No Amino Acid Substitution in HBV Pres1, HDAg, Or NTCP Associated With Suboptimal Response to Bulevirtide in Combination With Pegylated Interferon Alfa-2a Treatment in Participants With Chronic Hepatitis Delta: Results From MYR204, a Phase 2b Study

SAT-371

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Conclusions

- Suboptimal on-treatment response to BLV in combination with Peg-IFNα as well as posttreatment viral relapse or rebound were not associated with amino acid substitutions in HBV PreS1, HDAg, or NTCP
- No evidence of resistance was detected in participants with suboptimal response in MYR204

Plain Language Summary

- We evaluated viral resistance in participants over 96 weeks of treatment with bulevirtide, either with or without pegylated interferon alfa-2a, and for up to 48 weeks of follow-up
  - No hepatitis delta virus strains with a reduced susceptibility to bulevirtide were detected in vitro
  - At baseline, viruses from participants with different treatment responses had similar sensitivity to bulevirtide in vitro
  - The suboptimal treatment response to bulevirtide experienced by some participants could not be explained by viral resistance or genetic differences in the human NTCP gene

References: **1.** Ni Y, et al. *Gastroenterology*. 2014;146:1070-83. **2.** Yan H, et al. *eLife*. 2012;1:e00049. **3.** Wedemeyer H, et al. *N Engl J Med*. 2023;389(1):22-32. **4.** Wedemeyer H, et al. EASL 2023. Abstract OS-068. DOI:10.1016/S0168-8278(23)00522-6. **5.** Hollnberger H, et al. *J Hepatol*. 2023;79:657-65. **6.** Aleman S, et al. AASLD 2023. Abstract 1237-C. DOI: 10.1097/HEP.0000000000000580.

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Introduction

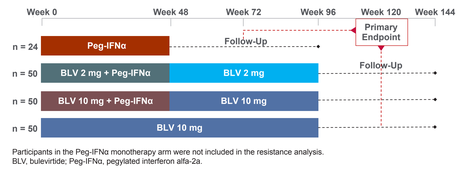
- Bulevirtide (BLV), a 47–amino-acid chemically synthesised lipopeptide, blocks entry of hepatitis delta virus (HDV) into hepatocytes via competitive inhibition of the interaction between the hepatitis B virus (HBV) preS1 domain and the sodium taurocholate cotransporting polypeptide (NTCP) receptor[1,2]
- BLV has been shown to be safe, well tolerated, and efficacious in treatment of chronic HDV infection (CHD). BLV 2 mg per day has been fully approved for the treatment of compensated CHD in the EU[3,4]
- Although BLV is a highly potent HDV inhibitor, some patients treated with BLV monotherapy had suboptimal virologic response that could not be explained by resistance[5,6]

Objective

- To perform a virologic analysis of participants with suboptimal response in the recently completed Phase 2b study MYR204, which evaluated finite treatment with BLV with or without pegylated interferon alfa-2a (Peg-IFNα) in participants with CHD

Methods

MYR204 Study Design

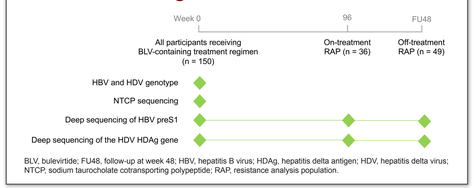


Participants in the Peg-IFNα monotherapy arm were not included in the resistance analysis.
BLV, bulevirtide; Peg-IFNα, pegylated interferon alfa-2a.

- On-treatment resistance analysis population (RAP): participants with suboptimal response to BLV, including the following 4 categories:
  - Nonresponder (NR): HDV RNA decline <1 $\log_{10}$ IU/mL from baseline (BL) through the end of treatment (EOT)
  - Virologic breakthrough (VB):
    - Confirmed[a] increase in HDV RNA ≥1 $\log_{10}$ IU/mL from the nadir under treatment, assuming the nadir was previously ≥1 $\log_{10}$ IU/mL below the BL HDV RNA value at 2 consecutive visits; or
    - Two consecutive HDV RNA values ≥lower limit of quantification (LLOQ) if HDV RNA was previously <LLOQ at ≥2 consecutive time points
  - Virologic blip at EOT (EOT Blip): met VB criteria for only 1 visit at EOT
  - Persistent viraemia (PV): HDV RNA >100 IU/mL through EOT
- Off-treatment RAP
  - Viral relapse: undetectable HDV RNA at EOT and detectable HDV RNA at follow-up week 48 (FU48)
  - Viral rebound: detectable HDV RNA at EOT and ≥2 $\log_{10}$ IU/mL increase in HDV RNA from EOT at FU48

[a]Values needed to meet criteria at 2 consecutive visits to be confirmed. HDV RNA levels determined using RoboGene® HDV RNA Quantification Kit 2.0 (LLOQ 50 IU/mL, lower limit of detection 6 IU/mL).

