The role of new viral biomarkers to assist the clinical development of HBV cure strategies: The industry perspective

Rob Elston

Lyon, 7th September 2023

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Disclaimer and Acknowledgments

- Rob Elston is an employee of GSK and holds stock/shares in the company
- Bepirovirsen is an investigational agent not approved for any indication anywhere in the world
- The information in this presentation is not intended to imply clinical safety or efficacy of bepirovirsen or that it will receive regulatory approval
- Editorial support was provided by Fishawack Indicia Ltd, UK, part of Fishawack Health, and was funded by GSK

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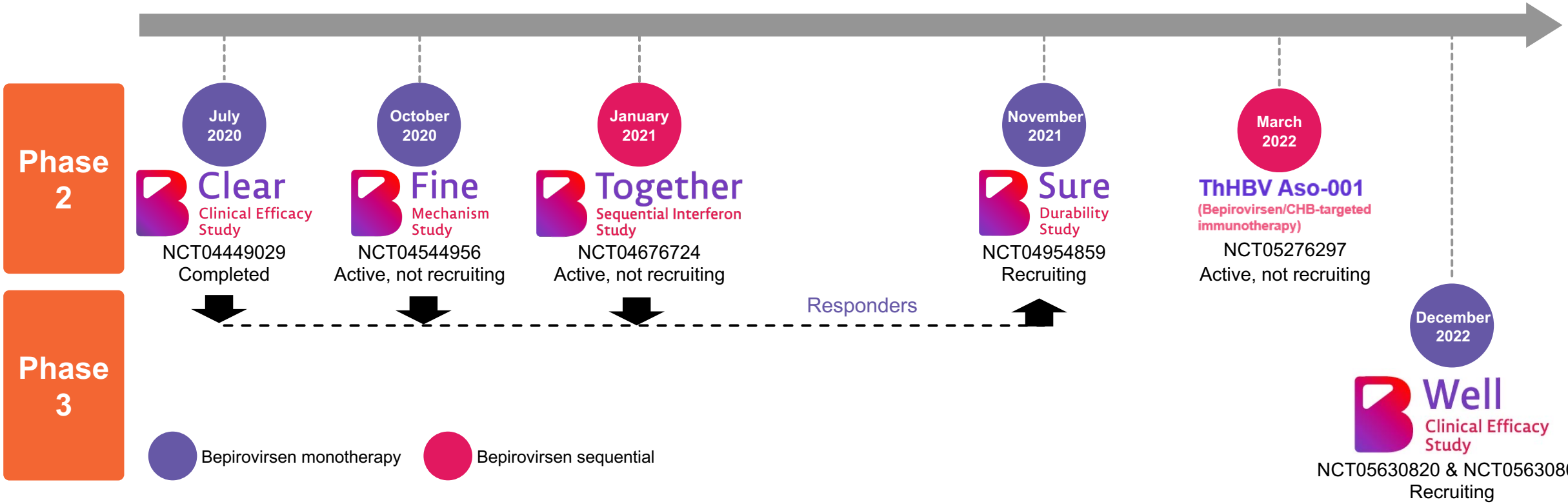
Bepirovirsen clinical trial programme

Bepirovirsen monotherapy and as a backbone

Bepirovirsen (BPV; GSK3228836) is an unconjugated antisense oligonucleotide that targets all HBV RNAs, including pregenomic RNA, via RNase H-mediated degradation resulting in a reduction of viral proteins such as HBsAg[1–3]



Phase 2b: B-Clear Study Design

B Clear Clinical Efficacy Study

Inclusion criteria

- Chronic HBV infection ≥6 months
- ALT ≤2 X ULN
- HBV DNA <90 IU/mL
- HBsAg >100 IU/mL

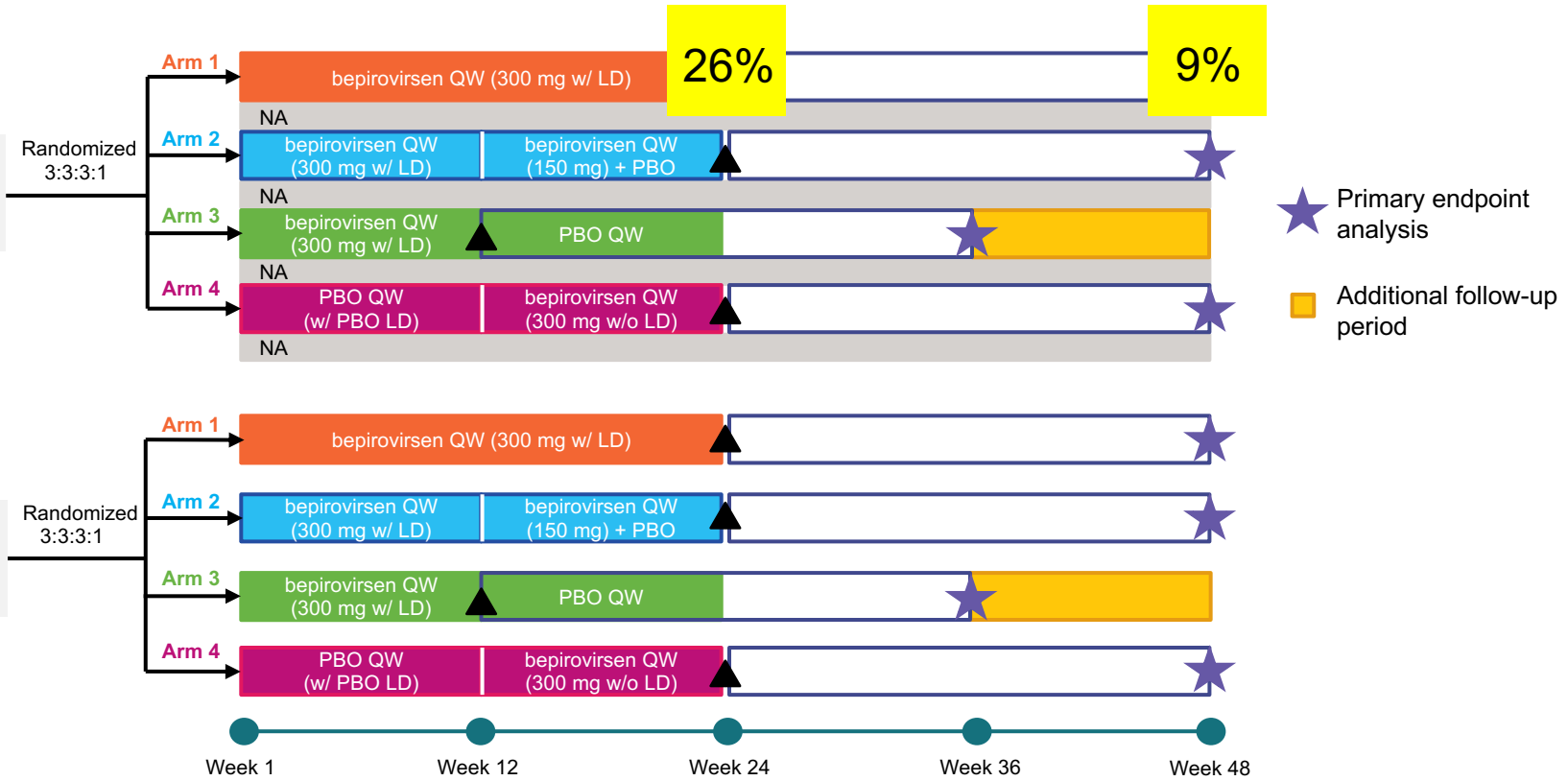
On-stable NA therapy n=227

- ALT <3 X ULN
- HBV DNA >2000 IU/mL
- HBsAg >100 IU/mL

Not currently on NA therapy n=230

Stratification

- HBeAg positive/negative
- HBsAg ≤3 log_{10} IU/mL or >3 log_{10} IU/mL



Primary endpoint (★): virologic response (HBsAg <LLOD [0.05 IU/mL] and HBV DNA <LLOQ [20 IU/mL]) sustained for 24 weeks from planned end of bepirovirsen treatment in the absence of rescue medication

Figure reproduced with permission from Yuen M-F, et al. Oral presentation at EASL 2022 (Publication No. 38817).
For the 150 mg dose, a placebo injection was added to maintain participant blinding. Participants on NA therapy at study start remained on their NA therapy throughout the duration of the study. Participants not currently on NA therapy had either never received HBV treatment or had ended NA therapy at least 6 months prior to the screening visit.
ALT, alanine aminotransferase; DNA, deoxyribonucleic acid; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LD, loading dose (Days 4 and 11); LLOD, lower limit of detection; LLOQ, lower limit of quantification; NA, nucleos(t)ide analog; PBO, placebo; QW, once a week; ULN, upper limit of normal; w, with; w/o, without.

