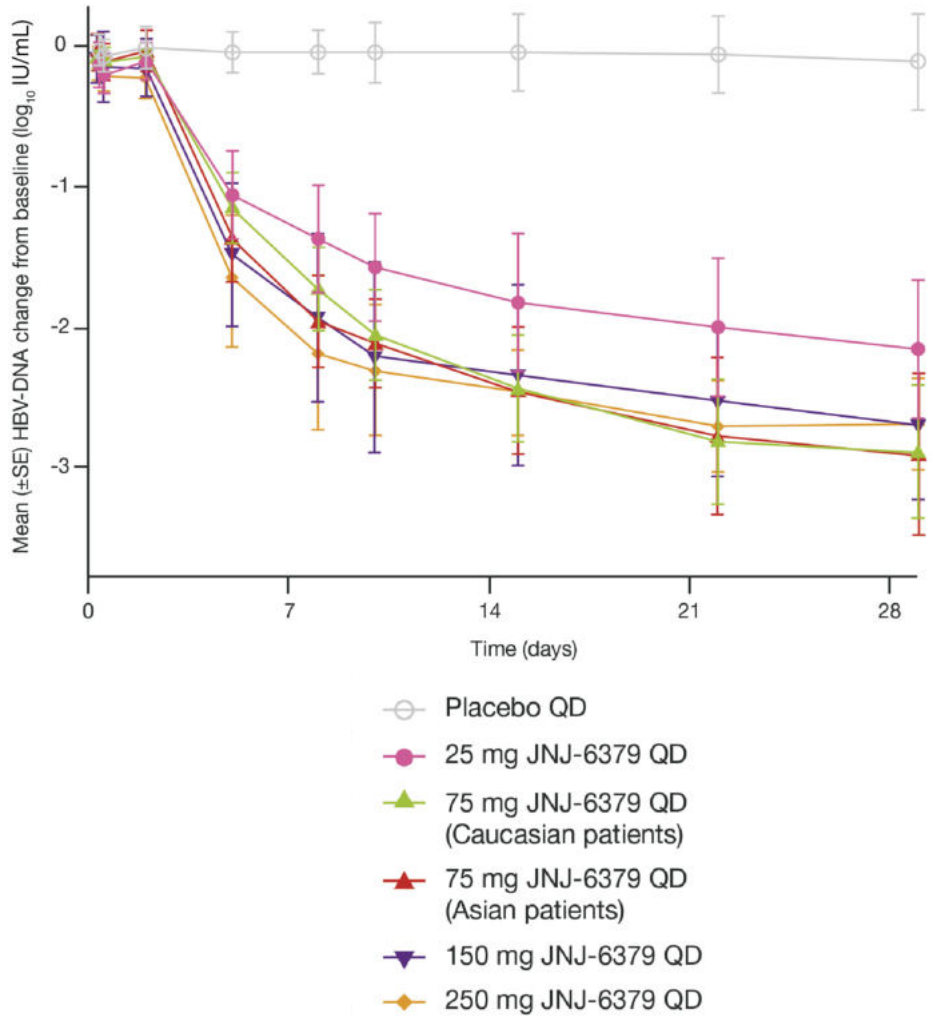
Gastroenterology
Volume 159, Issue 2, August 2020, Pages 521-533.e9

Original Research

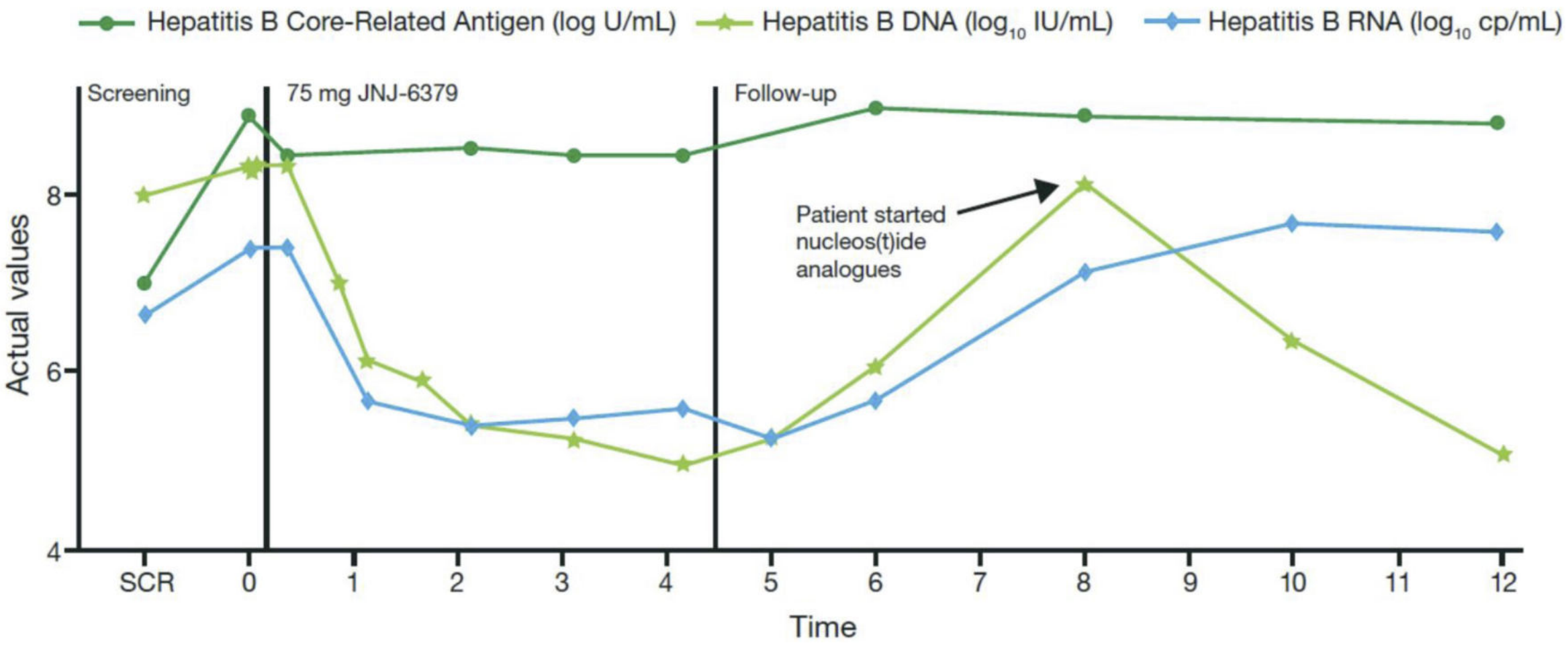
Full Report: Clinical—Liver

JNJ-56136379, an HBV Capsid Assembly Modulator, Is Well-Tolerated and Has Antiviral Activity in a Phase 1 Study of Patients With Chronic Infection

Fabien Zoulim [1 2], Oliver Lenz [3], Joris J. Vandenbossche [3], Willem Talloen [3], Thierry Verbinnen [3], Iurie Moscalu [4], Adrian Streinu-Cercel [5], Stefan Bourgeois [6], Maria Buti [7], Javier Crespo [8], Juan Manuel Pascasio [9], Christoph Sarrazin [10], Thomas Vanwolleghem [11 12], Umesh Shukla [13], John Fry [14], Jeysen Z. Yogaratnam [14]



Effect of CAM JNJ-56136379 on viral biomarkers in Phase 1



Gut

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Article Text

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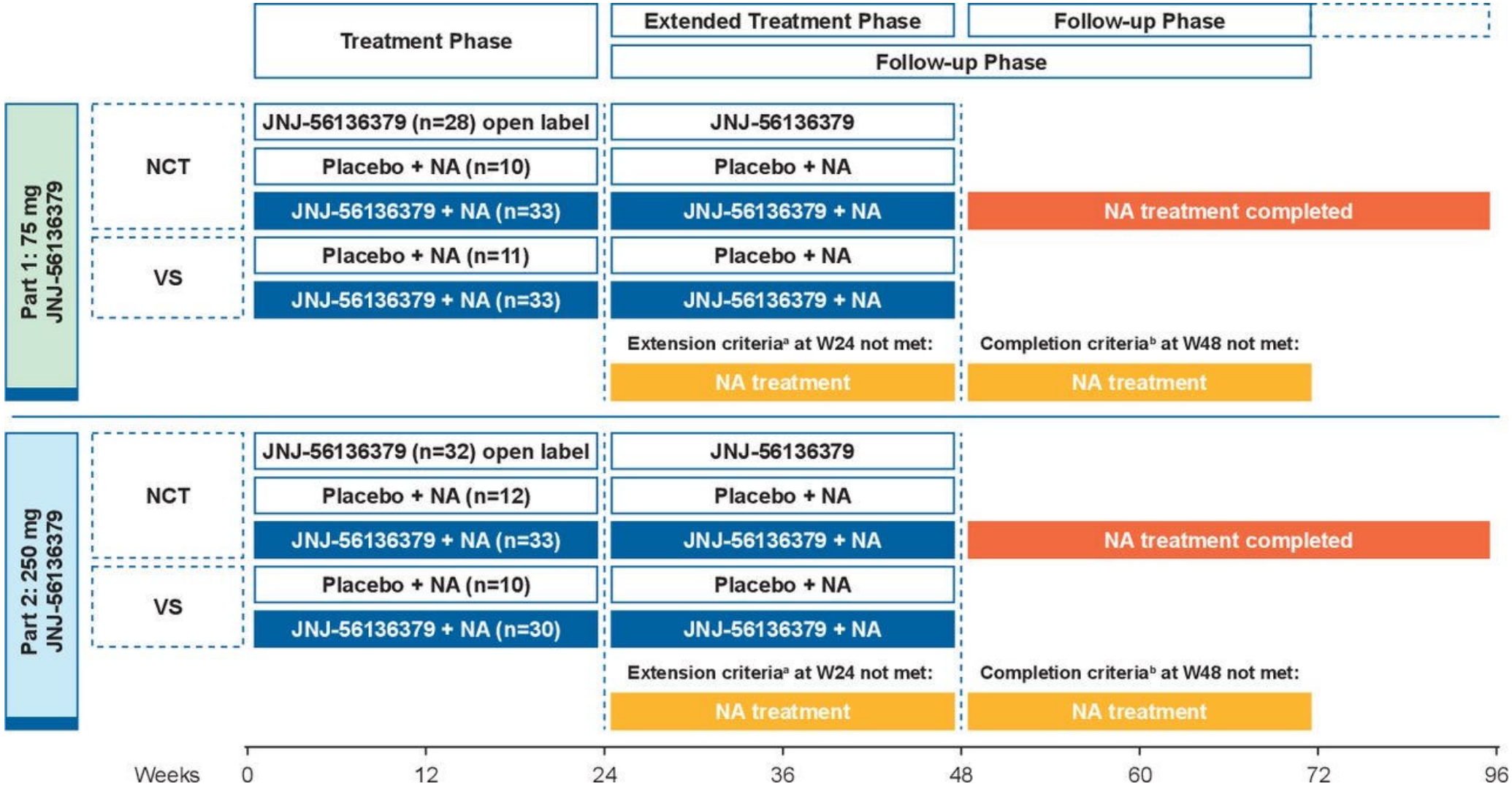
Hepatology
Original research

