Intrahepatic sodium taurocholate co-transporting polypeptide (NTCP) gene transcript and membrane-localized protein expression changes in chronic hepatitis D patients following 48 weeks of treatment with bulevirtide

SAT-373

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Conclusions

- Results suggest that BLV treatment does not alter the sodium taurocholate co-transporting polypeptide (NTCP) mRNA expression but may increase NTCP protein levels in the plasma membrane of hepatocytes.
- Baseline (BL) NTCP and changes in NTCP protein levels are not correlated with HDV viral load or bile acid (BA) and alanine transaminase (ALT) changes over 48 weeks (W48) of treatment.

Plain Language Summary

- BLV is an HDV inhibitor targeting the NTCP protein on liver cells.
- Patients with chronic HDV (CHD) infection enrolled in the MYR301 clinical trial were dosed with BLV 2 or 10 mg or were untreated (delayed treatment, Arm A).
- NTCP mRNA and protein levels were evaluated in BL and W48 liver biopsy samples.
- While BLV did not change NTCP mRNA expression, there was an on-treatment increase in NTCP protein levels compared to BL. These changes were not associated with virologic or biochemical readouts.

Introduction

- Bulevirtide (BLV) is a 47 amino acid N-terminally myristoylated, HBV large envelope protein-derived, synthesized lipopeptide indicated for treatment of CHD infection.
- BLV binds specifically to NTCP, a BA transporter and entry receptor for HBV and HDV, and acts as a potent, highly selective entry inhibitor of HDV into hepatocytes.
- In the ongoing MYR301 phase 3 trial in participants with CHD, the primary endpoint is a combined response at W48 of normalization of ALT levels and undetectable HDV RNA or a reduction of ≥2 $\log_{10}$ IU/mL from BL, achieved in 45%, 48%, and 2% of participants in the 2 mg and 10 mg BLV and delayed treatment arms, respectively[1].
  - The secondary endpoint, to achieve undetectable HDV RNA levels at week 48 in BLV-treated arms, was achieved in 12% and 20% of participants in the 2 mg and 10 mg BLV arms, respectively[1].
  - In addition, ALT levels were normalized in 51%, 56%, and 12% of patients in the 2 mg and 10 mg BLV and delayed treatment arms, respectively[1].
  - Given the mechanism of action of BLV and NTCP's biological role in transporting BAs, asymptomatic dose-dependent plasma BA elevations were observed[1].
- Here, we investigated whether treatment with BLV for W48 affects NTCP gene and protein expression. Further, we investigated if the NTCP mRNA expression and protein levels at BL and following W48 of BLV treatment affect the virologic and biochemical clinical outcomes.

References: **1.** H. Wedemeyer, S., et al. *NEJM*. 2023; 389(1):22-32. **2**. Aggarwal, A., et al. *JHep*. 2023; 5(4):100664.

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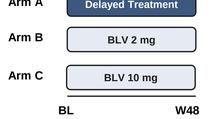
Methods

- Participants from the MYR301 Phase 3 study were randomized in a 1:1:1 ratio to receive 2 or 10 mg/day of BLV or no anti-HDV treatment (delayed treatment) for W48 (ClinicalTrials.gov Identifier: NCT03852719).
- Total bile acid, ALT, and HDV RNA were assessed at all time points including BL and W48.
- Virologic responder (VR): undetectable HDV RNA (<LLOD) or a decline in HDV RNA from BL of ≥2 $\log_{10}$ IU/mL at W48.
- ALT normalization status: those with ALT≤ULN at W48 (yes) and those that had ALT>ULN (no); ULN: ≤31 U/L for females and ≤41 U/L for males at Russian sites and as ≤34 U/L for females and ≤49 U/L for males at all other sites.
- A core needle liver biopsy was collected on a subset of consenting study participants at the screening visit (BL) and at study visit week 48.
  - For RNAseq,100bp sequencing reads were aligned to the human genome (GRCh38) and annotation GENCODE v38.
  - The remaining biopsy was formalin-fixed and paraffin embedded following standard procedures for a multiplex immunofluorescence assay including NTCP, sodium–potassium ATPase for cell membrane location and DAPI nuclear stain.
  - The image analysis[2], limited to hepatocytes, measured the median intensity of the NTCP membrane signal.
- A two-sided Wilcoxon signed rank test was used to test if fold-changes are significantly different than 1 and a non-parametric test was used for all comparisons.

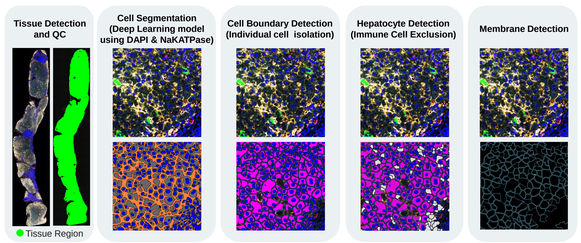
Liver biopsies from the MYR301 clinical study were used to evaluate NTCP gene and protein expression



- MYR301 is a multicenter, open-label, randomized phase 3 clinical study to assess efficacy and safety of bulevirtide in patients with chronic hepatitis delta.
- A portion of the biopsy was used for gene expression analysis by RNASeq (n=48[a]) and the remaining tissues were fixed and processed for multiple immunofluorescence staining (n=162[b]).

[a]A subset of biopsy samples, selected based on sample availability and RNA quality, were processed for RNASeq analysis.
[b]All available paired biopsies from consenting participants were processed for mIF staining.
BL, baseline; BLV, bulevirtide; mIF, multiplex immunofluorescence; W48, week 48 of treatment.