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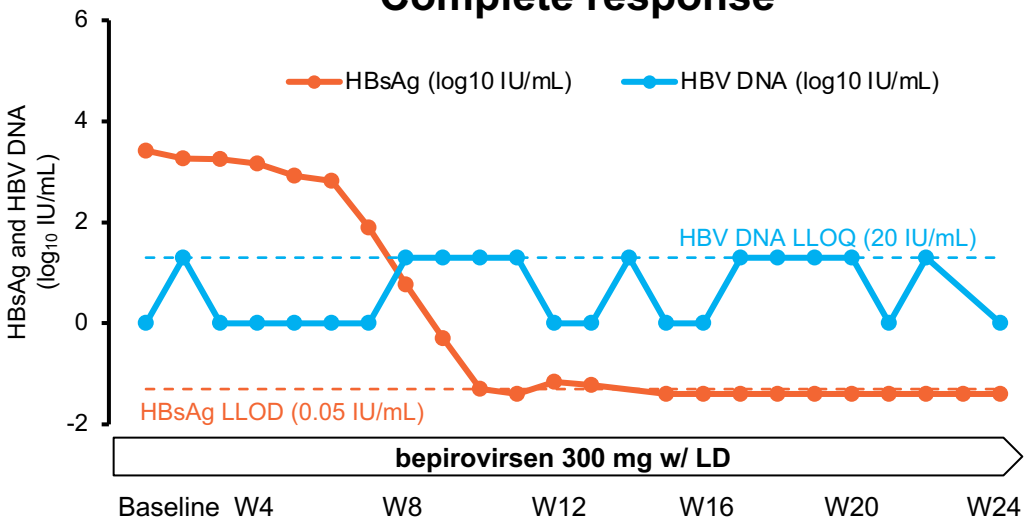
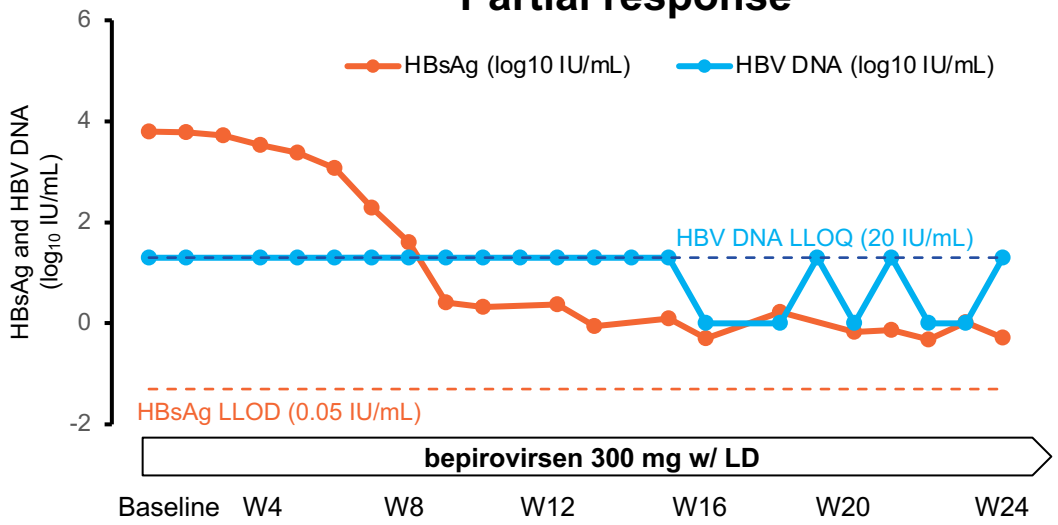
Variable bepirovirsen on-treatment responses were observed