Randomised phase 2 study (JADE) of the HBV capsid assembly modulator JNJ-56136379 with or without a nucleos(t)ide analogue in patients with chronic hepatitis B infection

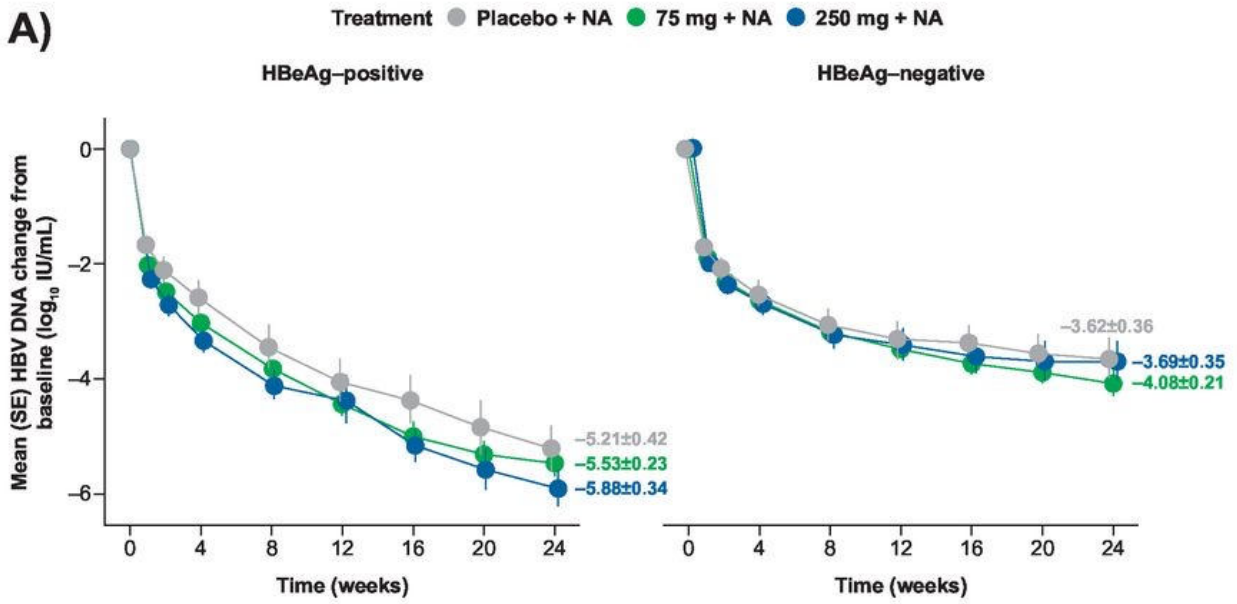
Harry L A Janssen [1, 2], Jinlin Hou [3], Tarik Asselah [4], Henry L Y Chan [5], Fabien Zoulim [6], Yasuhito Tanaka [7], Ewa Janczewska [8], Ronald G Nahass [9], Stefan Bourgeois [10], Maria Buti [11], Pietro Lampertico [12, 13], Oliver Lenz [14], Thierry Verbinnen [14], Joris Vandenbossche [14], Willem Talloen [14], Ronald Kalmeijer [15], Maria Beumont [15], Michael Biermer [14], Umesh Shukla [15]

JADE clinical trial design

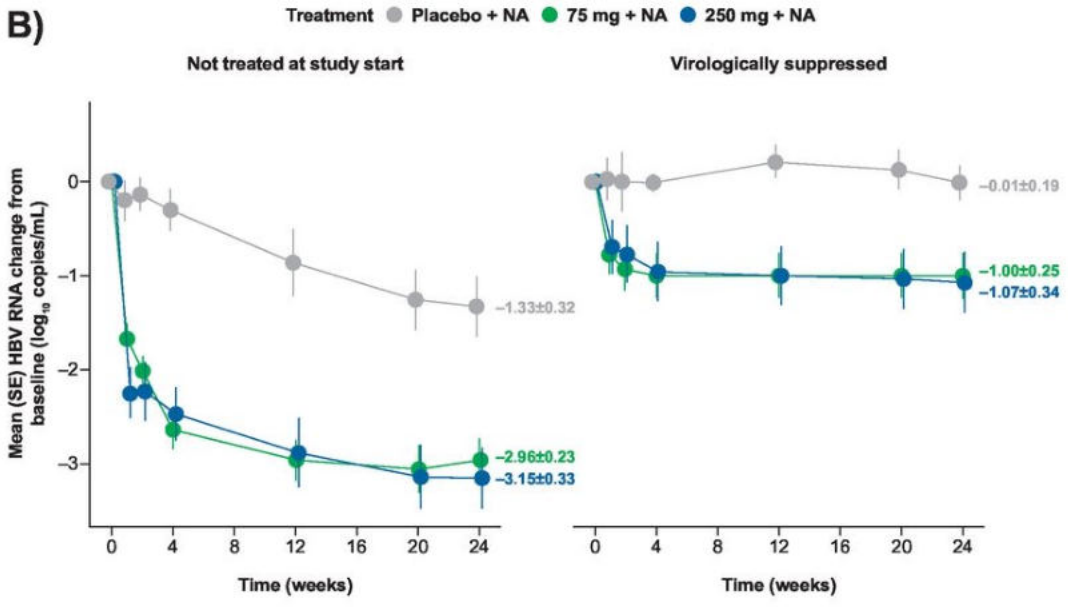


Mean change from baseline in HBV DNA and HBV RNA over 24 weeks of treatment

(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)

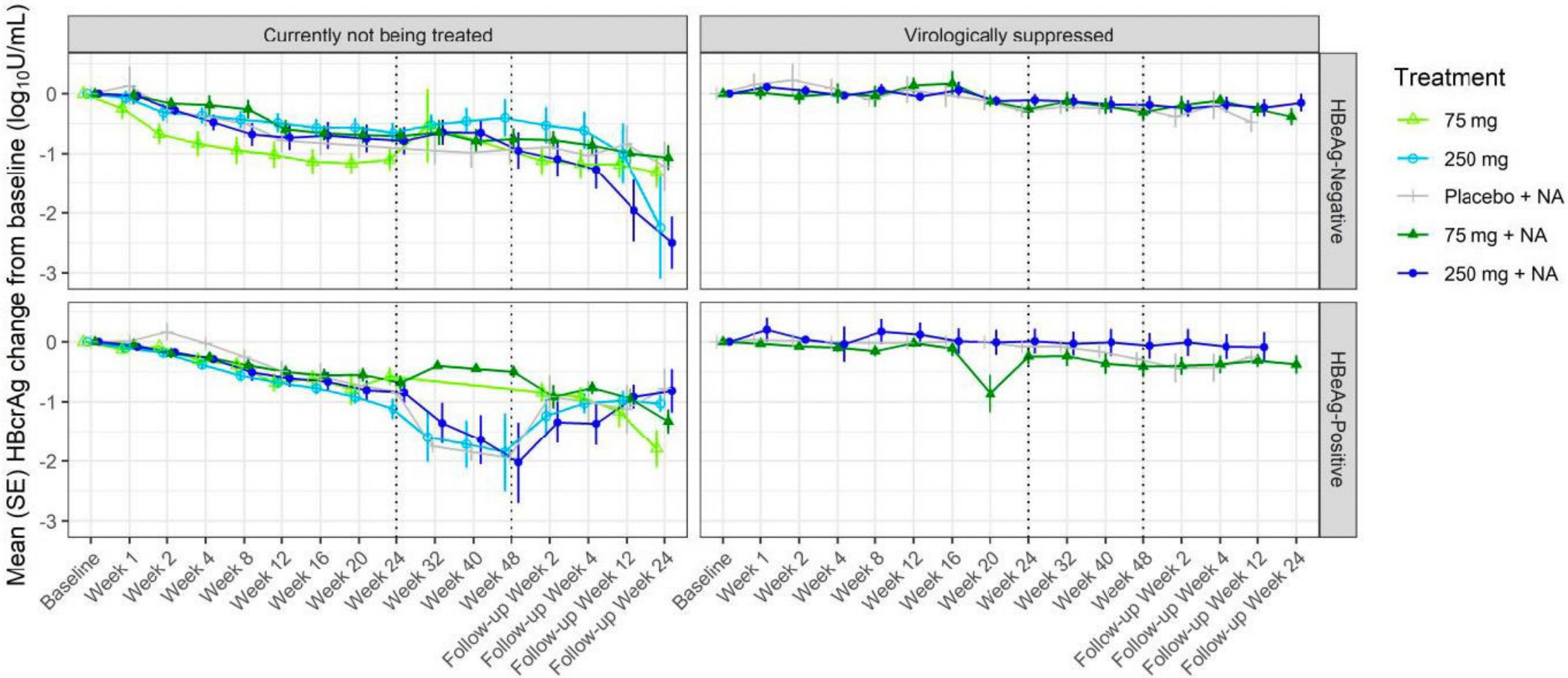


Patients with HBV RNA TND at Week 24, n (%)	JNJ-56136379 75 mg + NA		JNJ-56136379 250 mg + NA		Placebo + NA	
	NCT	VS	NCT	VS	NCT	VS
HBeAg–positive	3/12 (25)	9/9 (100)	4/11 (36)	10/10 (100)	0/8	1/5 (20)
HBeAg–negative	16/21 (76)	24/24 (100)	19/19 (100)	18/18 (100)	9/13 (69)	10/15 (67)

GUT

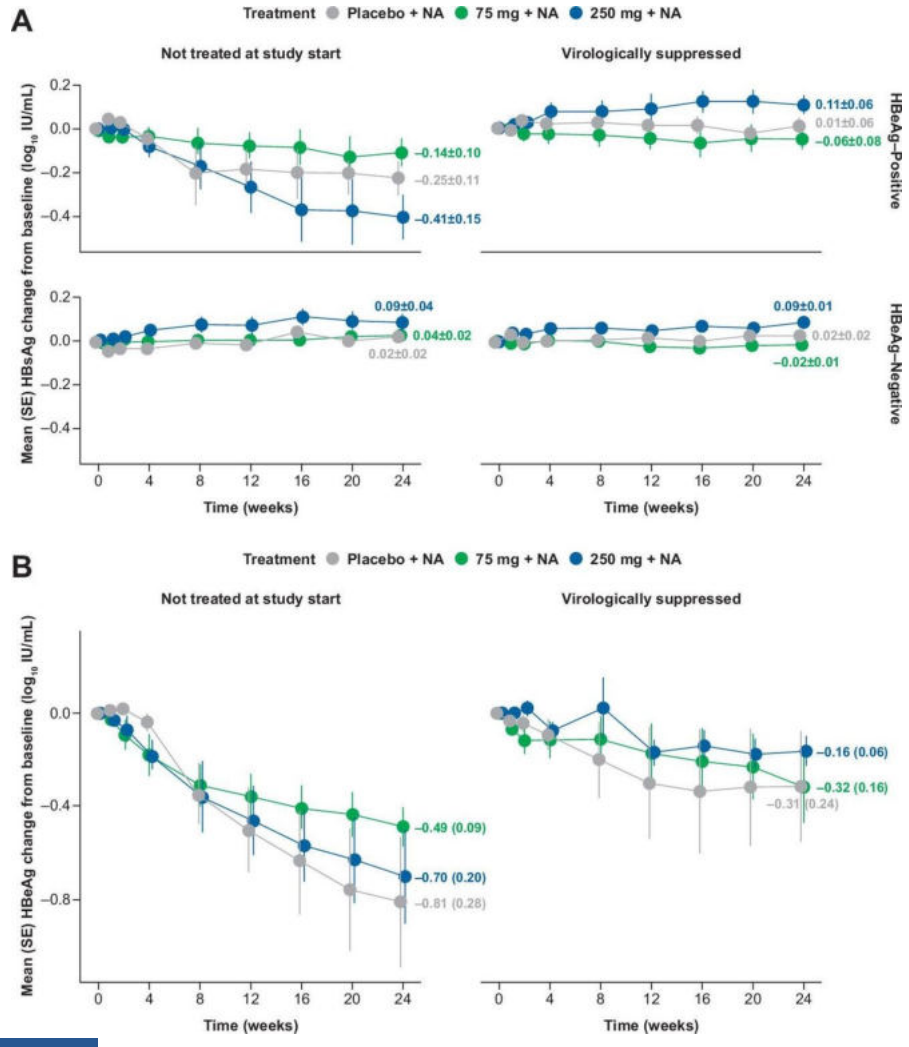
Harry L A Janssen et al. Gut 2023;72:1385-1398

