Development of a Direct RNA Interference Therapy for Hepatitis Delta Virus Infection

Xin Ye, Nicholas M. Snead, Trisha R. Barnard, Emily P. Thi, Ian MacLachlan, Amy C.H. Lee
Arbutus Biopharma, Burnaby, BC, Canada

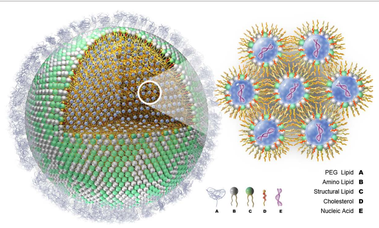
ABSTRACT

HDV infection causes fulminant hepatitis and aggravates the clinical course of liver damage and failure in patients with chronic Hepatitis B infection (CHB). The lack of approved HDV therapies urges the development of effective and specific treatment against HDV infection.

RNA interference is a gene silencing strategy triggered by introduction of small interfering RNA (siRNA), which allows direct and specific destruction of targeted RNAs. Arbutus Biopharma's clinically-validated lipid nanoparticle (LNP) platform enables safe and effective delivery of siRNAs into the body.

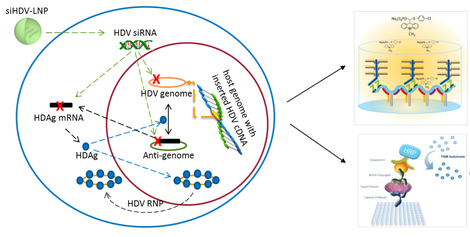
HDV targeting siRNAs delivered through the LNP platform achieve rapid and durable reduction of all HDV RNAs and antigen proteins, providing a novel opportunity to alleviate HDV pathogenesis.

INTRODUCTION



Lipid Nanoparticles (LNP) are comprised of neutral, cationic and PEG-lipids that can be formulated into a variety of compositions to confer desired pharmacokinetic and pharmacodynamic properties. LNPs protect the nucleic acid payload against nuclease degradation in the bloodstream, and enable effective delivery to the target hepatocytes. To date, 9 LNP drug products have entered clinical trials with over 250 patients treated, some have received repeated doses for over 1 year duration. LNP enabled RNAi drugs have strong clinical validation.

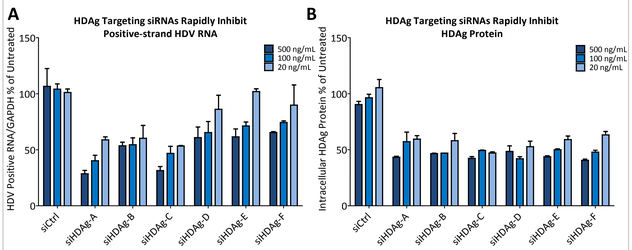
MATERIALS & METHODS



HDV stable cell line Huh7-D12 cells (1) were treated with a single dose of HDV siRNA-LNP. HDV RNAs were measured by QuantiGene branched DNA (bDNA) assay and HDV antigen proteins were quantified by ELISA.

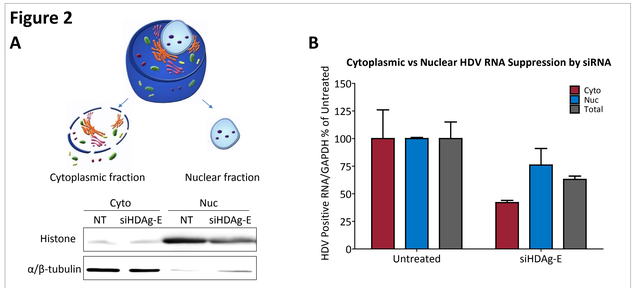
RESULTS

Figure 1



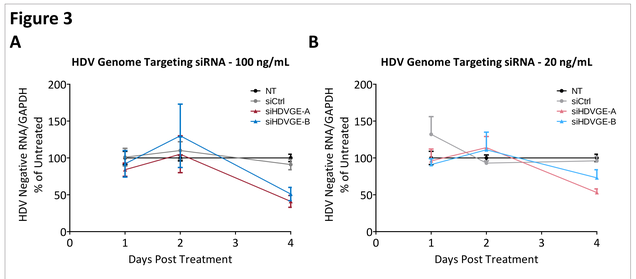
HDAg Targeting siRNAs Rapidly Suppress HDV RNAs and Proteins. Huh7-D12 cells were treated with a single dose of siRNA-LNP targeting different sites in HDAg mRNA region. Immediate and dose-dependent reduction of HDV positive-strand RNAs (**A**) and HDAg protein (**B**) was observed 24 h post treatment. (20 ng/mL=1.5 nM)

Figure 2



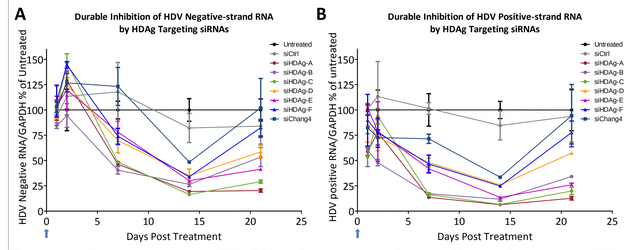
Cytoplasmic HDV RNAs Are More Susceptible to siRNA-LNP Targeting. Huh7-D12 cells were treated with 20 ng/mL of siHDAg-E-LNP and harvested at 24 h post treatment. Cytoplasmic and nuclear fractions of the cells were separated and subjected to Western Blot analysis of indicated protein markers (**A**) and bDNA quantification of HDV positive-strand RNAs (**B**). NT: Untreated.