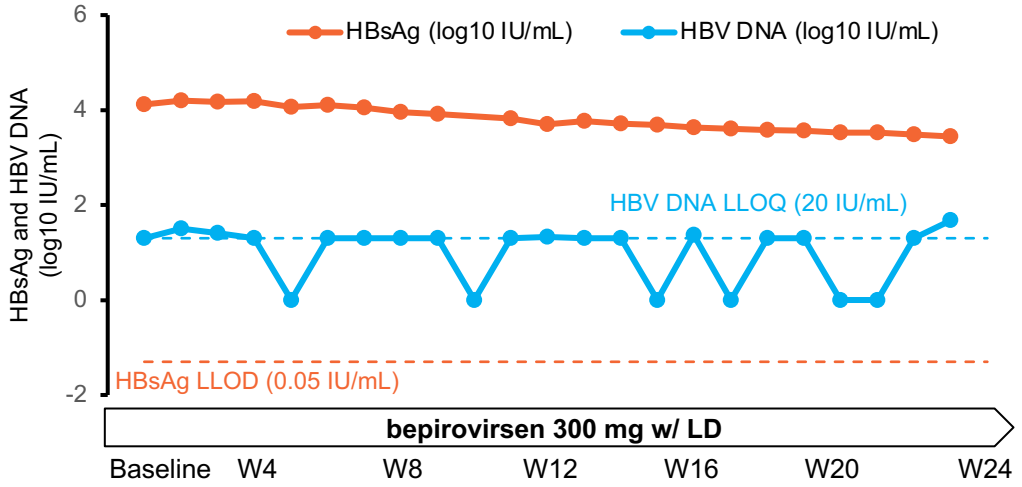
Complete response



Partial response

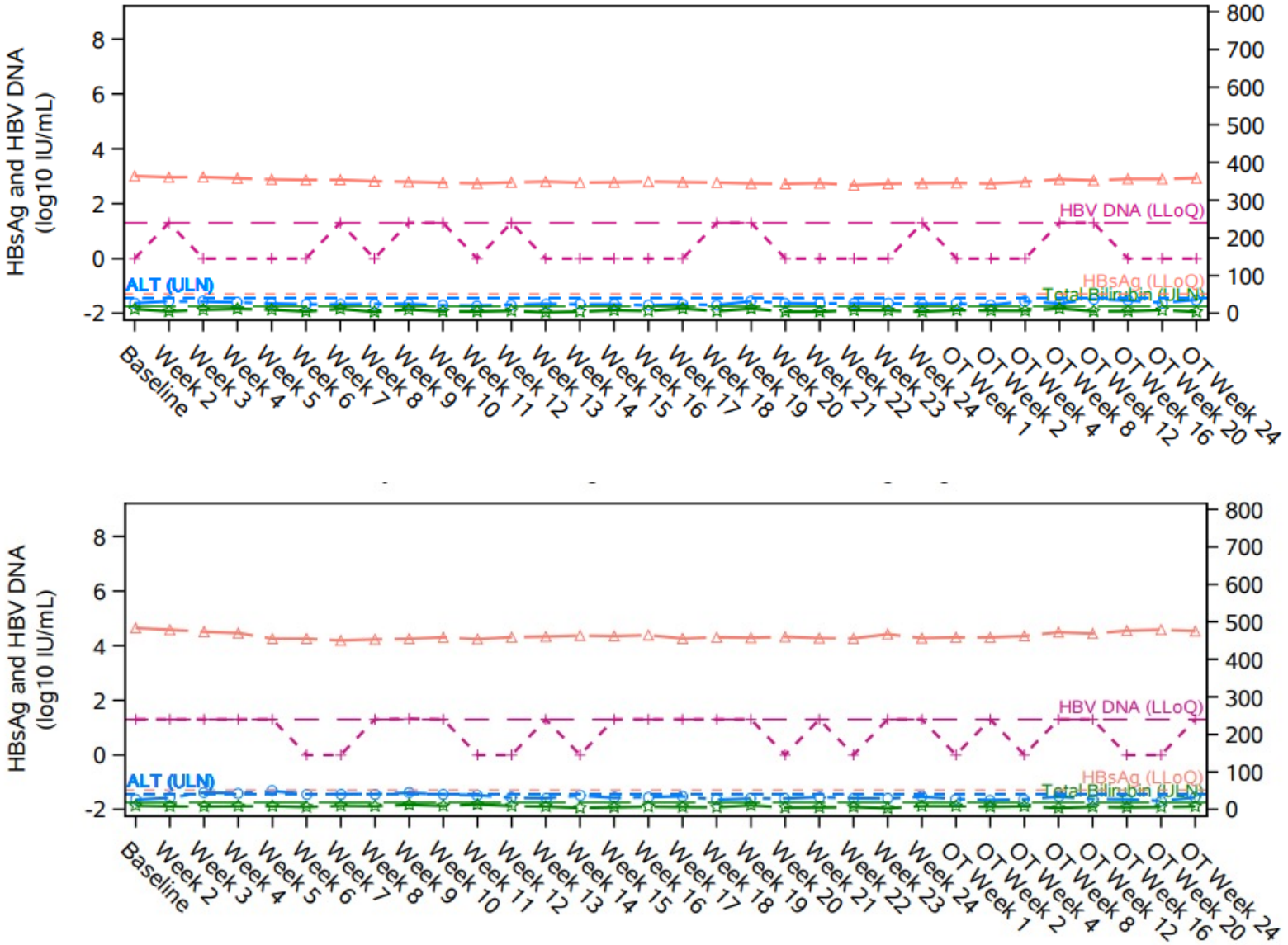


Non response



Figures were independently created by GSK.
DNA, deoxyribonucleic acid; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LD, loading dose (Days 4 and 11); LLOD, lower limit of detection; LLOQ, lower limit of quantification; NA, nucleos(t)ide analog; W, week; w/, with.

How we define Non or Partial Response may be important



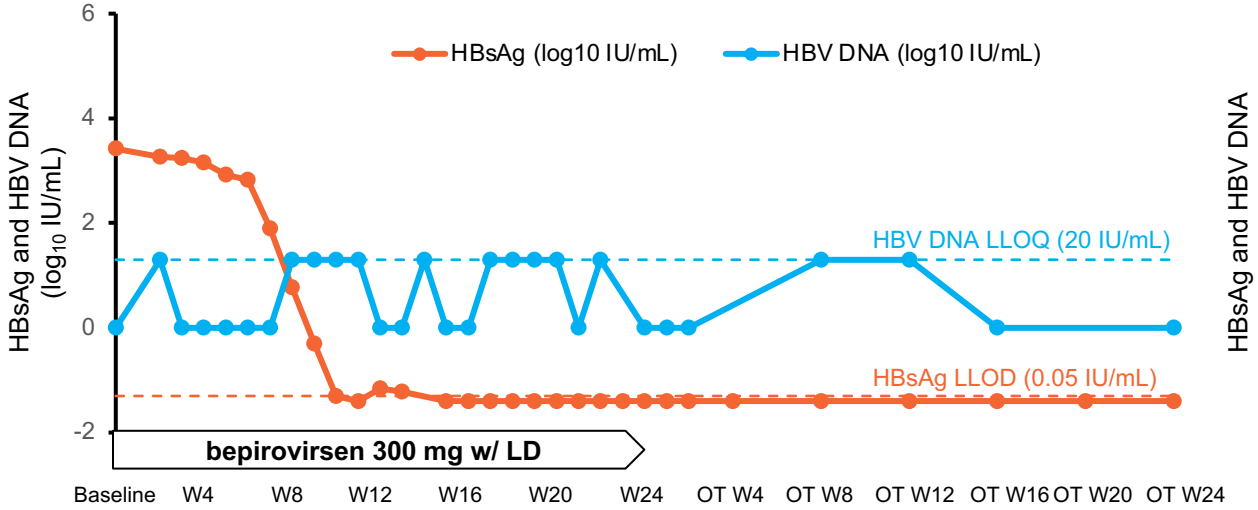
Baseline HBsAg = 1,018 IU/mL
Week 24 = 557 IU/mL
Reduction = -460 IU/mL
Log reduction = -0.26 IU/mL

Both patients have similar log reduction in HBsAg
but are they the same ?

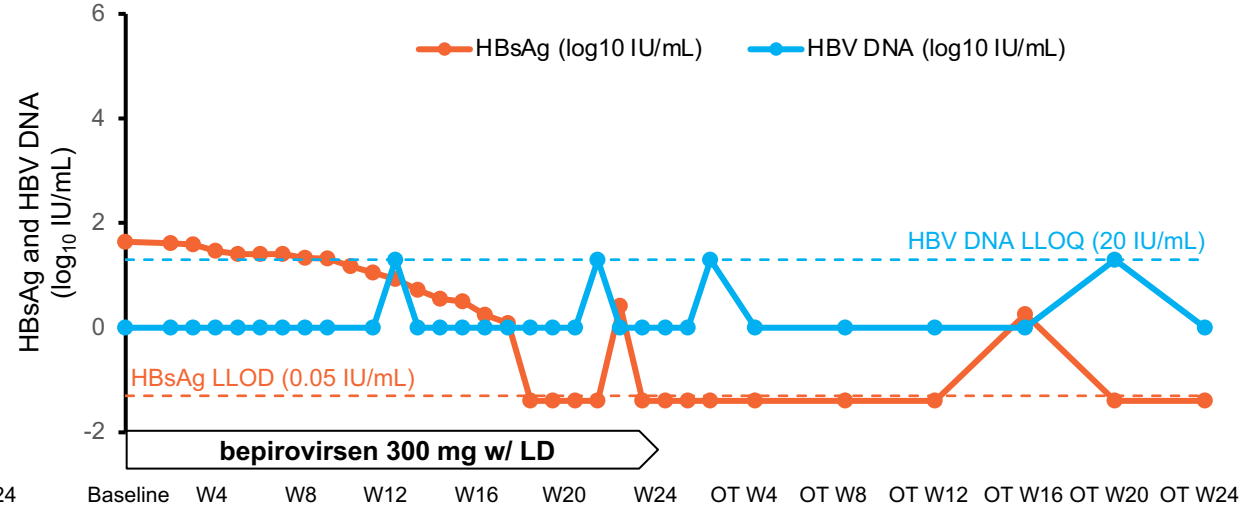
Baseline HBsAg = 44,442 IU/mL
Week 24 = 18,991 IU/mL
Reduction = -25,984 IU/mL
Log reduction = -0.37 IU/mL