Mean change from baseline in HBcrAg throughout the study by prior treatment and HBeAg status

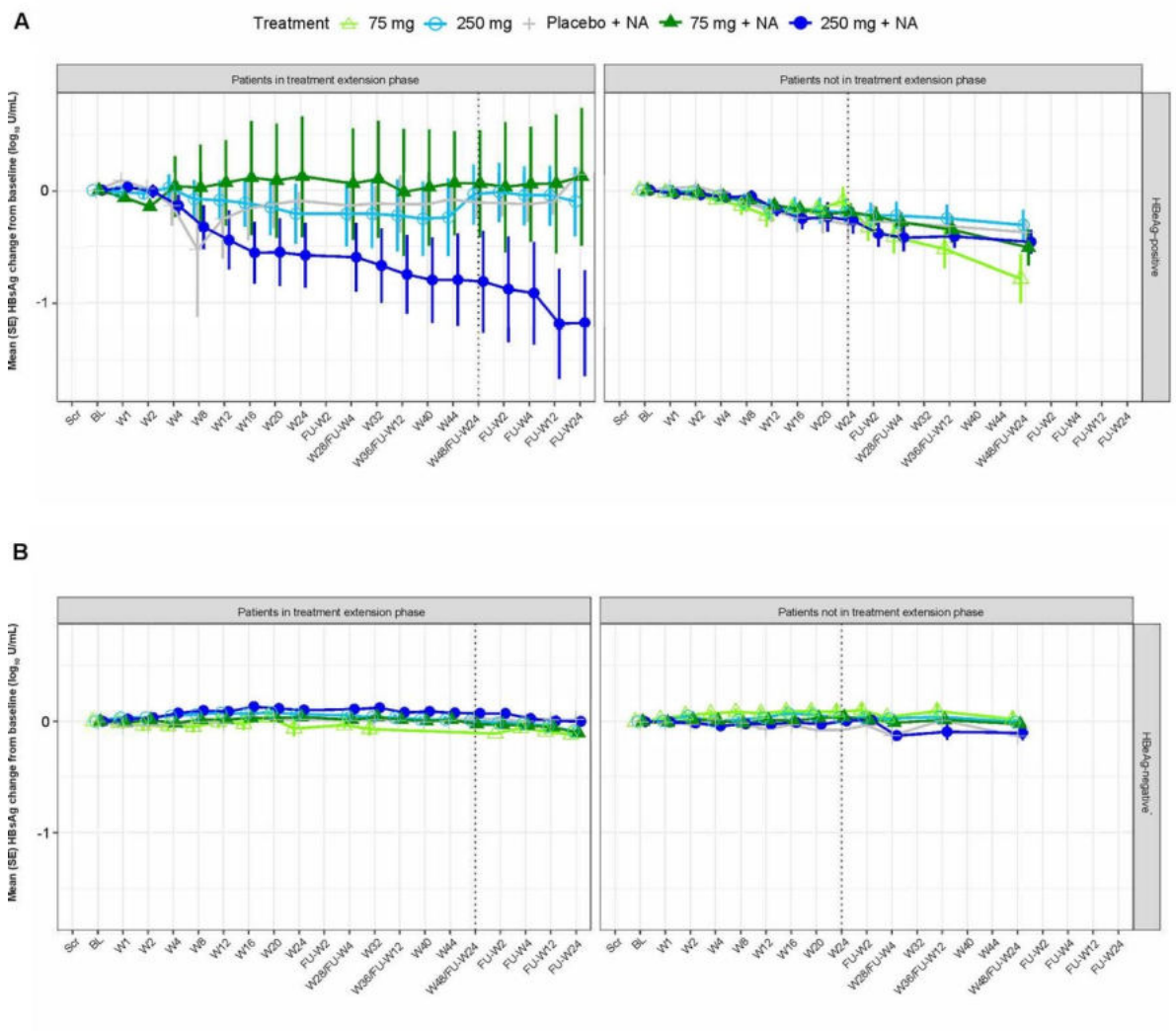


Mean change from baseline in HBsAg in the JADE trial

Over 24 weeks of treatment



Throughout the study in NCT patients, by HBeAg status.



GUT

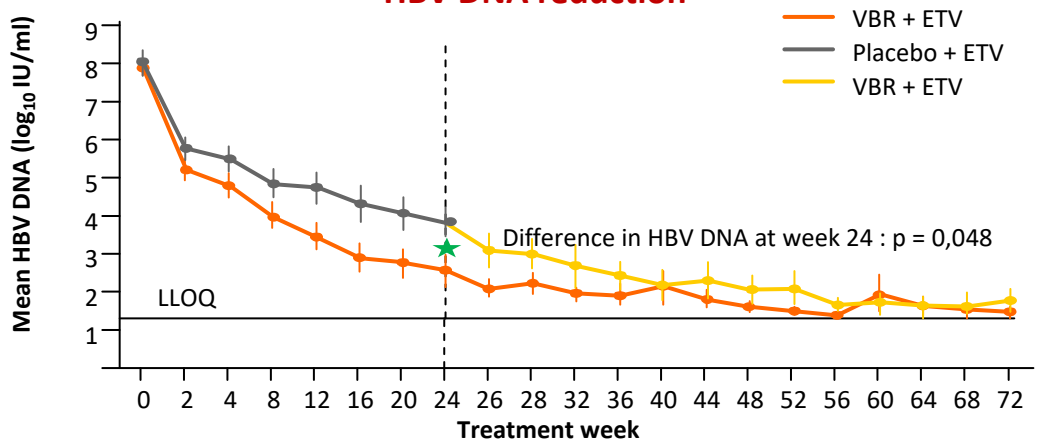
Harry L A Janssen et al. Gut 2023;72:1385-1398

Target engagement is not equal to reaching endpoint!

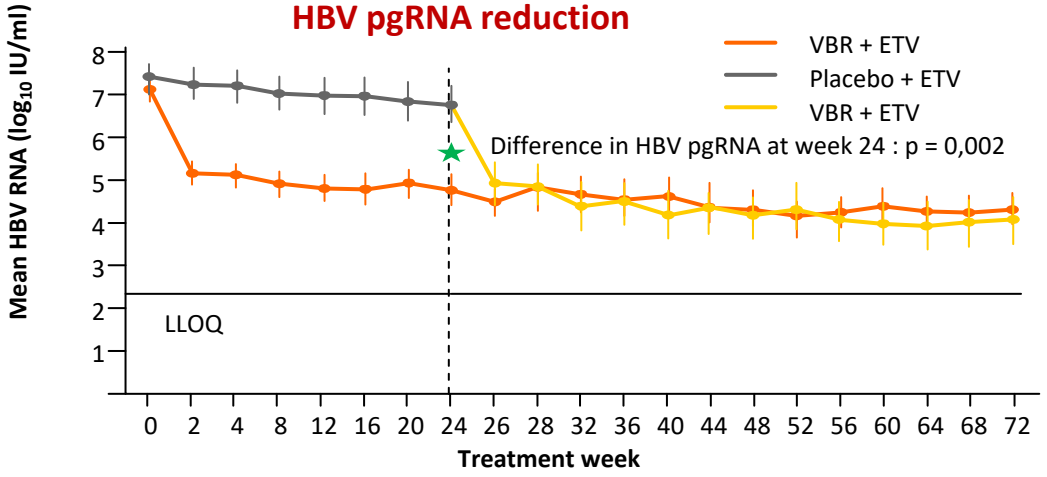
Entecavir + CAM ABI-H0731 (Vebicorvir) Phase 2 long term extension study

