092

A Bicyclic Synthetic Ligand for ASGR Is a Safe Alternative to GalNAc for Effective Hepatocyte-Specific Delivery in Mouse Models

Murali V. P. Nadella, Vincent Aduda, Luciano Apponi, John W. Davis II, Naoki Iwamoto, Genliang Lu, Sethu Menon, Lynnelle Pittet, Stephany Standley, Hailin Yang, Yuan Yin, Jason Zhang, Zhong Zhong, Chandra Vargeese

Wave Life Sciences, Cambridge, MA, USA

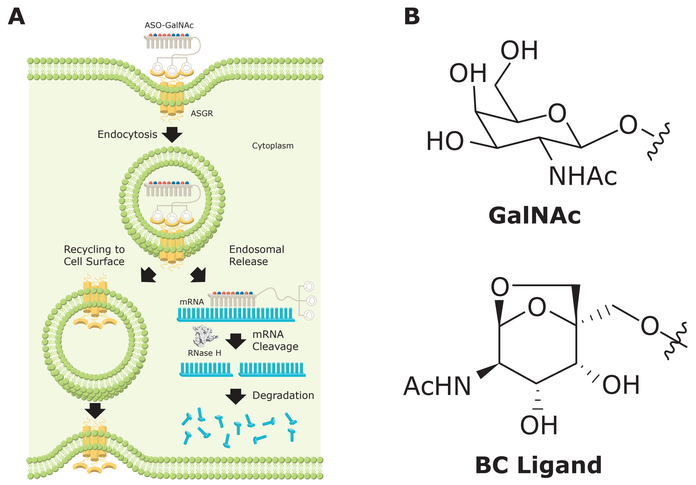
Summary

- Bicyclic compound-antisense oligonucleotide (BC-ASO) and N-acetylgalactosamine-ASO (GalNAc-ASO) conjugates of the same valency (mono-, bis-, or triantennary) comparably decreased serum apolipoprotein C-III (ApoC-III) levels.
- Bivalent and trivalent conjugates yielded more potent and durable knockdown than monovalent conjugates but were comparable to each other.
- In subacute toxicology studies in mice, trivalent BC-ASO (BC-3-ASO) yielded clinical chemistry, histologic, and hematologic profiles comparable to trivalent GalNAc-ASO (GalNAc-3-ASO), demonstrating that the safety profiles for BC ligands are comparable to those for GalNAc in mice.
- BC is a safe ligand, equivalent to GalNAc, and mediates effective delivery of a stereopure ASO to hepatocytes in mouse models.

ASGR Ligands for Hepatic Drug Delivery

- Asialoglycoprotein receptor (ASGR) is abundantly expressed in the liver, internalizes ligand-receptor complexes into hepatocytes, releases ligand and associated ASO in cells, and recycles to the cell surface where it is able to repeat the cycle (**Figure 1A**).
- Conjugation to GalNAc has improved the potency of ASO drugs ~30-fold.[1]
- A synthetic BC demonstrated superior ligand efficiency and binding affinity for ASGR compared with GalNAc. In cultured cells and mouse models, BC mediated productive hepatocyte uptake of various payloads.[2]
- BC is an alternative ligand for delivery of ASOs to hepatocytes (**Figure 1B**).

Figure 1. (A) The ASGR receptor mediates effective delivery of ASOs to hepatocytes; (B) BC is an alternative ligand to GalNAc for delivery of ASOs to hepatocytes



ASGR=asialoglycoprotein receptor; ASO=antisense oligonucleotide; BC=bicyclic compound; GalNAc=N-acetylgalactosamine; mRNA=messenger RNA.

Apolipoprotein C-III

- ApoC-III, encoded by *APOC3*, is produced by hepatocytes and plays a key role in the regulation of plasma triglycerides.
- ApoC-III is a component of lipoprotein particles in serum, and elevated plasma levels have been associated with hypertriglyceridemia and cardiovascular disease.[3]
- *APOC3* messenger RNA (mRNA) is expressed by hepatocytes.
- We developed a stereopure ASO that promotes RNase H-mediated degradation of *APOC3* mRNA and leads to more potent and durable knockdown of ApoC-III serum protein levels in transgenic mice expressing human *APOC3* than stereorandom ASOs of the same sequence.
- We investigated whether BC is a safe alternative to GalNAc for achieving effective hepatocyte delivery of our stereopure ASO.