Variable bepirovirsen off-treatment responses were observed

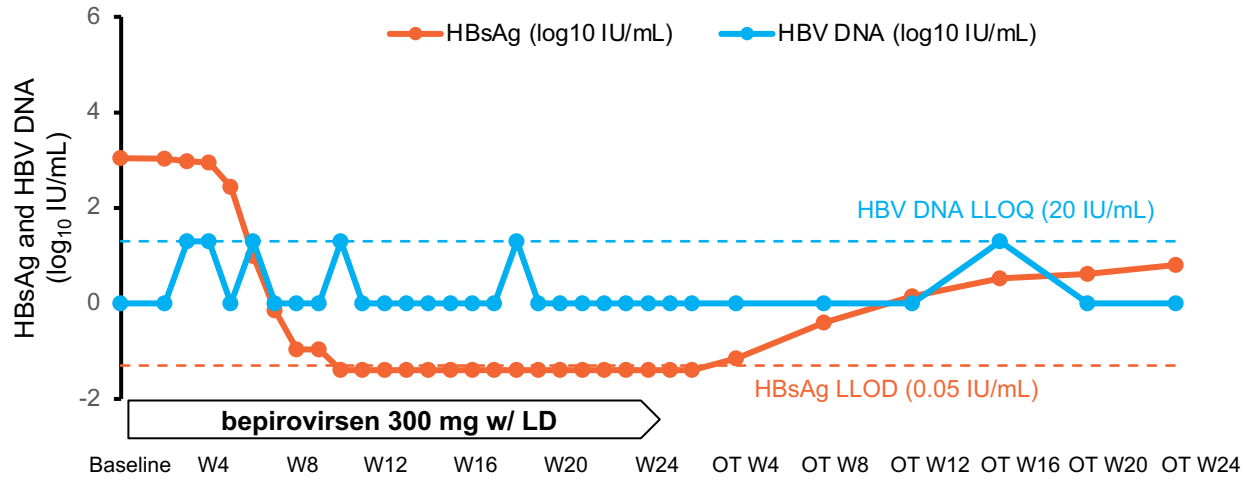
Responder



Responder ? Blip



Relapser



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DNA, deoxyribonucleic acid; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LD, loading dose (Days 4 and 11); LLOD, lower limit of detection; LLOQ, lower limit of quantification;
NA, nucleos(t)ide analog; W, week; w/, with.

Will a More Sensitive HBsAg Assay Be Needed?

Simulated HBsAg profiles following 300 mg QW dosing for 24 weeks

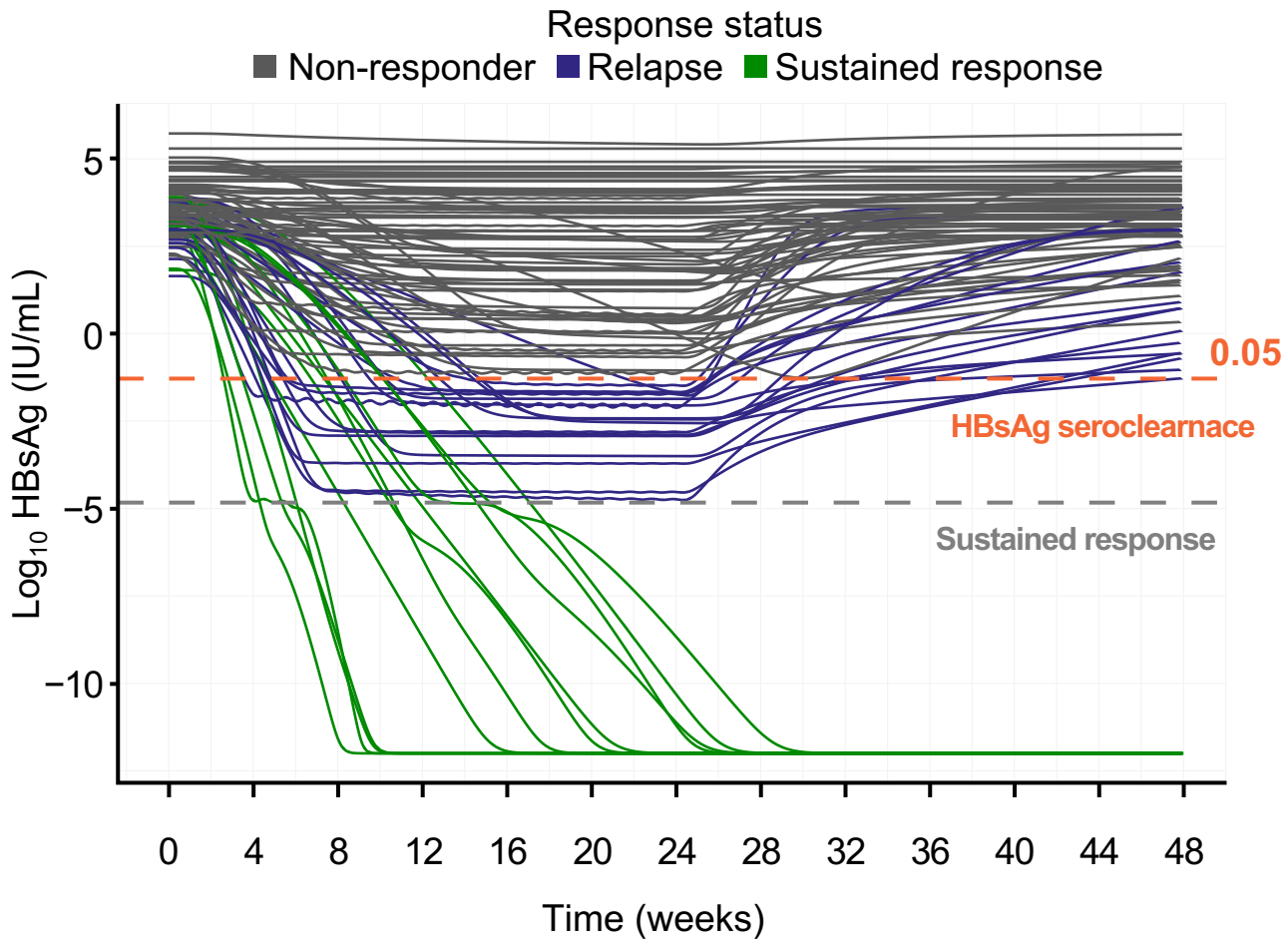
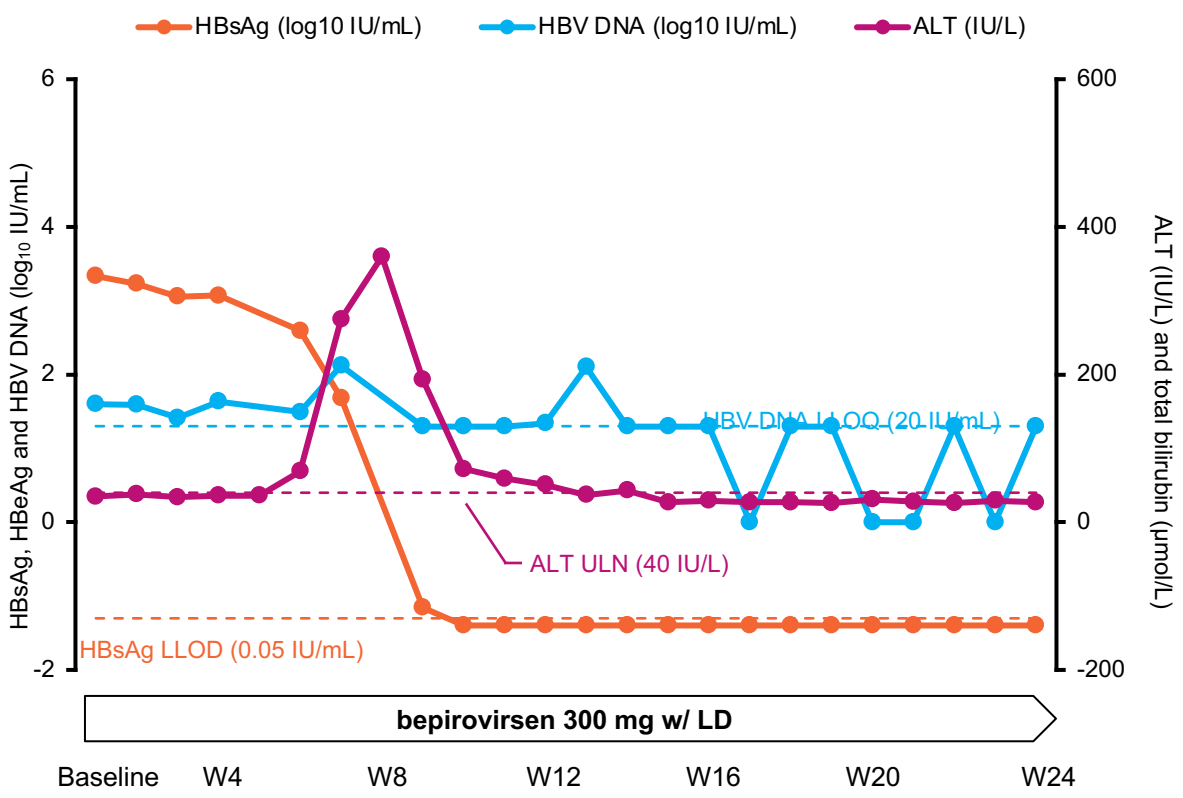