During treatment

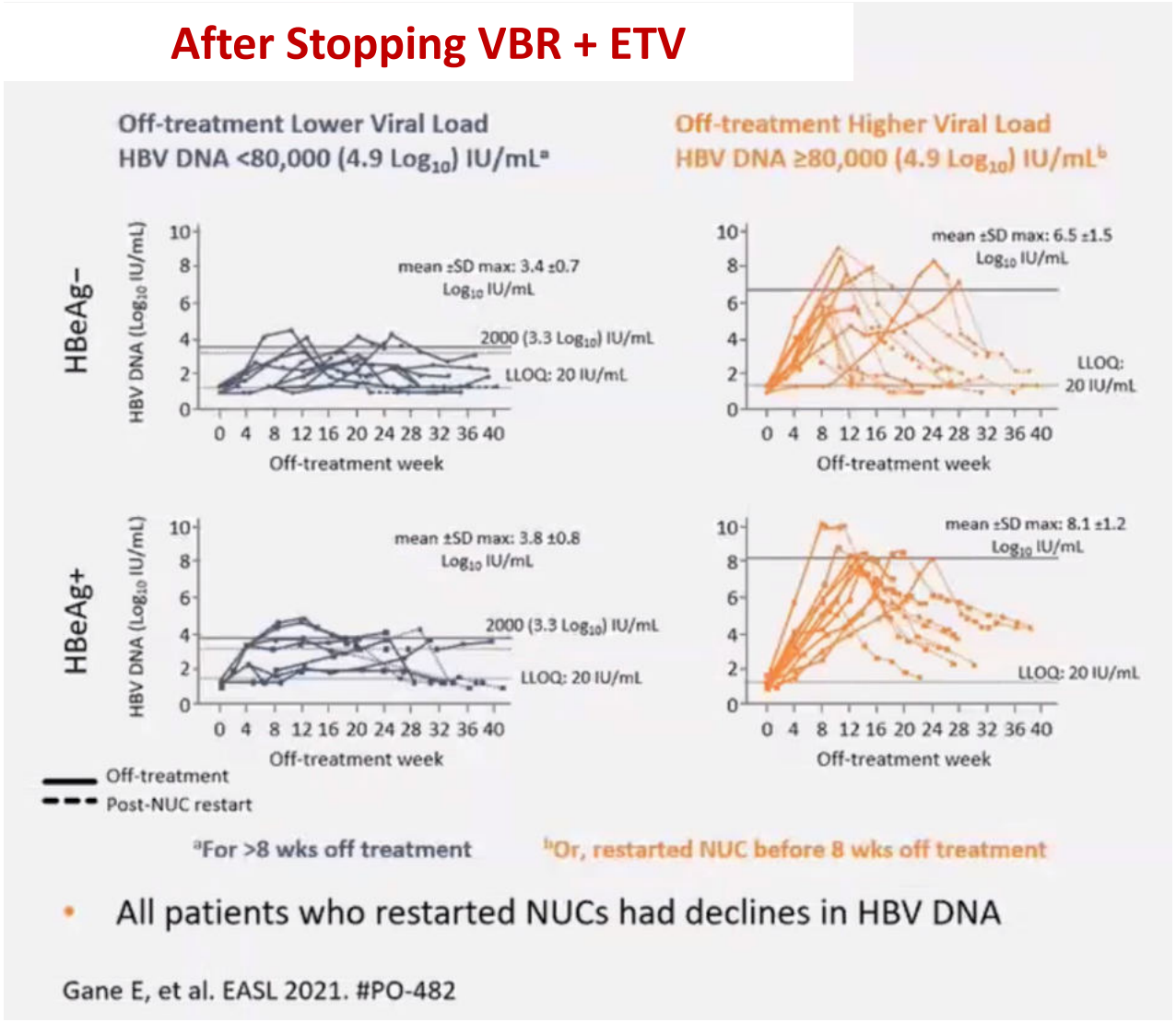
HBV DNA reduction



HBV pgRNA reduction



After Stopping VBR + ETV



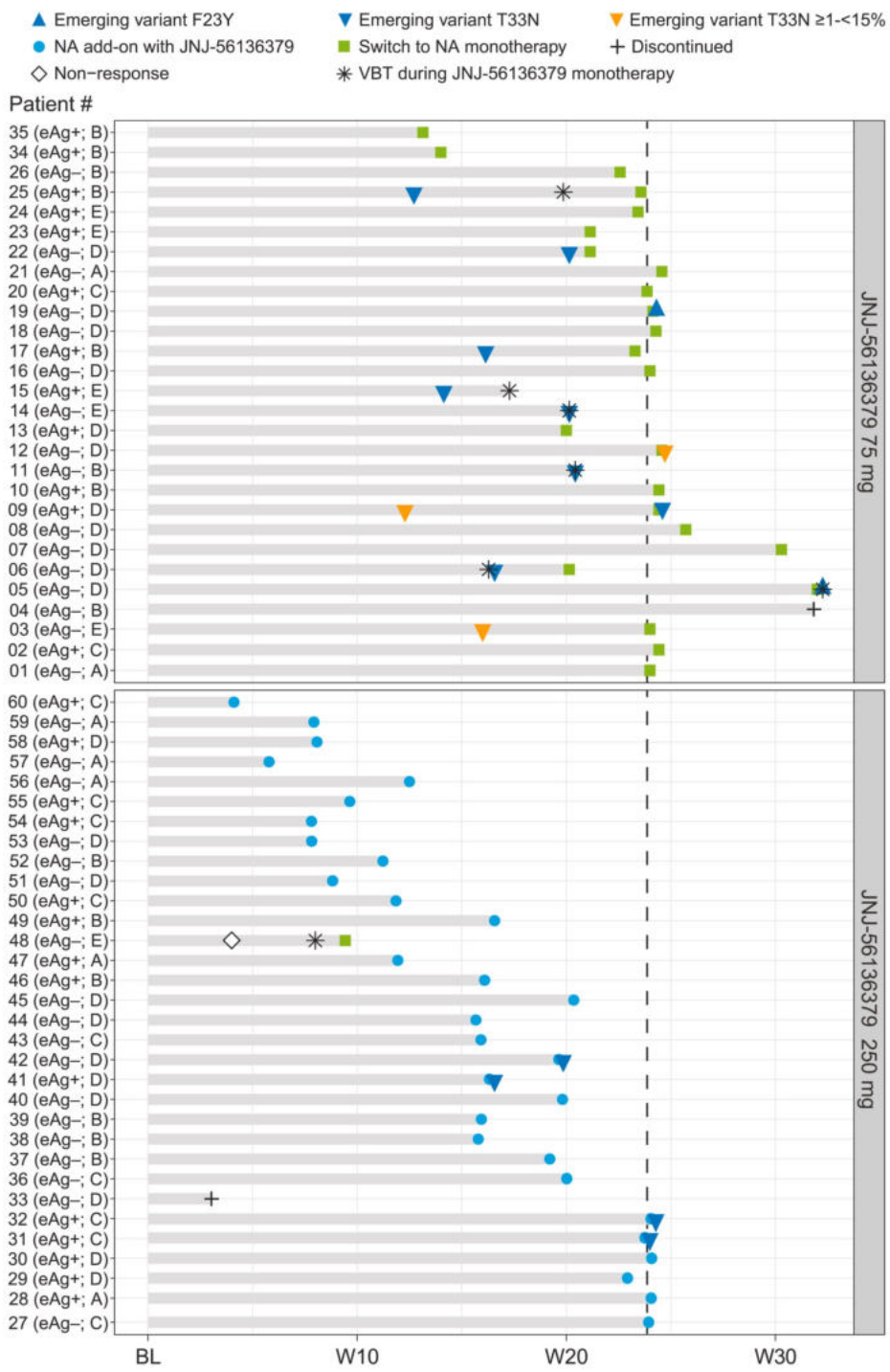
Yuen MF et al. EASL 2020, Abs. LP30; Yuen at al J Hepatol 2022; Sulkowski et al J Hepatol 2022

Antiviral Research
Volume 216, August 2023, 105660

Viral sequence analysis of chronic hepatitis B patients treated with the capsid assembly modulator JNJ-56136379 in the JADE phase 2a study

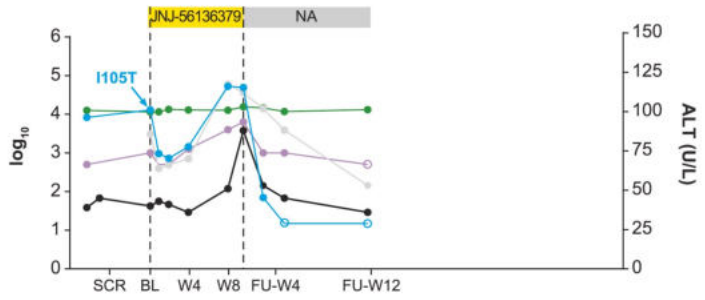
Thierry Verbinnen [a], Willem Talloen [a], Harry L.A. Janssen [b c], Fabien Zoulim [d], Umesh Shukla [e], Joris J. Vandenbossche [a], Michael Biermer [a], Sandra De Meyer [a], Oliver Lenz [a]

Pattern of antiviral drug resistance with JnJ 56136379

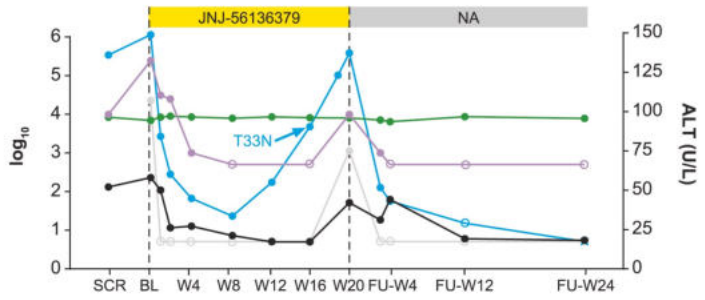


JnJ56136379 resistance: kinetics of viral biomarkers

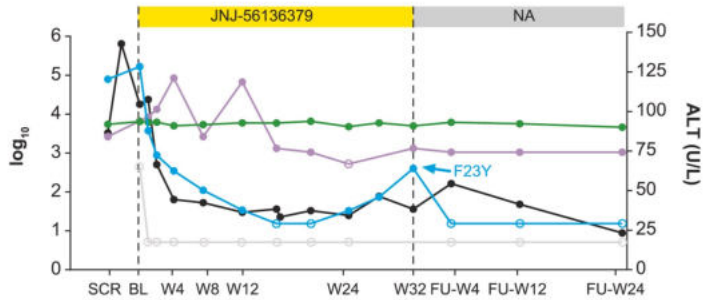
A. Patient #48: 250 mg, HBV genotype-E, HBeAg-negative, VBT at Week 8



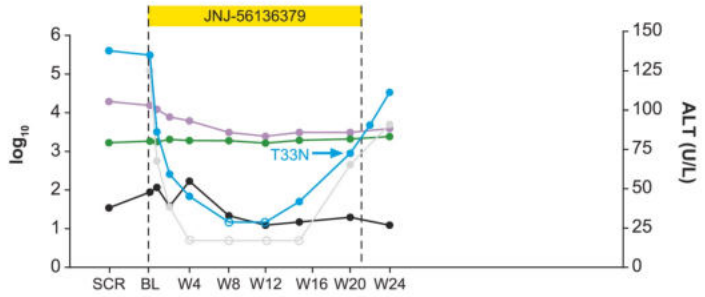
B. Patient #6: 75 mg, HBV genotype-D, HBeAg-negative, VBT at Week 16



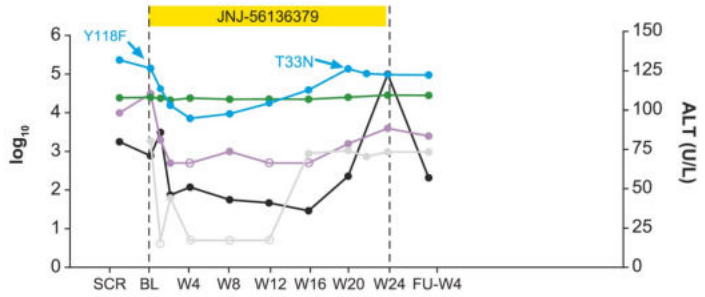
G. Patient #5: 75 mg, HBV genotype-D, HBeAg-negative, VBT at Week 32



C. Patient #11: 75 mg, HBV genotype-B, HBeAg-negative, VBT at Week 20

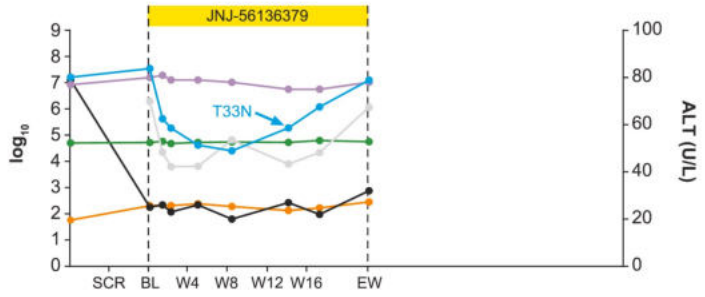


D. Patient #14: 75 mg, HBV genotype-E, HBeAg-negative, VBT at Week 20

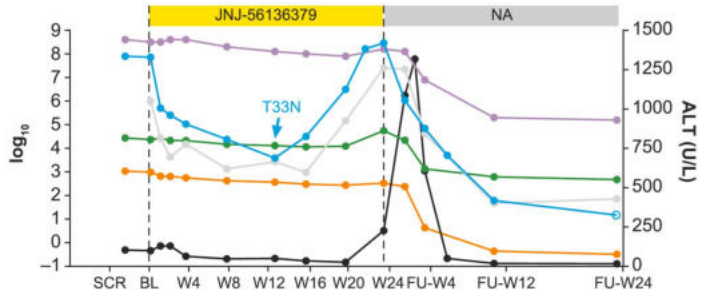


HBV DNA (IU/mL) HBV RNA (cp/mL) HBcrAg (U/mL)
HBsAg (IU/mL) ALT (U/L) HBeAg (IU/mL)