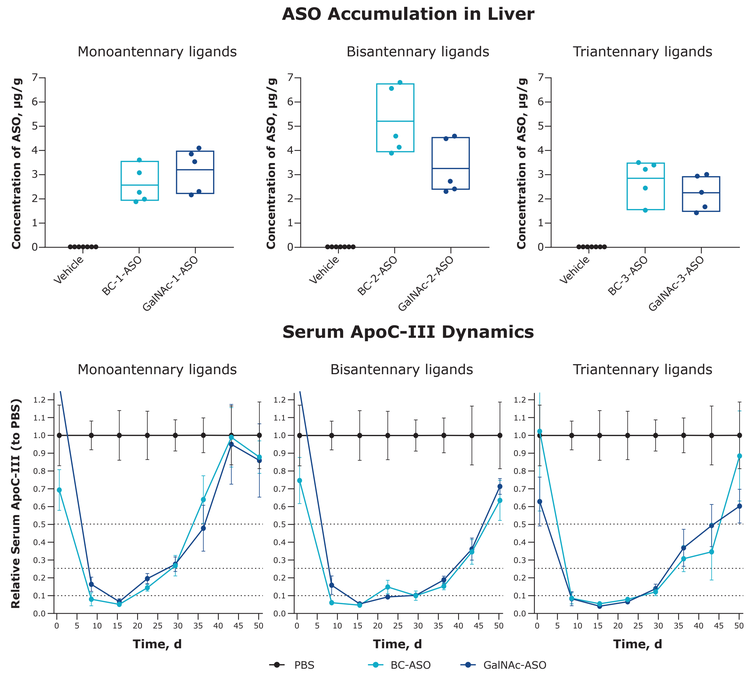
Methods

- Using Wave's stereopure ASOs targeting *APOC3*, we compared safety and efficacy profiles for BC-ASO and GalNAc-ASO conjugates.
- We investigated the pharmacokinetic-pharmacodynamic relationship for multiple BC-ASO conjugates (monovalent [-1], bivalent [-2], or trivalent [-3] ligand) in comparison with comparable GalNAc-ASO conjugates after subcutaneous administration in mice.
  - Transgenic mice expressing *APOC3* were treated with phosphate-buffered saline (PBS) or a single 3 mg/kg subcutaneous dose of an ASO-ligand conjugate (n=10 per group).
  - Livers were harvested 30 minutes (data not shown) or 8 days postdose.
- We also investigated the safety profile of BC-3-ASO in subacute toxicology studies in mice by comparing it with GalNAc-3-ASO.
  - Male CD1 mice were subcutaneously dosed with vehicle (PBS) or test article (BC-3-ASO or GalNAc-3-ASO) at 5, 25, or 50 mg/kg twice weekly for 2 weeks (n=5 per group).
  - Tissues were collected 3 days after the final dose.
  - Liver tissues were embedded, sectioned, and stained with hematoxylin and eosin for histologic analysis.

Results

- Stereopure ASOs rapidly accumulated in the liver and persisted for ≥8 days.
- BC-ASO and GalNAc-ASO conjugates of comparable valency (mono-, bis-, or triantennary) yielded comparable ASO accumulation at day 8 (**Figure 2**).
  - BC-ASO and GalNAc-ASO conjugates of the same valency (mono-, bis-, or triantennary) yielded comparable decreases in ApoC-III serum protein levels that persisted for the same period of time. No observed differences between conjugates with the same valency were statistically significant.
  - Bivalent and trivalent conjugates were more potent and yielded more durable effects than monovalent conjugates. No statistically significant differences were detected between bivalent and trivalent conjugates.

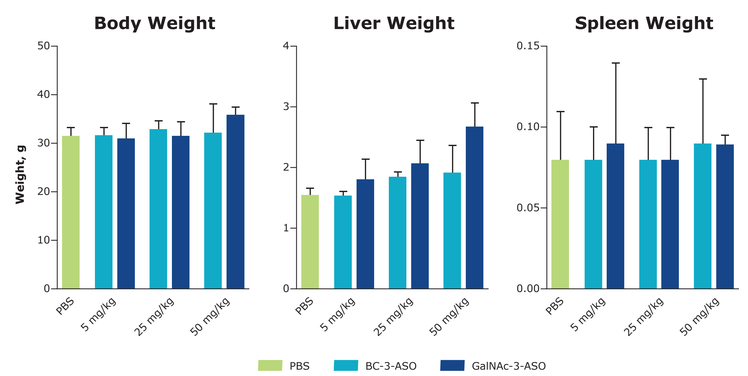
Figure 2. Mono-, bis-, and triantennary BC-ASO conjugates are comparable to GalNAc conjugates



ApoC-III=apolipoprotein C-III; ASO=antisense oligonucleotide; BC=bicyclic compound; GalNAc=N-acetylgalactosamine; PBS=phosphate-buffered saline.
Dots in the top panel represent individual mice.

- Body, liver, and spleen weights were not substantially affected by GalNAc-3-ASO or BC-3-ASO at any dose tested (**Figure 3**).