Figure amended with persmission from Youssef A, et al. Poster presented at EASL 2022 (Poster No. SAT441).
Chronic Hepatitis B Virus Infection: Developing Drugs for Treatment – Guidance for Industry. FDA CDER. April 2022.
FDA, Food and Drug Administration; HBsAg, hepatitis B surface antigen; QW, once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

HBsAg Seroclearance can Occur With or Without an ALT Flare

On-NA: 300 mg 24 week



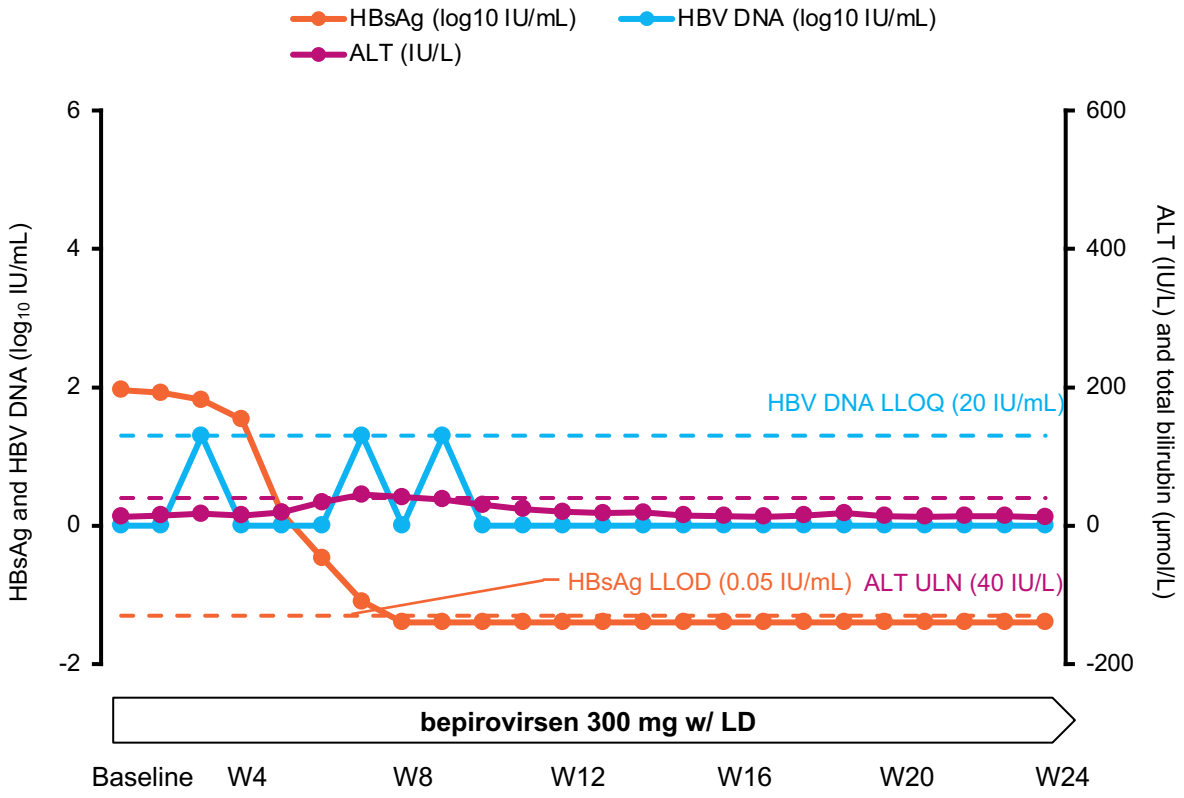
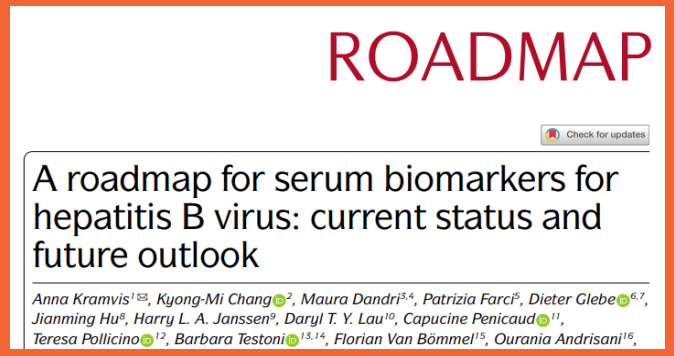


Figure to left reproduced with permission from Yuen M-F, et al. Poster presented at EASL 2022 (Poster No. SAT453). Figure to right independently created by GSK.
+ and − symbols indicate positive and negative anti-HBeAg status, respectively, at the relevant time point.
ALT, alanine aminotransferase; DNA, deoxyribonucleic acid; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LD, loading dose (Days 4 and 11); LLOD, lower limit of detection; LLOQ, lower limit of quantification; NA, nucleos(t)ide analog; ULN, upper limit of normal; W, week; w/, with.

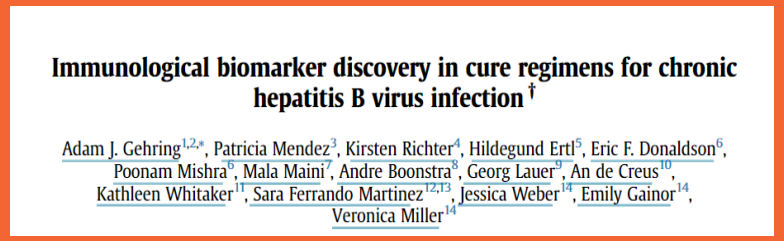
Biomarkers Hold the Key to Differentiation of Response


ROADMAP

Check for updates

A roadmap for serum biomarkers for hepatitis B virus: current status and future outlook

Anna Kramvis[1], Kyong-Mi Chang[2], Maura Dandri[3,4], Patrizia Farci[5], Dieter Glebe[6,7], Jianming Hu[8], Harry L. A. Janssen[9], Daryl T. Y. Lau[10], Capucine Penicaud[11], Teresa Pollicino[12], Barbara Testoni[13,14], Florian Van Bömmel[15], Ourania Andrisani[16],


Immunological biomarker discovery in cure regimens for chronic hepatitis B virus infection †

Adam J. Gehring[1,2,*], Patricia Mendez[3], Kirsten Richter[4], Hildegund Ertl[5], Eric F. Donaldson[6], Poonam Mishra[6], Mala Maini[7], Andre Boonstra[8], Georg Lauer[9], An de Creus[10], Kathleen Whitaker[11], Sara Ferrando Martinez[12,13], Jessica Weber[14], Emily Gainor[14], Veronica Miller[14]