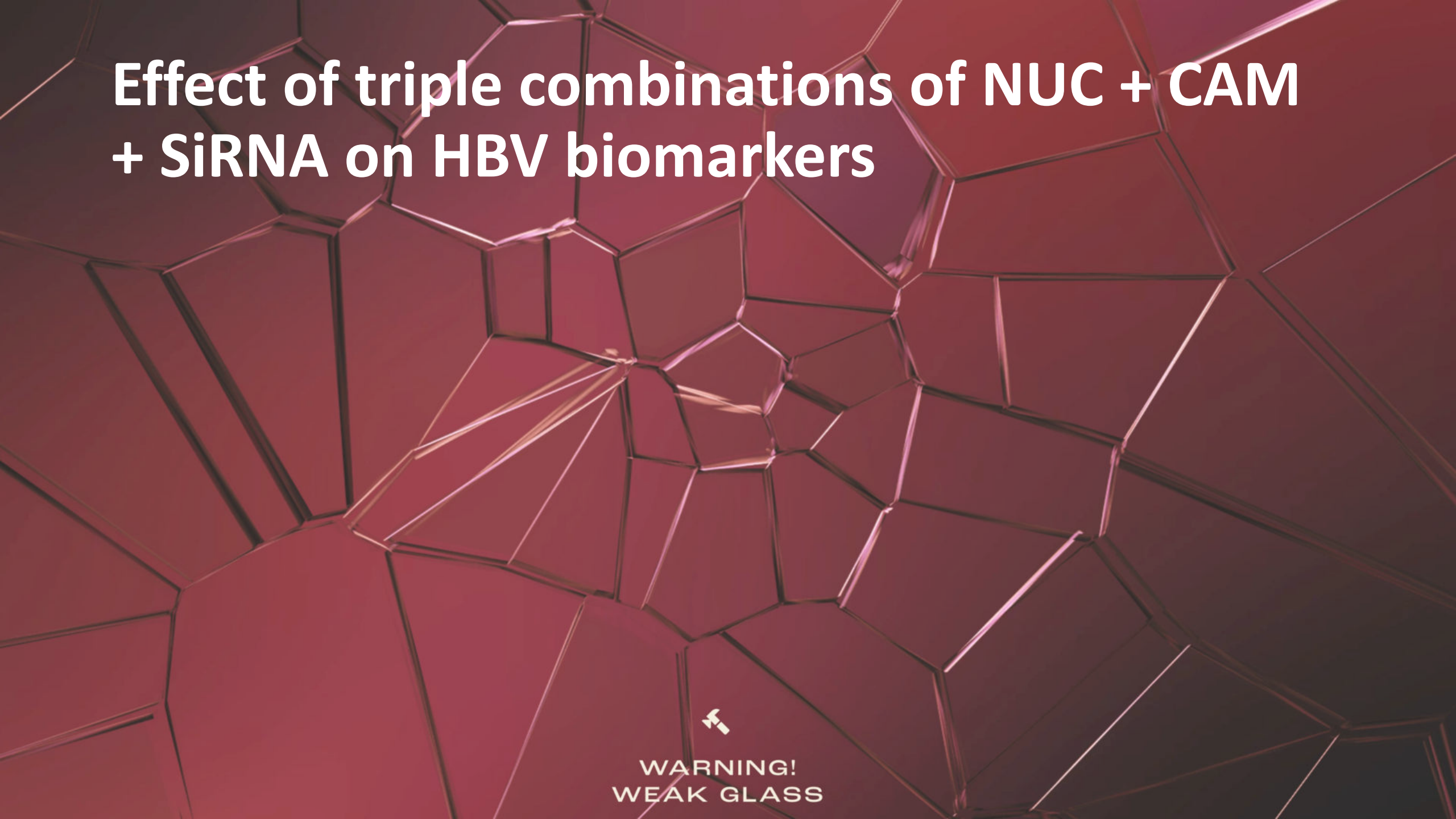
E. Patient #15: 75 mg, HBV genotype-E, HBeAg-positive, VBT at Week 16



F. Patient #25: 75 mg, HBV genotype-B, HBeAg-positive, VBT at Week 20



Effect of triple combinations of NUC + CAM + SiRNA on HBV biomarkers



THE LANCET
Gastroenterology & Hepatology

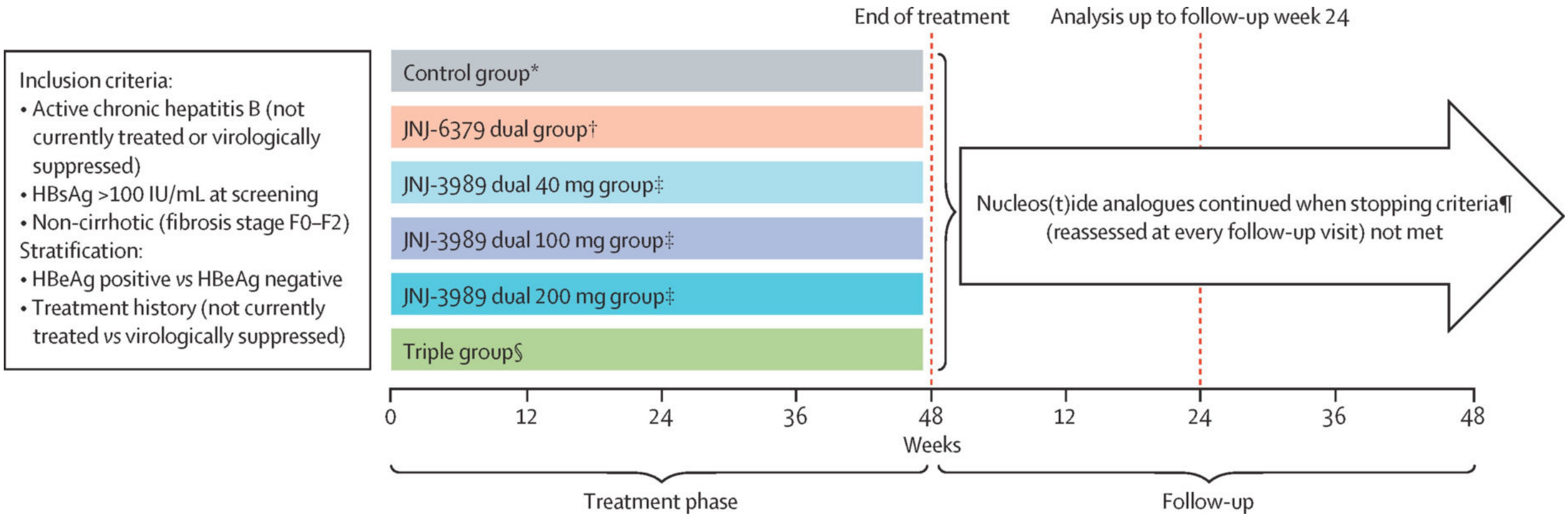
Volume 8, Issue 9, September 2023, Pages 790-802

Articles

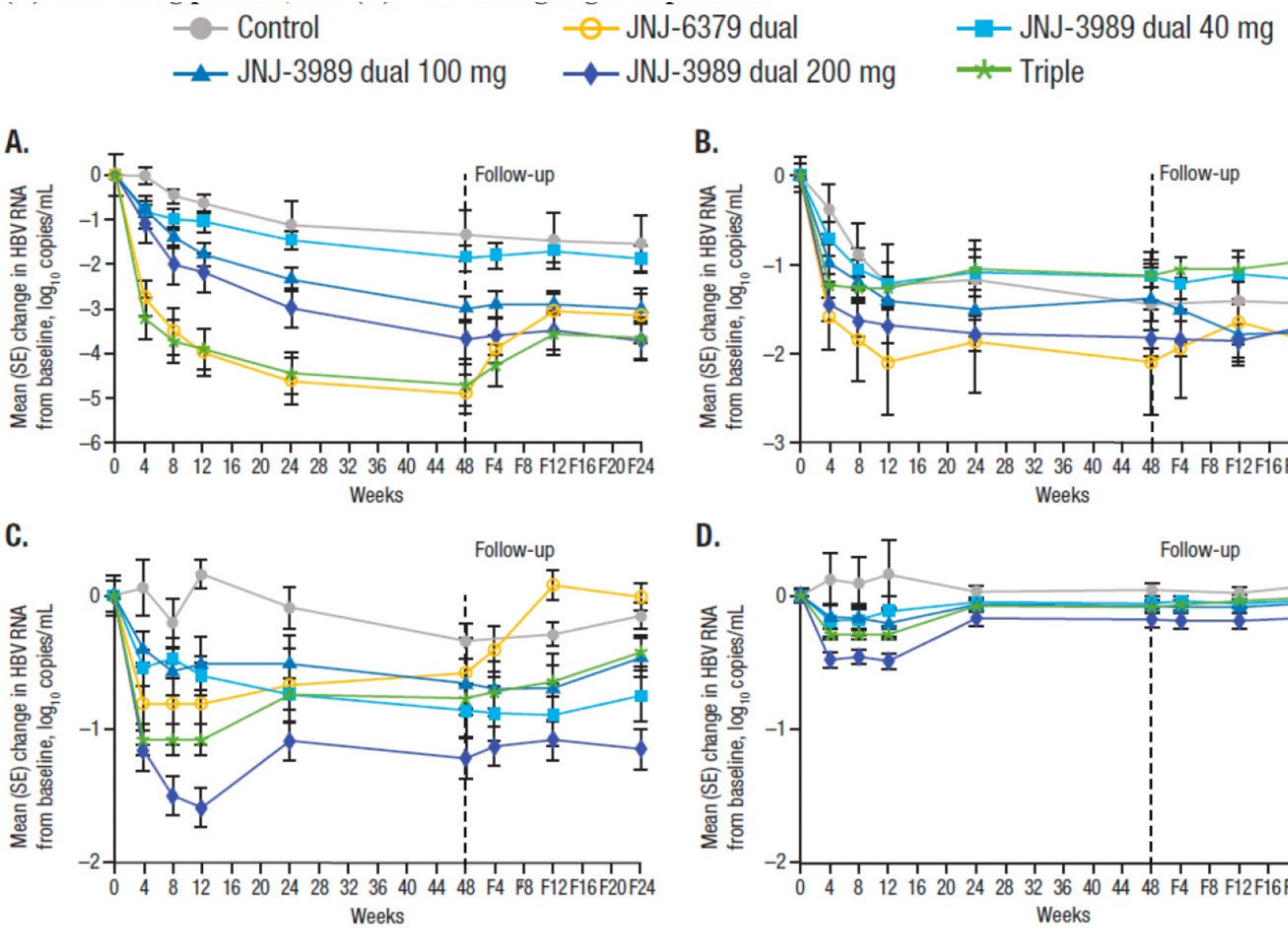
Efficacy and safety of the siRNA JNJ-73763989 and the capsid assembly modulator JNJ-56136379 (bersacapavir) with nucleos(t)ide analogues for the treatment of chronic hepatitis B virus infection (REEF-1): a multicentre, double-blind, active-controlled, randomised, phase 2b trial