Resistance Testing Methods



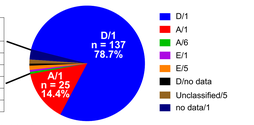
BLV, bulevirtide; FU48, follow-up at week 48; HBV, hepatitis B virus; HDAg, hepatitis delta antigen; HDV, hepatitis delta virus; NTCP, sodium taurocholate cotransporting polypeptide; RAP, resistance analysis population.

- HBV and HDV genotype: amplification of the HBV or HDV genome followed by sequencing analysis to determine genotype
- NTCP sequencing: participant whole blood was processed for whole-exome sequencing followed by analysis of single-nucleotide polymorphisms/small insertions and deletions and variants in the coding region of the *NTCP* gene[5]
- HBV preS1 deep sequencing: total HBV nucleic acids were extracted from plasma followed by complementary DNA (cDNA) synthesis; polymerase chain reaction was conducted using both DNA and cDNA to increase assay sensitivity[5]
- HDV hepatitis delta antigen (HDAg) deep sequencing: HDV RNA was extracted from plasma followed by cDNA synthesis and HDV full-genome amplification with 2 overlapping fragments[5]
- Phenotyping: primary human hepatocytes were pretreated with BLV and then infected with plasma; after 5 days, immunofluorescence staining was performed to determine cells that were positive for HDAg, and half-maximal effective concentration (EC_{50}) was obtained[5]

Results

HBV/HDV Genotypes of Participants in MYR204

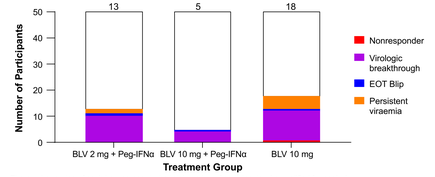
HBV/HDV GT	Participant Number	%
A/6	1	0.6%
E/1	1	0.6%
E/5	2	1.1%
D/no data	1	0.6%
Unclassified/5	2	1.1%
No data/1	5	2.9%



GT, genotype; HBV, hepatitis B virus; HDV, hepatitis delta virus.

- The majority of participants had HBV/HDV genotypes D/1 followed by A/1

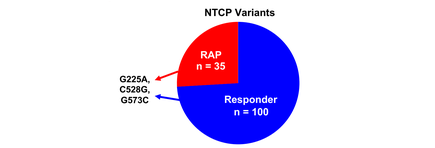
Participants Who Qualified for Resistance Analysis in Each Treatment Group at EOT



The number on top of each bar represents the total number of participants who qualified for analysis.
BLV, bulevirtide; EOT, end of treatment; EOT Blip, virologic blip at EOT; Peg-IFNα, pegylated interferon alfa-2a.

- At EOT, a total of 36 of 150 (24%) participants qualified for resistance testing, with the majority of participants (25/36, 69%) experiencing VB; only 1 NR was identified

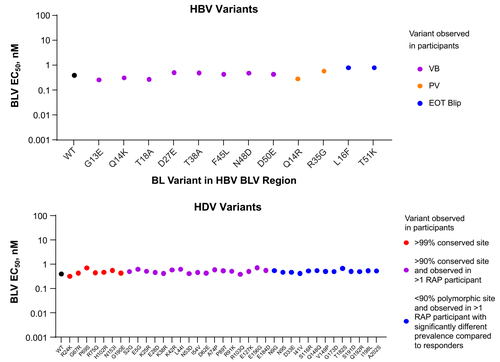
NTCP Variants Observed at BL From On-Treatment RAP Participants



Responder: >2 $\log_{10}$ decline in HDV RNA or undetectable HDV RNA at EOT and excluding participants experiencing VB, EOT Blip, or HDV RNA >100 IU/mL at EOT.
BL, baseline; EOT, end of treatment; EOT Blip, virologic blip at EOT; HDV, hepatitis delta virus; NTCP, sodium taurocholate co-transporting polypeptide; RAP, resistance analysis population; VB, virological breakthrough.

- The BL NTCP sequence was obtained from 35 of 36 on-treatment RAP participants, with 3 synonymous nucleotide variants detected, none of which were at the NTCP binding region; the G225A variant was also observed in responders with similar prevalence; G528C and G573C variants were rare and were each detected in a single participant in this study

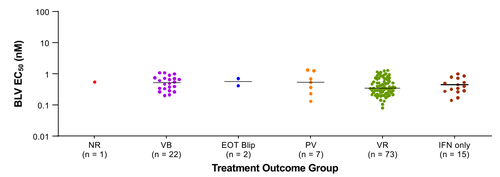
HBV and HDV Variants Observed at BL From On-Treatment RAP Participants



Responder: >2 $\log_{10}$ decline in HDV RNA or undetectable HDV RNA at EOT and excluding participants experiencing VB, EOT Blip, or HDV RNA >100 IU/mL at EOT.
BL, baseline; BLV, bulevirtide; EC_{50}, half-maximal effective concentration; EOT, end of treatment; EOT Blip, virologic blip at EOT; HBV, hepatitis B virus; HDAg, hepatitis delta antigen; HDV, hepatitis delta virus; PV, persistent viraemia; RAP, resistance analysis population; VB, virological breakthrough; WT, wild-type.