Virology

Standard assays

- ✓ HBV DNA
- ✓ HBsAg
- ✓ HBeAg
- ✓ anti-HBsAg
- ✓ anti-HBeAg

Exploratory assays

- ✓ HBcrAg
- ✓ HBV RNA
- ✓ HBV genotype
- ✓ HBV mutation profiling

Immunology

- ✓ Cytokine profiles
- ✓ B and T cell immune profiling – Flow
- ✓ Measures of exhaustion/activation
- ✓ Functional HBV specific T cell assay
- ✓ HBV specific B cells
- ✓ NK cell phenotyping

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Kramvis A, et al. *Nat Rev Gastroenterol Hepatol.* 2022;19(11):727–45; Gehring AJ, et al. *J Hepatol.* 2022;77(2): 525−38.
Anti-HBsAg, hepatitis B surface antibody; anti-HBeAg, hepatitis B e antibody; DNA, deoxyribonucleic acid; HBcrAg, hepatitis B core-related antigen; HBeAg; hepatitis B e-antigen HBsAg; hepatitis B surface antigen; HBV, hepatitis B virus; NK, natural killer; RNA, ribonucleic acid.

Some Challenges

- Monitoring peripheral biomarkers versus site of action in the liver
- Operational considerations:
  - Timing of sample collection
  - Timing of analysis
  - Blood volumes
  - Isolation of quality PBMCs
  - “Big Data” integration
- Sensitivity of assays – for example, HBcrAg, HBV RNA
- Lack of commercial assays – HBV RNA
- Assessing genotype in patients on-NA

HBcrAg, hepatitis B core-related antigen; HBV, hepatitis B virus; NA, nucleos(t)ide analog; PBMC, peripheral blood mononuclear cell; RNA, ribonucleic acid.

In On-NA patients many virology biomarkers have limited availability

- Many HBV Phase 2 studies have recruited patients either on stable NA or initiated NA as part of the trial
- Consequently less virological biomarker information is available

Virology

Standard assays

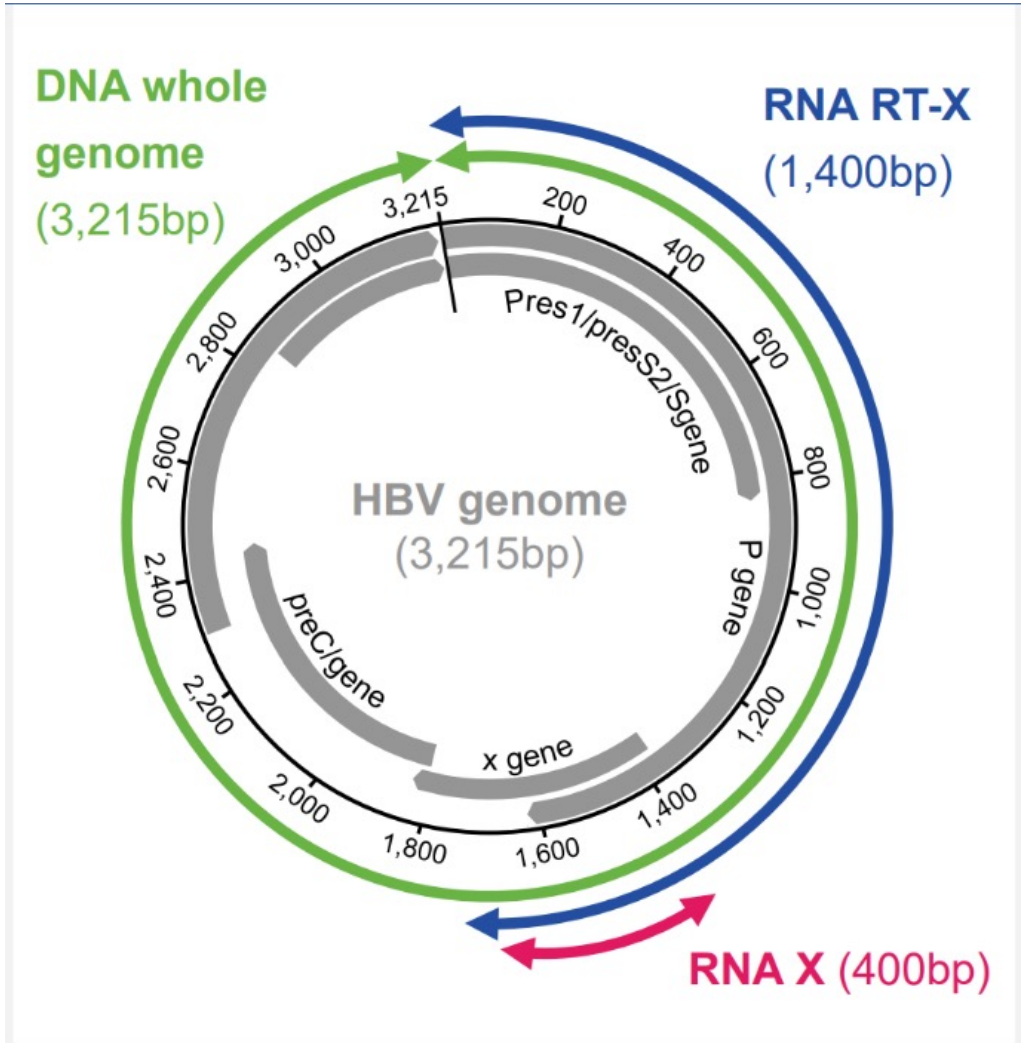
- ✓ ~~HBV DNA~~
- ✓ HBsAg
- ✓ ~~HBeAg~~
- ✓ anti-HBsAg
- ✓ anti-HBeAg

Exploratory assays

- ✓ ~~HBcrAg~~
- ✓ ~~HBV RNA~~
- ✓ ~~HBV genotype~~
- ✓ ~~HBV mutation profiling~~