Prof Man-Fung Yuen DSc [a], Prof Tarik Asselah MD [b], Prof Ira M Jacobson MD [c], Prof Maurizia Rossana Brunetto MD [d], Prof Harry L A Janssen MD [e f], Prof Tetsuo Takehara MD [g], Prof Jin Lin Hou MD [h], Thomas N Kakuda PharmD [i], Tom Lambrecht MSc [j], Maria Beumont MD [k], Ronald Kalmeijer MD [k], Carine Guinard-Azadian MD [j], Cristiana Mayer PhD [k], John Jezorwski MS [k], Thierry Verbinnen PhD [j], Oliver Lenz PhD [j], Umesh Shukla PhD [k], Michael Biermer MD [j]
REEF-1 Study Group†

REEF-1 study design



Mean change in HBV RNA in the REEF-1 study



NCT HBeAg+

VS HBeAg+

NCT HBeAg-

VS HBeAg-

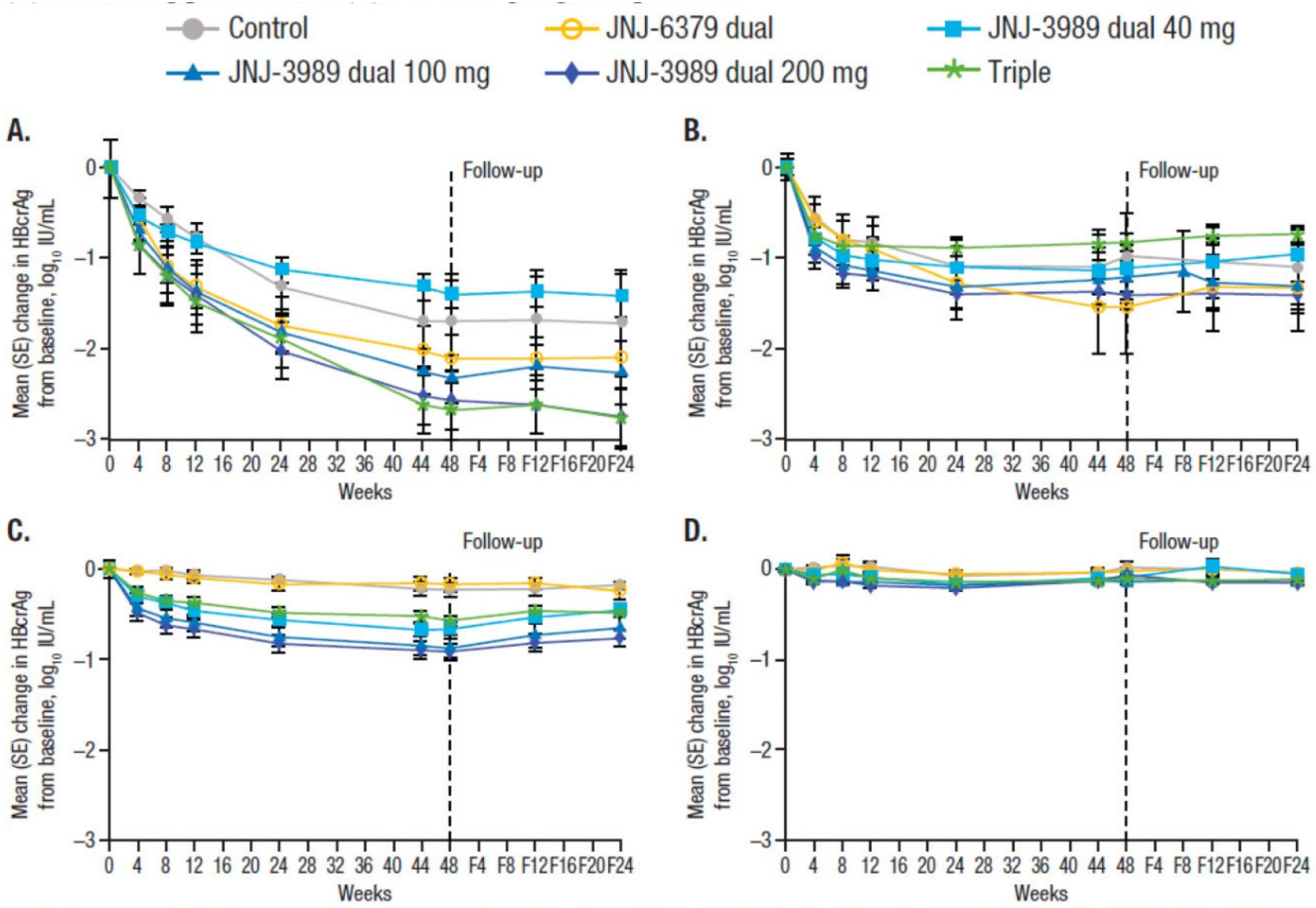
Mean change in HBcrAg in the REEF-1 study

NCT HBeAg+

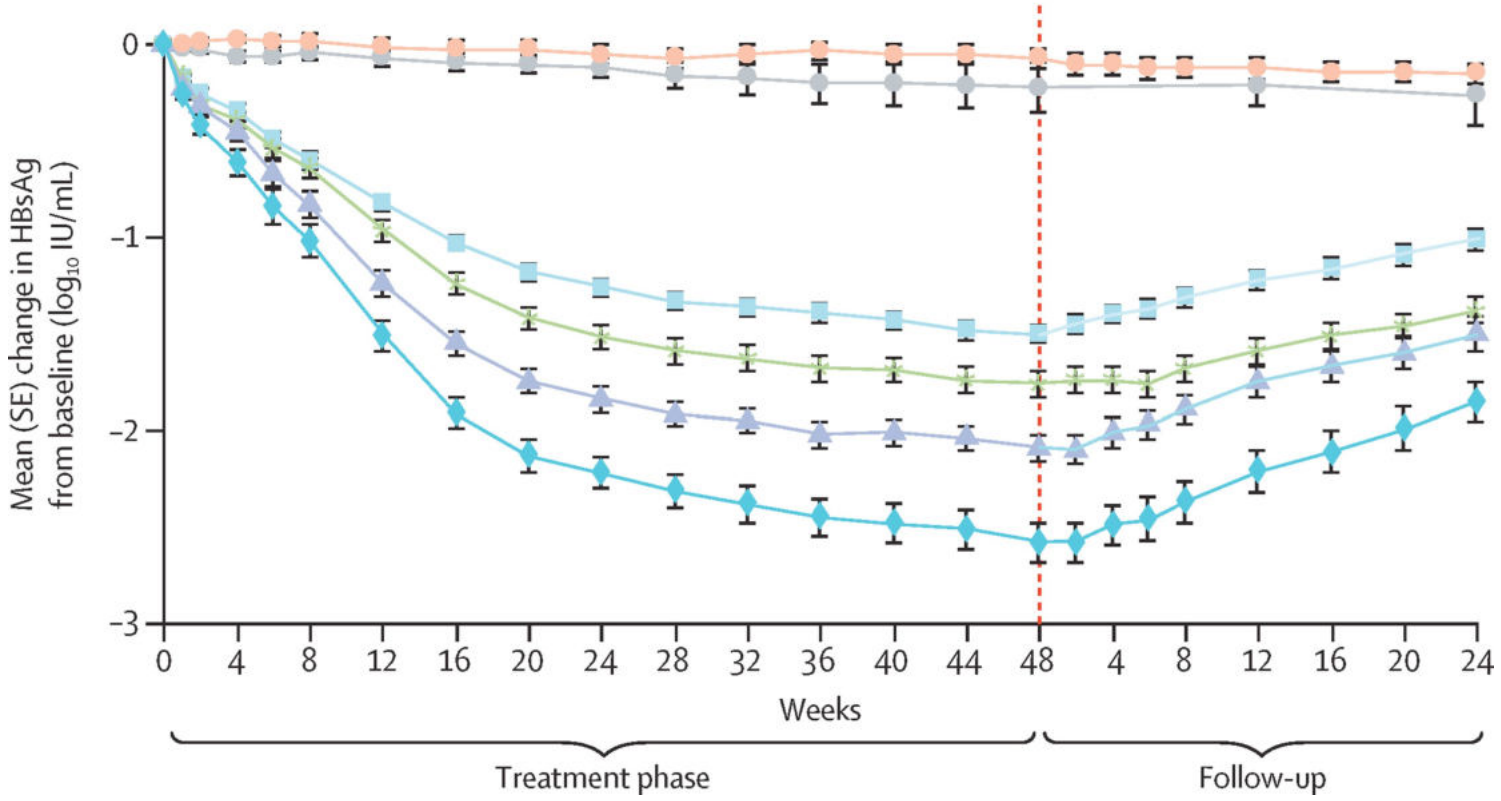
VS HBeAg+

NCT HBeAg-

VS HBeAg-



Mean change in HBsAg in the REEF-1 study



	Mean (SE) change in HBsAg from baseline, log_{10} IU/mL	
	Week 48	Follow-up week 24
Control group	−0·22 (0·13)	−0·26 (0·15)
JNJ-6379 dual group	−0·07 (0·05)	−0·15 (0·05)
JNJ-3989 dual 40 mg group	−1·5 (0·05)	−1·0 (0·05)
JNJ-3989 dual 100 mg group	−2·1 (0·07)	−1·5 (0·09)
JNJ-3989 dual 200 mg group	−2·6 (0·10)	−1·9 (0·10)
Triple group	−1·8 (0·07)	−1·4 (0·07)