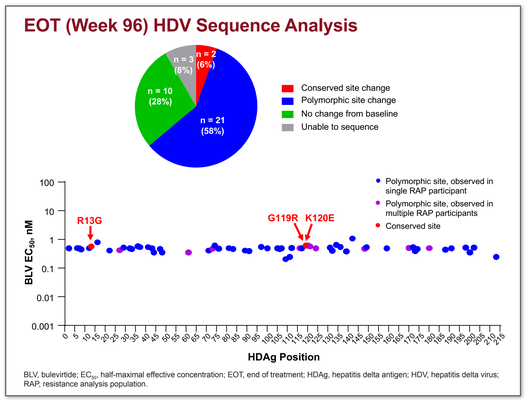
- All HBV variants remained susceptible to BLV in vitro; Q14R was also observed in 3 responders
- All HDV variants remained susceptible to BLV in vitro; 29 of 37 (78%) variants were also observed in responders

No Difference in BLV Sensitivity at BL Across Different Treatment Outcome Groups



Circles represent individual data; *horizontal lines* represent median. VR was defined as >2 $\log_{10}$ decline in HDV RNA or undetectable HDV RNA at EOT and excluding participants experiencing VB, EOT Blip, or HDV RNA >100 IU/mL at EOT.
BL, baseline; BLV, bulevirtide; EC_{50}, half-maximal effective concentration; EOT, end of treatment; EOT Blip, virologic blip at EOT; HDV, hepatitis delta virus; IFN, interferon; NR, nonresponder; PV, persistent viraemia; VB, virologic breakthrough; VR, virologic responder.

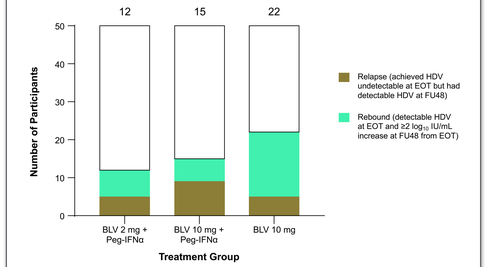
EOT (Week 96) HDV Sequence Analysis



BLV, bulevirtide; EC_{50}, half-maximal effective concentration; EOT, end of treatment; HDAg, hepatitis delta antigen; HDV, hepatitis delta virus; RAP, resistance analysis population.

- Change from BL in the HDV sequence at EOT
  - 33 of 36 (92%) participants had both BL and week 96 HDAg sequence data available
  - Conserved site changes were only observed in 2 participants
  - Virus carrying amino acid substitutions in HDAg at EOT remained sensitive to BLV in vitro
- Change from BL in the HBV sequence at EOT
  - The HBV PreS1 sequence was not obtained from 34 of 36 RAP participants at EOT due to extremely low HBV DNA levels
  - 2 participants had both BL and week 96 HBV BLV region sequence data available, and both had no sequence change from BL

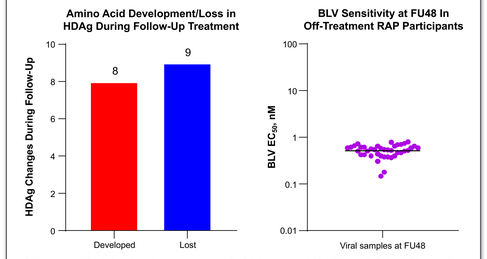
Participants With Viral Relapse or Rebound in Follow-Up Period in Each Treatment Group



The number on top of each bar represents the total number of participants who qualified for analysis.
BLV, bulevirtide; EOT, end of treatment; FU48, follow-up at week 48; HDV, hepatitis delta virus; Peg-IFNα, pegylated interferon alfa-2a.

- At FU48, a total of 49 of 150 (33%) participants experienced viral relapse or rebound, with the highest number of rebounds observed in the BLV 10 mg monotherapy group
- More participants had viral relapse in the BLV 10 mg + Peg-IFNα group (more participants in this group achieved undetectable HDV RNA at EOT, hence having the potential for relapse)

HDV/HBV Sequence Analysis in Follow-Up Period



BLV, bulevirtide; EC_{50}, half-maximal effective concentration; FU48, follow-up at week 48; HBV, hepatitis B virus; HDAg, hepatitis delta antigen; HDV, hepatitis delta virus; RAP, resistance analysis population.

- HDV
  - 12 participants had both on-treatment and follow-up treatment sequences available
  - The numbers of amino acid changes over follow-up were almost the same between development (n = 8) and loss (n = 9), suggesting the substitutions were driven by natural viral variation, consistent with the high diversity of natural sequence variation in HDV
  - None of the lost/developed amino acid substitutions occurred in more than 1 participant, and viruses with the amino acid changes remained sensitive to BLV in vitro
- HBV
  - The HBV PreS1 sequence at FU48 was only obtained from 3 of 49 off-treatment RAP participants without amino acid development/ loss observed