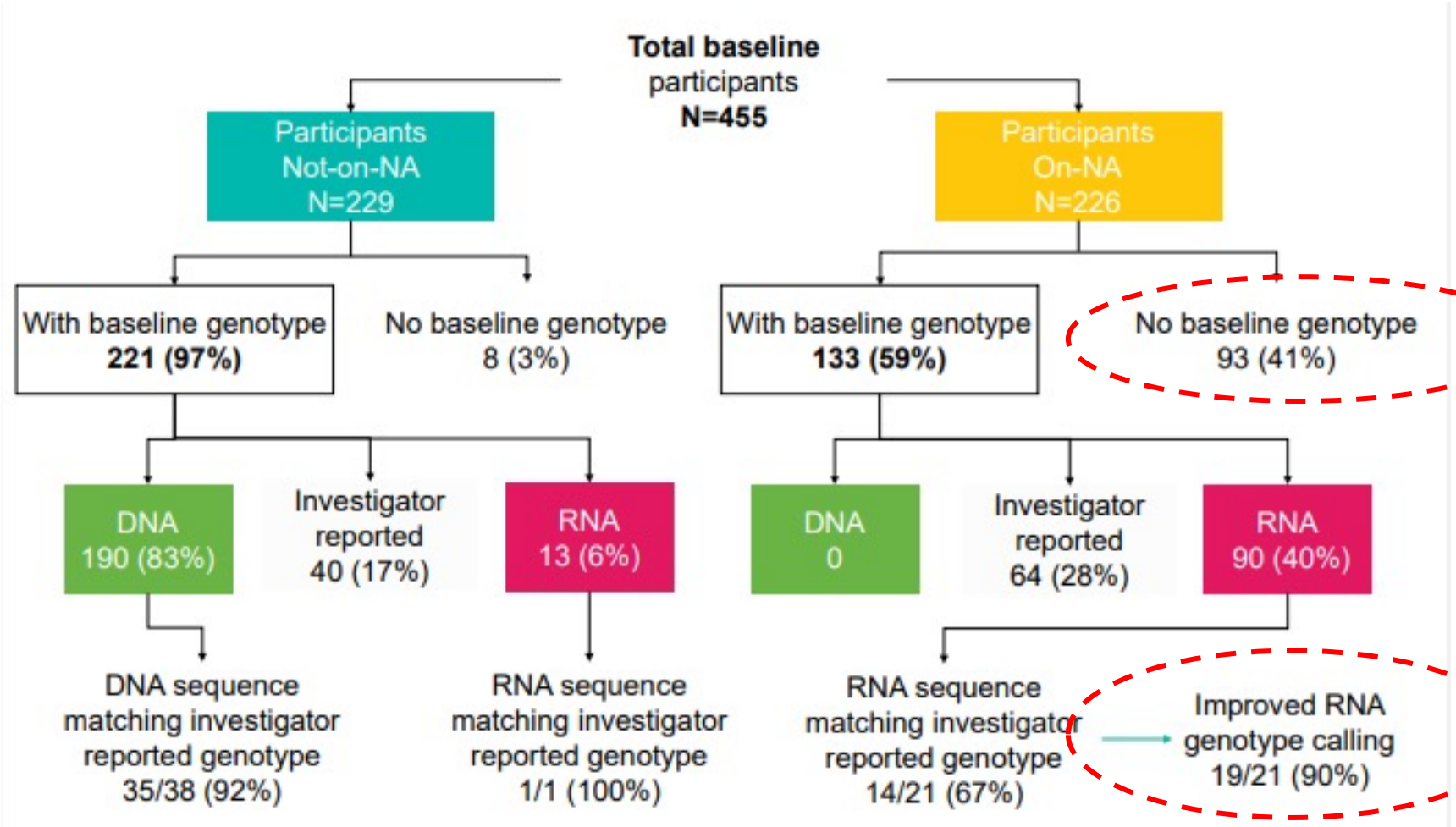
Anti-HBsAg, hepatitis B surface antibody; anti-HBeAg, hepatitis B e antibody; DNA, deoxyribonucleic acid; HBcrAg, hepatitis B core-related antigen; HBeAg; hepatitis B e-antigen HBsAg; hepatitis B surface antigen; HBV, hepatitis B virus; RNA, ribonucleic acid.

HBV DNA and RNA levels impact assessment of baseline polymorphisms/genotype



- HBV DNA whole genome sequencing was attempted if HBV DNA >1000 IU/mL
- Samples with HBV DNA <1000IU/mL, but HBV RNA >1000 copies/mL were amplified HBV RT-X gene.
- Samples with HBV RNA <1000 copies/mL were amplified for the X region only

B-Clear HBV Genotype Assessment

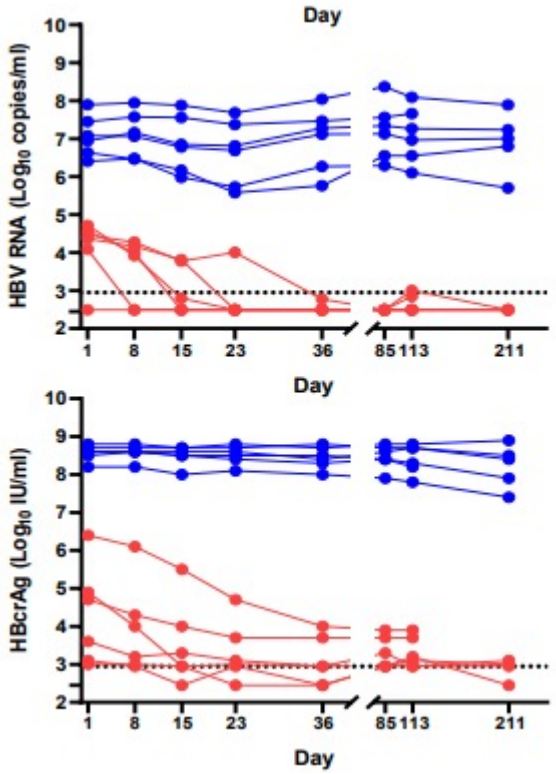


Need methods to increase genotypic data – such as serology

Validation of new methods

HBcrAg and HBV RNA Assessment

- In Phase 2a, Reductions in HBcrAg and HBV RNA were observed



- In B-Clear, patients on-NA often had low/undetectable levels of both biomarkers at baseline

	<LLoD	%
HBV RNA	170/226	75%
HBcrAg	74/225	32%

Biomarker Data Will Provide a Better Understanding to Inform:

PS

Patient Stratification

Understanding patient populations allows for selection of the right subjects

NPR

Path forward in non or partial responders

Understanding what biology is missing

MD

Mechanistic Differentiation

Understanding mechanistic differences between investigational agents

PM

Patient Management

Defining biomarkers as tools for response guided therapy, NA discontinuation, prediction of durable response

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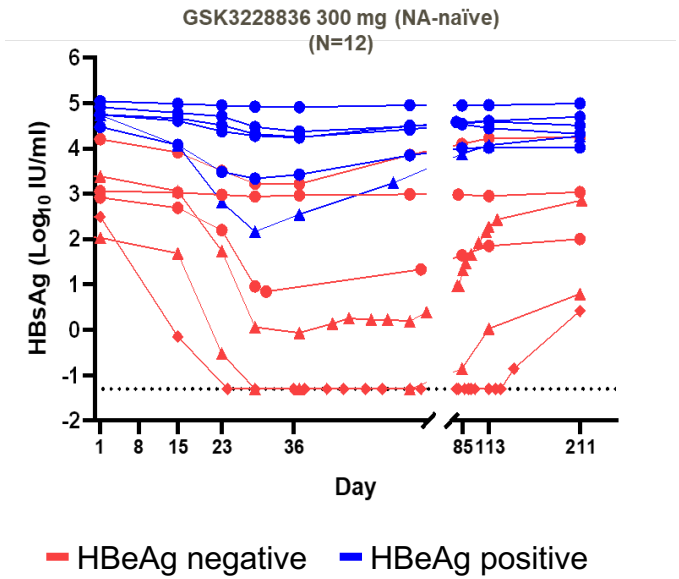
Patient Stratification: Signal to Selection

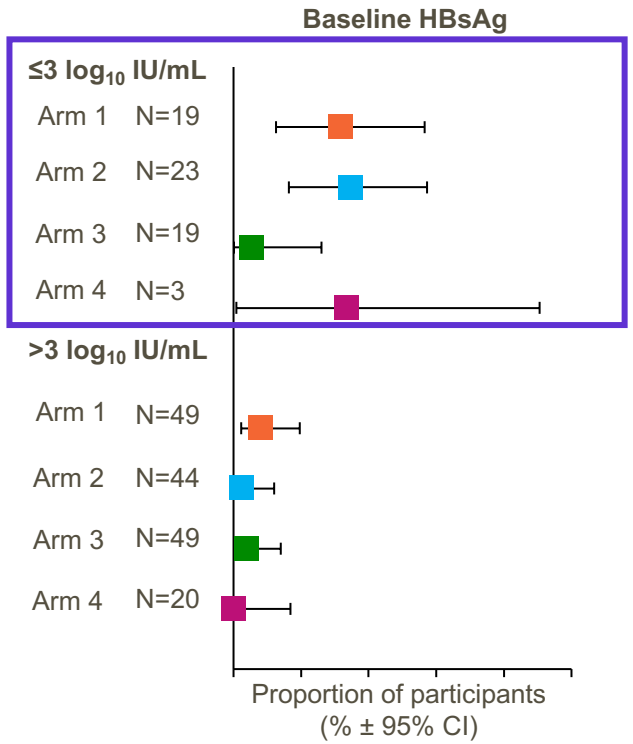
1. **Find** signal that differentiates between responders and non-responders in initial study