Other emerging treatment regimens

Gastroenterology
Volume 158, Issue 8, June 2020, Pages 2180-2194

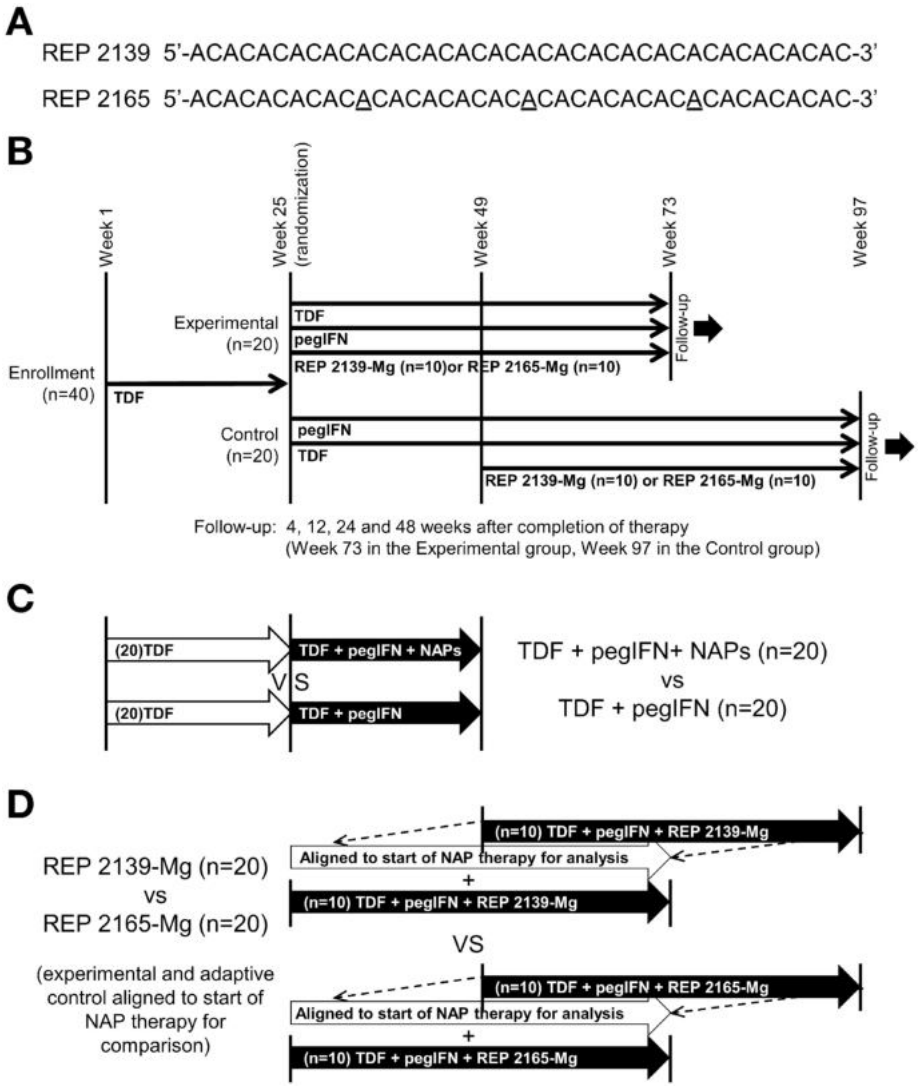
Original Research

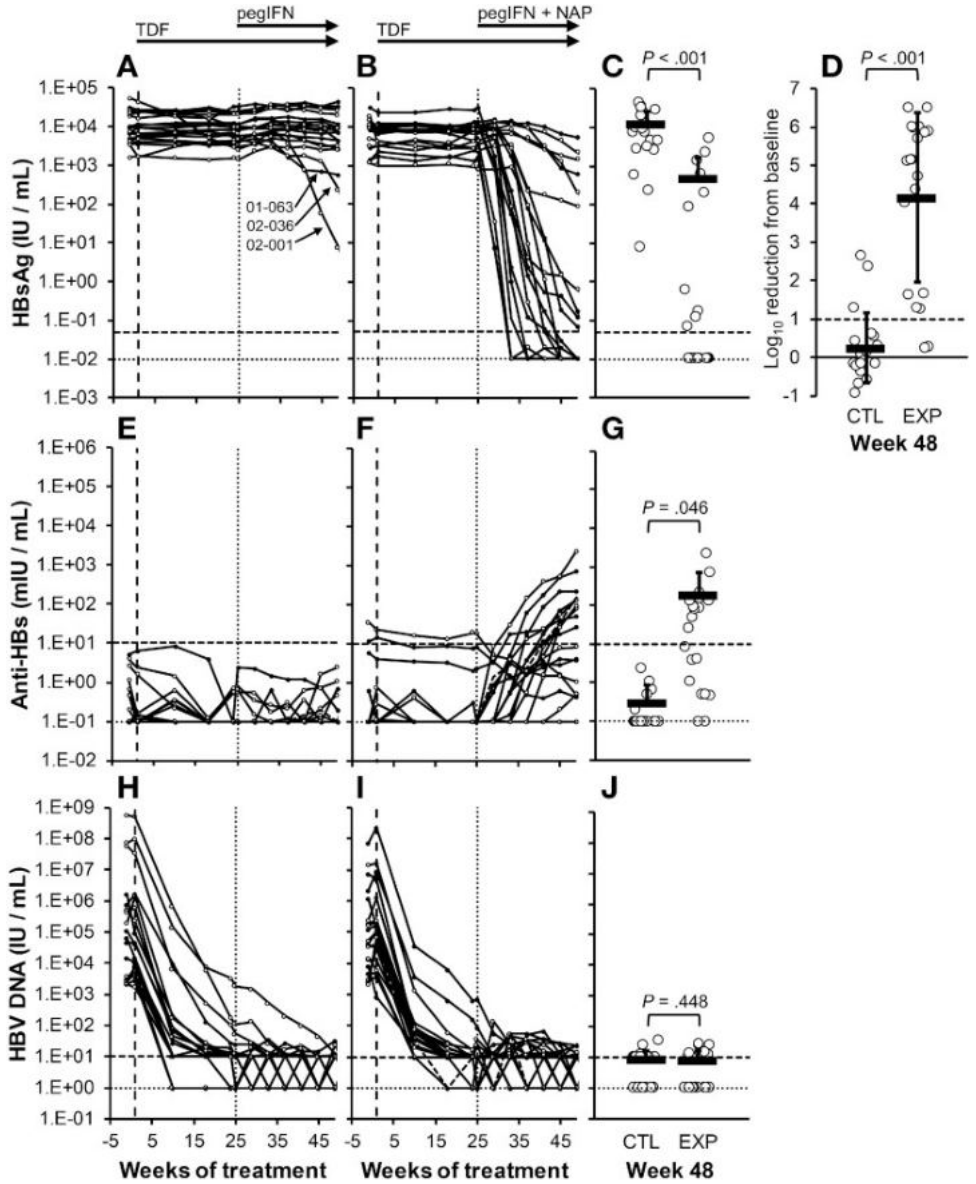
Full Report: Clinical—Liver

Safety and Efficacy of 48 Weeks REP 2139 or REP 2165, Tenofovir Disoproxil, and Pegylated Interferon Alfa-2a in Patients With Chronic HBV Infection Naïve to Nucleos(t)ide Therapy

Michel Bazinet [1], Victor Pântea [2], Gheorghe Placinta [2], Iurie Moscalu [3], Valentin Cebotarescu [2], Lilia Cojuhari [2], Pavlina Jimbei [4], Liviu Iarovoi [2], Valentina Smesnoi [4], Tatiana Musteata [4], Alina Jucov [2 3], Ulf Dittmer [5], Adalbert Krawczyk [5 6], Andrew Vaillant [1]

Kinetics of HBsAg in patients with REP 2139/2165 + TDF + Peg-IFN combination





HBsAg decline in Phase II studies of Bepirovirsen

nature medicine

ARTICLES

https://doi.org/10.1038/s41591-021-01513-4

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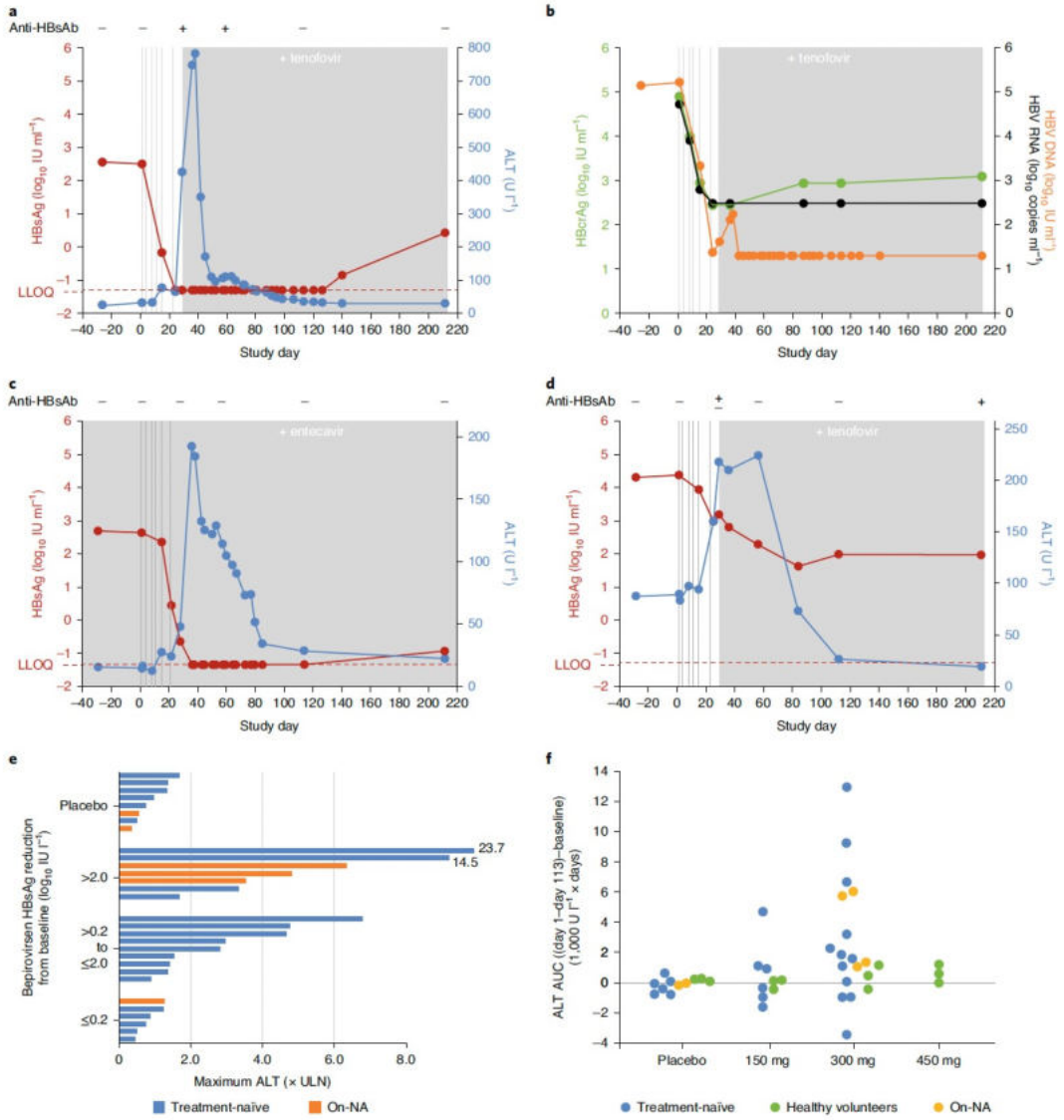
OPEN

Safety, tolerability and antiviral activity of the antisense oligonucleotide bepirovirsen in patients with chronic hepatitis B: a phase 2 randomized controlled trial

Man-Fung Yuen[1]✉, Jeong Heo[2], Jeong-Won Jang[3], Jung-Hwan Yoon[4], Young-Oh Kweon[5], Sung-Jae Park[6], Yvonne Tami[7], Shihyun You[8], Phillip Yates[9], Yu Tao[8], Jennifer Cremer[10], Fiona Campbell[9], Robert Elston[9], Dickens Theodore[10], Melanie Paff[8], C. Frank Bennett[7] and T. Jesse Kwoh[7]