Figure 3



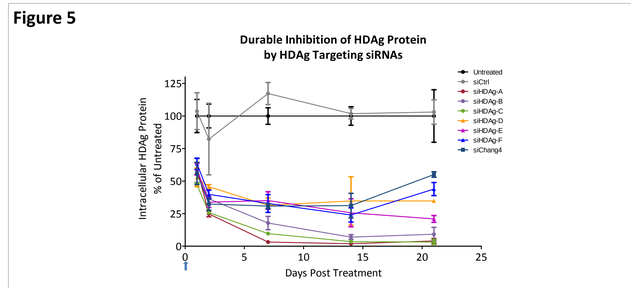
HDV Genome Targeting siRNAs Show No Effect Until 4 Days Post-Treatment. Huh7-D12 cells were treated with a single dose of siRNA-LNP targeting different sites in the negative-strand HDV genomic RNA. No reduction of HDV genomic RNA was observed until Day 4 post LNP treatment, distinct from the HDAg mRNA targeting siRNAs. This effect was dose-responsive.

Figure 4



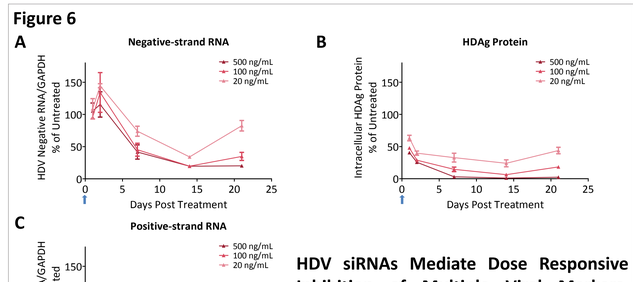
Durable Reduction of HDV RNA Observed with a Single Dose of HDAg Targeting siRNA. Huh7-D12 cells were treated with 20 ng/mL single dose of siRNA-LNP targeting different sites in HDAg mRNA region. Inhibition of HDV negative-strand (**A**) and positive-strand (**B**) RNA levels was observed throughout the 21-day duration of the study.

Figure 5



Durable Reduction of HDAg Protein with a Single Dose of HDAg Targeting siRNAs. Huh7-D12 cells were treated with 20 ng/mL single dose of siRNA-LNP targeting different sites in HDAg mRNA region. Inhibition on HDAg protein levels was observed throughout the 21-day duration of the study. These siRNAs are more potent than siChang4, a benchmark siRNA published in (2).

Figure 6



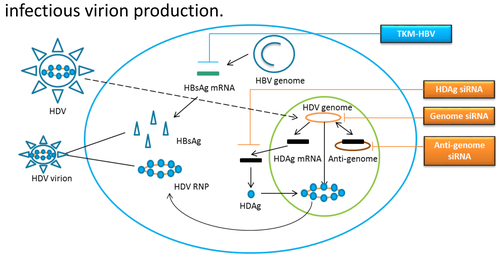
HDV siRNAs Mediate Dose Responsive Inhibition of Multiple Viral Markers. Huh7-D12 cells were treated with various doses of siHDAg-F-LNP. Dose-dependent inhibition on HDV RNAs (**A, B**) and protein (**C**) was observed.

CONCLUSIONS

- The LNP platform successfully delivers siRNA in vitro.
- HDAg mRNA targeting siRNAs rapidly inhibit HDV RNAs and proteins with long-lasting duration of activity.
- Effect of HDV genome targeting siRNAs is delayed compared to HDAg targeting siRNAs.
- Cytoplasmic HDV RNAs are more susceptible to siRNA-LNP targeting than nuclear HDV RNAs.
- HDV targeting siRNAs delivered through the in vivo validated LNP platform effectively suppress HDV transcripts and antigens, providing a promising therapeutic strategy for HDV infection.

FUTURE DIRECTIONS

- Validating the efficacy of HDV siRNA in a true-infection model with a complete HDV life cycle.
- Testing the effect of HBV siRNA (TKM-HBV) on HDV viral replication. HBV siRNA inhibits HBsAg, an essential component required for HDV infectious virion production.



ACKNOWLEDGEMENTS

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CONTACT INFORMATION

Xin Ye : xye@arbutusbio.com, telephone: 604.419.3280
Amy Lee: alee@arbutusbio.com
Arbutus Biopharma Corp.
100 - 8900 Glenlyon Parkway
Burnaby, British Columbia, Canada V5J 5J8