Phase 2a: Best HBsAg log IU/mL reduction in low HBsAg patients



2. **Verify** observation in independent study

Phase 2b: Baseline HBsAg predicts log IU/mL response



3. **Implement** assay fit for patient selection in next study

Phase 3: Use of HBsAg for patient selection

Well 1 Clinical Efficacy Study

Well 2 Clinical Efficacy Study

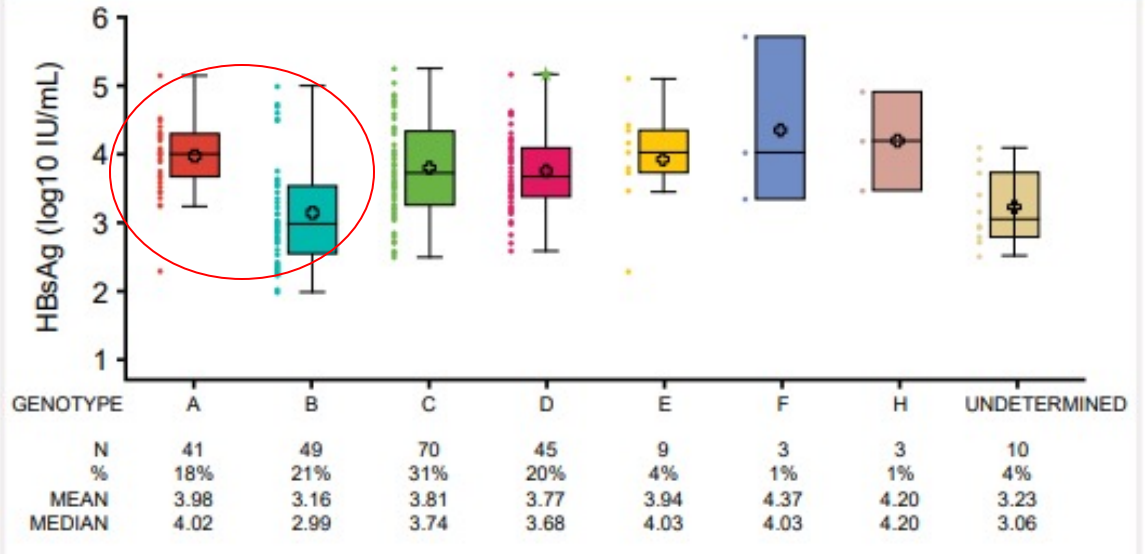
B-Well 1 and 2: **Only** enrolling patients with HBsAg concentration >100 IU/mL, but ≤3000 IU/mL

Figure reproduced with permission from Yuen M-F, et al. *Nat Med*. 2021;27(10):1725–34, as permitted under the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/) and Figure reproduced with permission from Yuen M-F, et al. Oral presentation at EASL 2020 (AS067).
CI, confidence interval; HBsAg, hepatitis B surface antigen; NA, nucleos(t)ide analog.

Challenging to dissect out influence of other factors once HBsAg level taken into account.

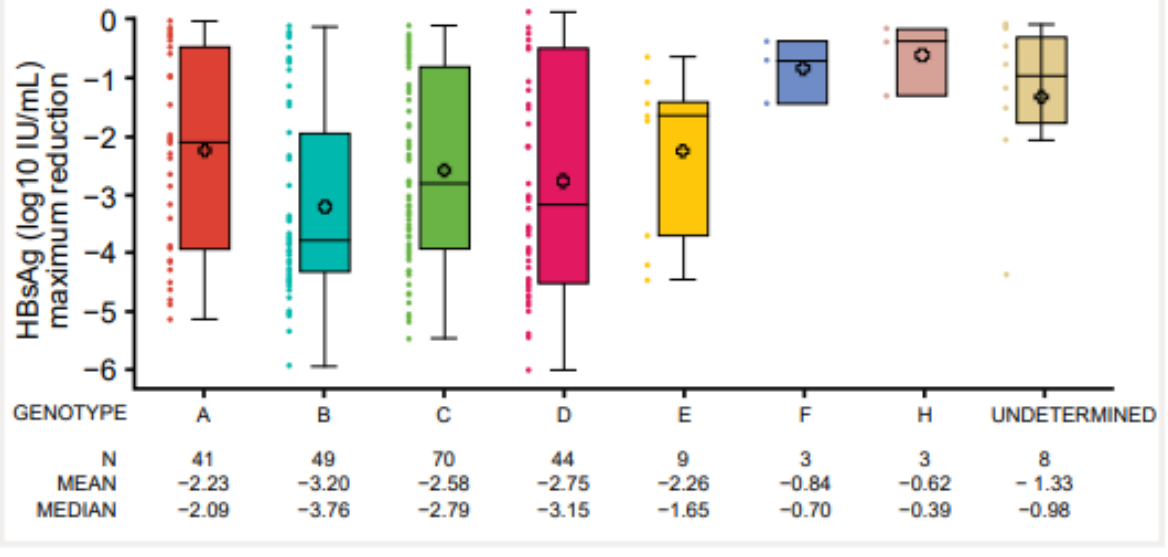
Example: Baseline HBsAg levels not evenly distributed over genotypes

Figure 4. Baseline HBsAg by genotype (Not-on-NA population)



GENOTYPE	A	B	C	D	E	F	H	UNDETERMINED
N	41	49	70	45	9	3	3	10
%	18%	21%	31%	20%	4%	1%	1%	4%
MEAN	3.98	3.16	3.81	3.77	3.94	4.37	4.20	3.23
MEDIAN	4.02	2.99	3.74	3.68	4.03	4.03	4.20	3.06

Figure 5. EOS HBsAg reduction by genotype (Not-on-NA population)



GENOTYPE	A	B	C	D	E	F	H	UNDETERMINED
N	41	49	70	44	9	3	3	8
MEAN	−2.23	−3.20	−2.58	−2.75	−2.26	−0.84	−0.62	− 1.33
MEDIAN	−2.09	−3.76	−2.79	−3.15	−1.65	−0.70	−0.39	−0.98

Figure amended with persmission from Jerome Bouquet, et al. Poster presented at EASL 2023 (Poster No. SAT-176 (abstract No. 3451).

Summary: Biomarkers Hold the Key to Differentiation of Response

…..but there are opportunities to improve

Virology

Standard assays

- ✓ HBV DNA
- ✓ HBsAg – FDA approved quantitative assays, more sensitive assays ?
- ✓ HBeAg
- ✓ anti-HBsAg
- ✓ anti-HBeAg

Exploratory assays

- ✓ HBcrAg – more sensitive assays
- ✓ HBV RNA - commercial assays – more sensitive assays
- ✓ HBV genotype - ability to genotype patients on nucleos(t)ide – serology assays
- ✓ HBV mutation profiling – understanding clinical relevant levels

Other assays

- ➢ HBsAb-Ag complexes
- ➢ HBsAg isoform specific assays
- ➢ Ability to differentiate integrated transcripts
- ➢ Anti-HBc
- ➢ …….

Kramvis A, et al. *Nat Rev Gastroenterol Hepatol.* 2022;19(11):727–45; Gehring AJ, et al. *J Hepatol.* 2022;77(2): 525−38.
Anti-HBsAg, hepatitis B surface antibody; anti-HBeAg, hepatitis B e antibody; DNA, deoxyribonucleic acid; HBcrAg, hepatitis B core-related antigen; HBeAg; hepatitis B e-antigen HBsAg; hepatitis B surface antigen; HBV, hepatitis B virus; NK, natural killer; RNA, ribonucleic acid.