Chronic infection with hepatitis B virus (HBV) leads to an increased risk of death from cirrhosis and hepatocellular carcinoma. Functional cure rates are low with current treatment options (nucleos(t)ide analogs (NAs) and pegylated interferons). Bepirovirsen is an antisense oligonucleotide targeting all HBV messenger RNAs; in cell culture and animal models, bepirovirsen leads to reductions in HBV-derived RNAs, HBV DNA and viral proteins. This phase 2 double-blinded, randomized, placebo-controlled trial is the first evaluation of the safety and activity of an antisense oligonucleotide targeting HBV RNA in both treatment-naïve and virally suppressed individuals with chronic HBV infection. The primary objective was to assess the safety and tolerability of bepirovirsen in individuals with chronic hepatitis B (CHB) (NCT02981602). The secondary objective was to assess antiviral activity, including the change from baseline to day 29 in serum hepatitis B surface antigen (HBsAg) concentration. Participants with CHB infection ≥6 months and serum HBsAg ≥50 IU ml^{-1} were enrolled from seven centers across Hong Kong and the Republic of Korea and randomized (3:1 within each dose cohort) to receive bepirovirsen or placebo via subcutaneous injection twice weekly during weeks 1 and 2 (days 1, 4, 8 and 11) and once weekly during weeks 3 and 4 (days 15 and 22). Participants were then followed for 26 weeks. Twenty-four participants were treatment-naïve and seven were receiving stable NA therapy. Treatment-emergent adverse events were mostly mild/moderate (most commonly injection site reactions). Eleven (61.1%) and three (50.0%) treatment-naïve participants experienced one or more treatment-emergent adverse event in the bepirovirsen and placebo groups, respectively. In participants receiving NA therapy, the corresponding numbers were three (60.0%) and one (50.0%). Transient, self-resolving alanine aminotransferase flares (≥2× upper limit of normal) were observed in eight treatment-naïve participants and three participants on stable NA regimens in the bepirovirsen treatment arms. HBsAg reductions were observed and were significant versus placebo for treatment-naïve participants receiving bepirovirsen 300 mg ($P = 0.001$), but not for the bepirovirsen 150 mg group ($P = 0.245$) or participants receiving stable NA therapy ($P = 0.762$). Two participants in each of the 300 mg dose groups achieved HBsAg levels below the lower limit of quantitation by day 29 ($n = 3$) or day 36 ($n = 1$). Bepirovirsen had a favorable safety profile. These preliminary observations warrant further investigation of the safety and activity of bepirovirsen in a larger CHB patient population.



HBsAg decline in Phase II studies of Bepirovirsen

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RESEARCH SUMMARY

Efficacy and Safety of Bepirovirsen in Chronic Hepatitis B Infection

Yuen M-F et al. DOI: 10.1056/NEJMoa2210027

CLINICAL PROBLEM

Fewer than 5% of patients with chronic hepatitis B virus (HBV) infection have hepatitis B surface antigen (HBsAg) loss after nucleoside or nucleotide analogue (NA) treatment. Bepirovirsen, an antisense oligonucleotide that targets all HBV mRNAs, led to a rapid reduction in HBsAg levels in a phase 2a trial, but its potential to induce HBsAg loss is unclear.

CLINICAL TRIAL

Design: A phase 2b multinational, randomized, investigator-unblinded trial assessed the efficacy and safety of bepirovirsen in adults with chronic HBV infection.

Intervention: 457 participants were assigned (in a 3:3:3:1 ratio) to receive weekly subcutaneous injections of 300 mg of bepirovirsen for 24 weeks (group 1); 300 mg of bepirovirsen for 12 weeks followed by 150 mg for 12 weeks (group 2); 300 mg of bepirovirsen for 12 weeks followed by placebo for 12 weeks (group 3); or placebo for 12 weeks followed by 300 mg of bepirovirsen for 12 weeks (group 4). Half the participants were receiving stable NA therapy. The composite primary efficacy outcome, assessed in groups 1, 2, and 3, was an HBsAg level below the lower limit of detection and an HBV DNA level below the lower limit of quantification, maintained for 24 weeks after the end of treatment without new antiviral medication.

RESULTS

Efficacy: A primary-outcome event occurred in 9 to 10% of participants in group 1, depending on concurrent NA therapy; in 6 to 9% in group 2; and in 1 to 3% in group 3.

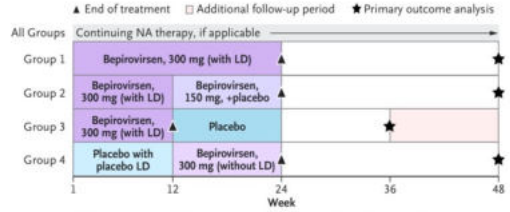
Safety: During weeks 1 through 12, adverse events, including injection-site reactions and increased levels of alanine aminotransferase, were more common with bepirovirsen than with placebo.

LIMITATIONS AND REMAINING QUESTIONS

- Larger and longer-term trials are needed to further assess the safety and efficacy of bepirovirsen, as well as the durability of its effect.

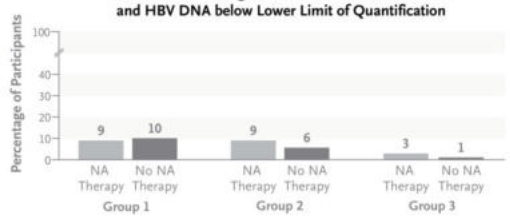
Links: Full Article | NEJM Quick Take | Editorial

Trial Design

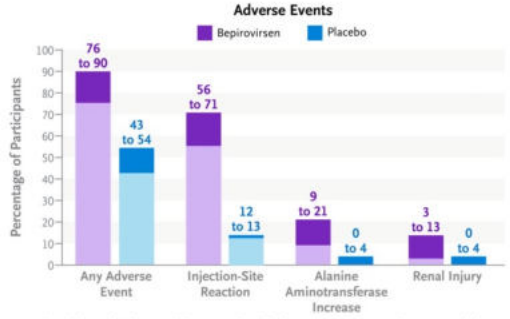


Loading dose (LD) of bepirovirsen (300 mg, in groups 1, 2, and 3) or placebo (in group 4) on days 4 and 11.

Patients with HBsAg below Lower Limit of Detection and HBV DNA below Lower Limit of Quantification



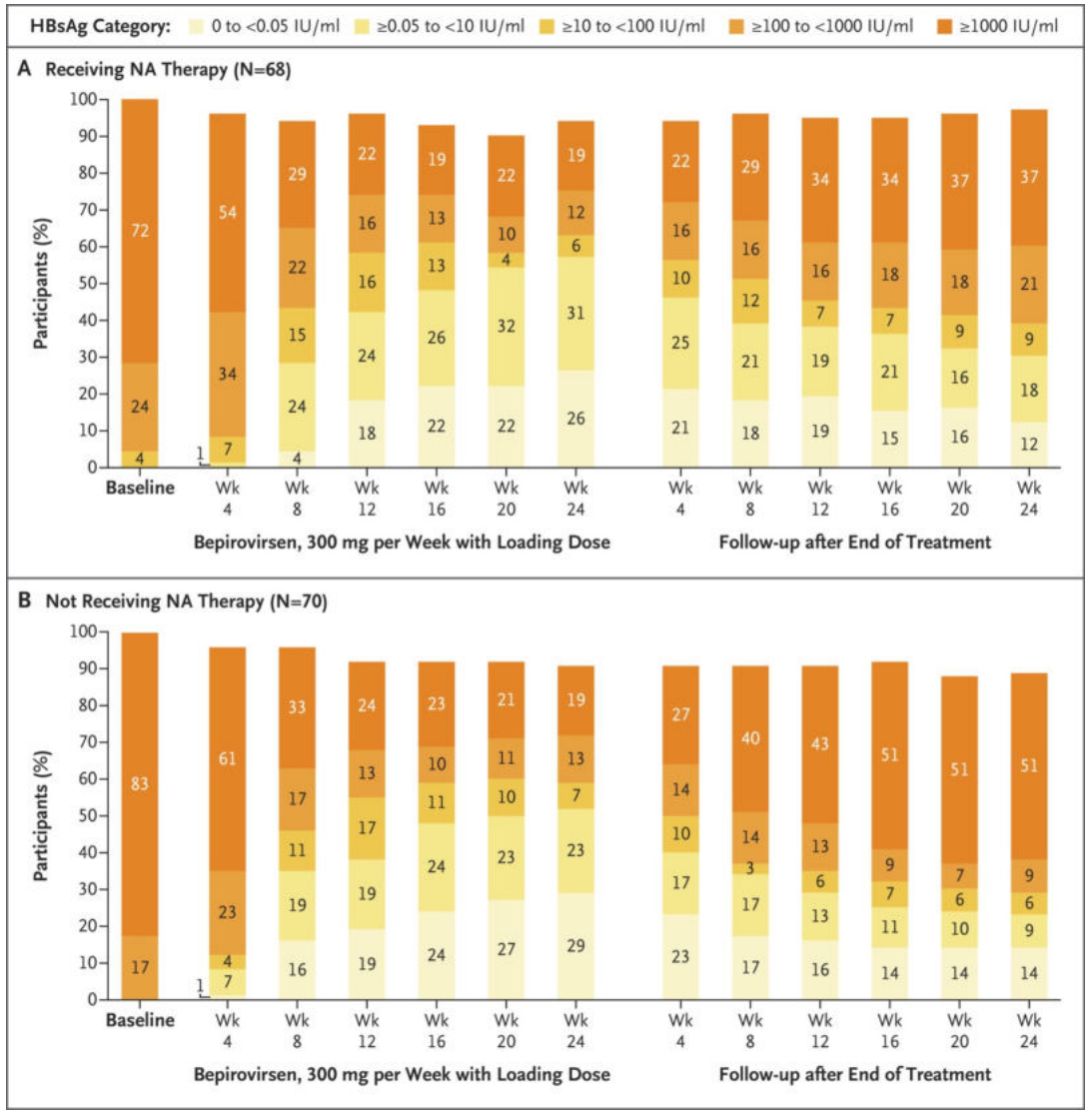
Adverse Events



The lighter shade in each bar extends to the lower percentage in the range, and the darker shade to the higher percentage.

CONCLUSIONS

In participants with chronic HBV infection, 24 weeks of treatment with 300 mg of bepirovirsen resulted in HBsAg and HBV DNA loss that was maintained for 24 weeks after treatment in 9 to 10% of participants.



Conclusion and open questions

- HBcrAg (HBc) and HBV RNA are important to assess target engagement in early phase clinical studies

- Role of HBc, HBcrAg, and HBV RNA in ASO containing regimens?

- Role of viral biomarkers in immunotherapy?

- Currently considered as exploratory biomarkers / endpoints

- Next questions to address:
    - Patient stratification?
    - Role in response guided therapy?
    - Role in predicting functional cure?
    - Role in predicting viral rebound?
    - Role in the clinical monitoring of patients?

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