Image Analysis Workflow



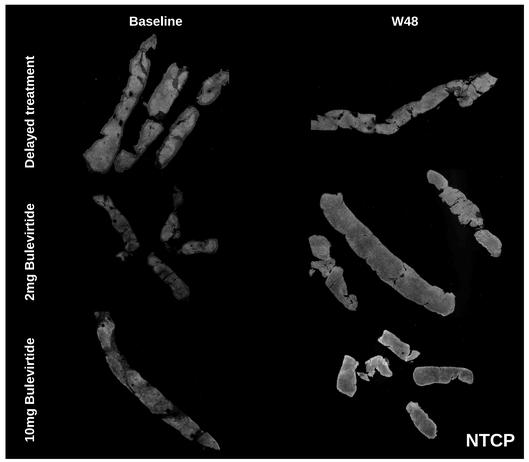
Preprocessing: Appropriate tissue regions were identified within the whole slide image and QC performed for artifact exclusion.

Image Analysis: Cell segmentation was performed using U-Net deep learning convolutional neural network mode[2], fine-tuned by using DAPI and NaKATPase.

Data extraction: Cell membrane area and the median intensity of NTCP protein on the cell membrane of the individual hepatocytes segmented was computed.

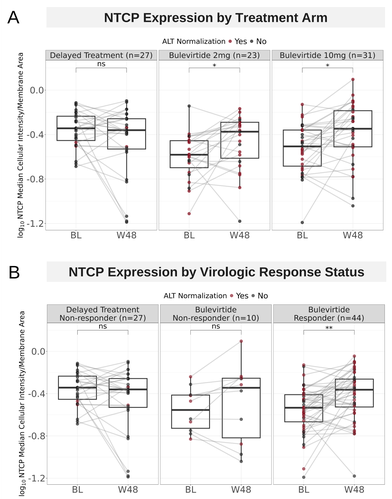
Results

Differential expression of NTCP protein between BL and W48 core liver biopsies



- Representative fluorescent- labelled images of longitudinal liver biopsies from participants treated with either BLV 2 or 10 mg or delayed treatment, stained using an anti-human NTCP antibody (white).

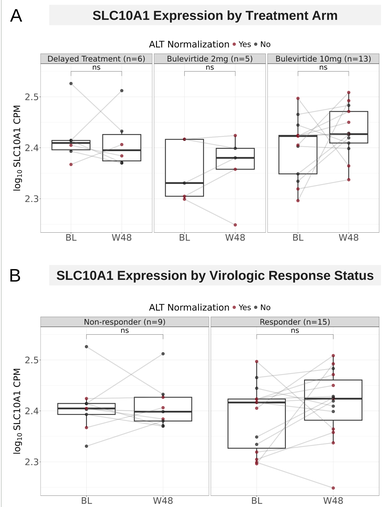
Membrane-localized NTCP protein expression is elevated in participants treated with BLV at W48 compared to BL



- BLV 2 mg and 10 mg treatment arms show elevated median intensity of intrahepatic NTCP protein at W48 compared to BL while no significant changes were observed in the delayed treatment arm (**A**).
- Stratifying by virologic response, the W48 biopsies showed significantly higher median NTCP intensity compared to BL, in virologic responder group only (**B**).
- Interpretation of results from the non-responder group is limited by the low sample size.
- Notably, NTCP expression is heterogenous across treatment arms and there is no difference based on ALT normalization status.

Data is presented as the $\log_{10}$ NTCP median cellular intensity normalized to the membrane area of hepatocytes compared between BL and W48 for A) treatment arms and B) virologic response status of pooled BLV-treated arms and delayed treatment.
BL, baseline; W48, week 48; n.s., non-significant (p>0.05); *p<0.05; **p<0.01

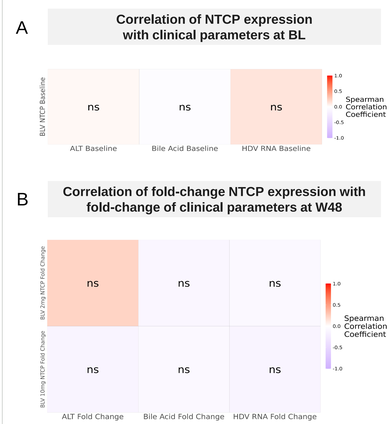
Transcriptomics did not reveal any differences in NTCP gene expression between treatment arms or virologic response groups



- Intrahepatic gene expression analysis of the *SLC10A1* gene encoding NTCP by RNA-seq does not show differences by treatment arm (**A**) or virologic response at W48 compared to BL (**B**).
- *SLC10A1* gene expression is heterogenous across treatment arms and there is no observable difference based on ALT normalization status.
- The low sample size across treatment arms and virological response group may limit the interpretation of the data.

Data is presented as A) The $\log_{10}$ of counts per million (CPM) of *SLC10A1* for each treatment arm and B) the $\log_{10}$ CPM of *SLC10A1* by virologic responder status.
BL, baseline; NS, non-significant; W48, week 48

NTCP membrane protein expression is not associated with clinical parameters (HDV RNA, bile acids and ALT)



- The median cellular intensity of NTCP protein in BLV-treated arms at BL did not correlate with the clinical parameters (HDV RNA, bile acids, and ALT) at BL (**A**).
- The fold change in median cellular intensity of NTCP in BLV-treated arms did not correlate with the fold-change of clinical parameters at W48 (**B**).

Data is presented as A) the Spearman correlation between the baseline median cellular intensity of NTCP protein and baseline clinical parameters (BA, ALT, and HDV RNA) and B) the Spearman correlation between the fold change of the median cellular intensity of NTCP protein and the fold change clinical parameters at W48. BA, bile acids, BL, baseline; n.s., non-significant (p>0.05); W48, week 48