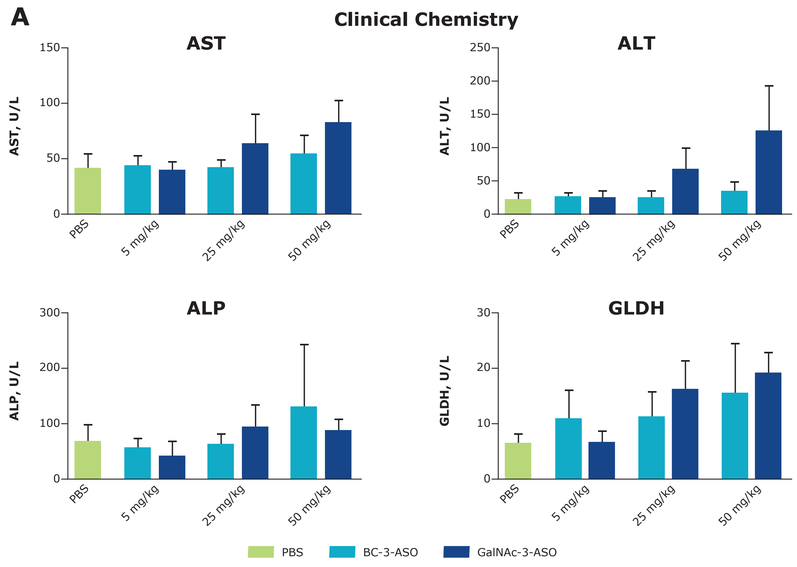
Figure 3. Total body and organ weights are not affected by treatment

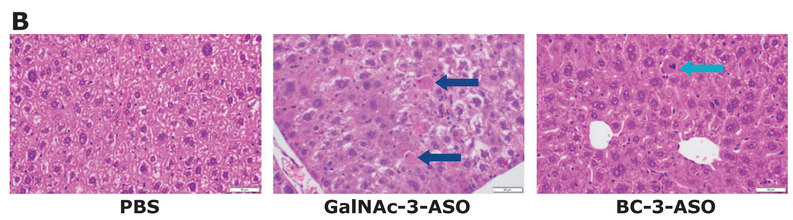


ASO=antisense oligonucleotide; BC=bicyclic compound; GalNAc=N-acetylgalactosamine; PBS=phosphate-buffered saline.

- Weights of other organs (e.g., kidney, thymus, and testes) were also unaffected by treatment (data not shown).
- We observed minimal increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and glutamate dehydrogenase (GLDH) for all test articles (**Figure 4A**). Changes were not statistically significant.
  - There were no substantial changes in alkaline phosphatase (ALP; **Figure 4A**) or total bilirubin (data not shown).
- In liver sections, we observed minimal but dose-dependent increased incidence of single-cell necrosis for GalNAc-3-ASO but not BC-3-ASO (**Figure 4B**).
  - We observed a dose-dependent increase in the incidence and/or severity of hepatocellular hypertrophy with GalNAc-3-ASO, whereas with BC-3-ASO, minimal hypertrophy was noted only at the highest dose (**Figure 4B**).
  - Minimal Kupffer cell hypertrophy was noted with both ligand-ASO conjugates and only at the highest dose.

Figure 4. (A) Conjugates led to minimal increases in liver enzymes for BC ligand and GalNAc; (B) liver histologic findings

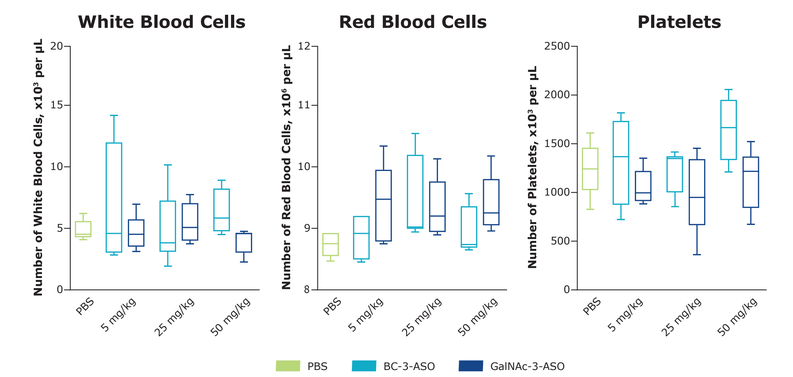




ALT=alanine aminotransferase; AST=aspartate aminotransferase; ALP=alkaline phosphatase; ASO=antisense oligonucleotide; BC=bicyclic compound; GalNAc=N-acetylgalactosamine; GLDH=glutamate dehydrogenase; PBS=phosphate-buffered saline.
Navy arrows denote necrotic cells in GalNAc-3-ASO–treated liver (50 mg/kg dose); aqua arrow denotes a mitotic cell in BC-3-ASO–treated liver (50 mg/kg dose).

- There were no statistically significant changes in white blood cell, red blood cell, or platelet counts for any test article (**Figure 5**).

Figure 5. Hematologic findings were not significant



ASO=antisense oligonucleotide; BC=bicyclic compound; GalNAc=N-acetylgalactosamine; PBS=phosphate-buffered saline.

References: 1. Viney NJ, et al. *Lancet*. 2016;388:2239-2253. 2. Sanhueza CA, et al. *J Am Chem Soc*. 2017;139:3528-3536. 3. Chan DC, et al. *Int J Clin Pract*. 2008;62:799-809. **Acknowledgments:** The authors thank collaborators at Pfizer. Editorial support was provided by ICON plc (North Wales, PA) and funded by Wave Life Sciences Ltd. **Disclosures:** All authors are employees of Wave Life Sciences Ltd.

Presented at Oligonucleotide Therapeutics Society; September 30–October 3, 2018; Seattle, WA

Supported by Wave Life Sciences Ltd, Cambridge, MA, USA.