TfR1-Targeted Bicyclic Peptide-Oligonucleotide Conjugates for Improved Potency and Enhanced Muscle Delivery

Antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs) are uniquely suited to address single-gene diseases affecting skeletal and cardiac muscles. While distributed broadly, they are more likely to accumulate in the liver and kidneys than muscles, thus limiting their treatment applicability. Additionally, the anticipated dose levels needed for unconjugated ASOs to reach these muscles are very high. Ionis Pharmaceuticals, in partnership with Bicycle Therapeutics, aims to change this.

The companies recently published a study in *Nucleic Acids Research*, demonstrating a delivery method that requires a small dose and improves the potency of oligonucleotide therapeutics for skeletal and cardiac muscle. This work was awarded the OTS Late Discovery Featured Paper of the Year.

Reaching muscle cells: the challenge and the solution

To be effective in muscle, ASOs and siRNAs must travel from the injection site to their RNA target, passing through the bloodstream, crossing capillaries' endothelium, reaching the tissue interstitium, and finally entering muscle cells (1). In organs like the liver or kidney, the blood vessel walls have larger openings, making it easy for ASOs to slip through. However, in muscle and heart tissue, the capillaries are tightly sealed, creating a significant barrier to drug entry (1).

Transferrin receptor 1 (TfR1) is a highly recycling receptor expressed on the surface of muscle, cardiac muscle, and endothelial cells, where its primary role is iron uptake. Previous studies have shown that attaching an antibody fragment to TfR1 enhances the activity of siRNA and ASOs in mice, demonstrating the potential for delivering oligonucleotide drugs to skeletal muscle (2, 3). However, using antibodies (mAb) and their fragments (Fab) for this purpose presents several challenges: potential off-target effects like anemia and TfR1 depletion caused

by anti-TfR1 antibodies (4, 5); the complex structure of antibodies complicates manufacturing (6, 7); their large size requires administering larger volumes, which may reduce tolerability and increase the risk of anti-drug antibody production; and the necessity for intravenous delivery (1, 8).

Bicycle molecules are peptides that possess attractive drug-like properties, such as high affinity and selectivity for their target, high plasma stability, tunable pharmacokinetics, and a more straightforward manufacturing process (1). Unlike conventional small molecules, Bicycle molecules can be readily conjugated to other chemical payloads without losing their pharmacology, states Bicycle Therapeutics. The study uses these peptides as part of Ionis's broader Ligand-Conjugated Antisense (LICA) strategy, which attaches targeting ligands to ASOs to enhance uptake into specific tissues. The Bicycle approach represents the newest addition to this LICA platform.

Michele Carrer, Ph.D., one of the lead authors of the paper, explained at the 2025 Oligonucleotide Therapeutics Society Annual Meeting that attaching ASOs and siRNAs to a high-affinity bicyclic peptide targeting TfR1 significantly increases their potency in skeletal and cardiac muscles. By linking the two, it naturally guides the antisense molecule into the cell (1).

Bicycle peptide enhances ASO activity in skeletal and cardiac muscle

To demonstrate that the bicycle-based delivery method could work in humans, the researchers created a mouse model expressing the human TfR1 open reading frame. Before selecting BCY17901 as the lead ligand, the team systematically screened 12 different Bicycle peptides with varying TfR1 affinities. By comparing these ligands head-to-head, they identified BCY17901 as the optimal balance of potency, receptor affinity, and intracellular release (1).

They then conjugated these different bicycle molecules to two ASOs: one targeting the Dmpk mRNA and the other targeting the Malat1 RNA. The mice were given either a high dose of the unconjugated ASO, a much lower dose of the Bicycle-ASO, or a benchmark ligand for comparison. After dosing, the researchers measured the extent to which the target gene was reduced in the quadricep muscles and the heart (1).

The results found that, despite being a 10-fold lower dose, the Bicycle ligand (BCY17901) made the ASOs much more effective, achieving more gene knockdown than the high-dose plain ASO in both the quadriceps and heart. When the same Bicycle-ASO was used in normal mice — with mouse TfR1 — delivery was significantly reduced, demonstrating its selectivity to human TfR1 (1).

Bicycle peptide shows robust knockdown, improved potency in muscle cells

To compare the potency of the Bicycle conjugate with that of the unconjugated ASO or lipid-conjugated siRNA molecules, the researchers performed dose-response experiments in mice. In multiple muscle types, the team measured a robust increase in potency for both the Dmpk and Malat1 Bicycle-conjugated ASOs compared to the respective unconjugated ASOs. Additionally, the bicycle conjugates had much lower ED50 values, meaning they required smaller doses to achieve the same effect as the unconjugated ASO (1).

The authors note that the administration of BCY17901-conjugated Malat1 ASO in the mice did not result in target RNA knockdown in the central nervous system, suggesting it did not cross the blood-brain barrier (1).

The researchers also measured how the Bicycle-based conjugates affected the liver. A key nuance is that ASOs and siRNAs behave differently when conjugated to the Bicycle ligand. For ASOs, BCY17901–Malat1 was only slightly more active in the liver relative to the unconjugated ASO. By contrast, for siRNAs, BCY17901–HPRT produced a 5.9-fold decrease in potency in the liver compared to a lipid (palmitate)-conjugated siRNA benchmark. In a separate study, a comparison with a benchmark TfR1-binding antibody fragment (OKT9 Fab') highlighted this. The antibody fragment drove nearly 90% knockdown in the liver, while the Bicycle–siRNA produced only 43%, demonstrating the clear liver-sparing advantage of small Bicycle ligands over antibody-based systems (1).

Bicycle peptide shows good target knockdown in skeletal and heart muscles of NHPs

The study also demonstrated that Bicycle-conjugated ASOs and siRNAs could efficiently reduce their target RNAs in the skeletal and cardiac muscles of non-human primates (NHPs) (1).

"Of note, BCY17901 has ~ tenfold lower affinity for the cynomolgus monkey TfR1 compared to the human receptor," the authors state (1).

Despite this lower affinity, conjugation of Malat1 ASO and HPRT siRNA to BCY17901 resulted in 71% and 86% target RNA knockdown, respectively, in quadricep muscles. In the heart, these Bicycle conjugates caused 63% and 75% target reduction, respectively. As seen in mice, conjugation of HPRT siRNA to BCY17901 exhibited a partial liver-sparing effect, resulting in only 11% target knockdown in hepatic tissue (1).

Advantages of Bicycle-ASO conjugates

The Bicycle peptides are well-suited for conjugation to ASOs and RNAs compared to antibody ligands, Carrer explained at OTS 2025.

The Bicycle peptides are also stable and chemically flexible, offering improved tissue penetration, potential lower total drug doses, reduced liver uptake, and easier manufacturing, and are broadly compatible with ASOs and siRNAs (1).

"Their small size potentially enables a lower total dose of drug that needs to be administered to patients," Carrer said.

Compared to the unconjugated Dmpk ASO, the Bicycle conjugate was 4.9-fold more potent in the quadriceps muscle and 5.8-fold more potent in the gastrocnemius muscle. In the heart, the bicycle ASO achieved a 50% reduction in the target mRNA at a much lower dose than the unconjugated ASO, which was less effective (1).

Limitations and unresolved questions

The authors note that their work has limitations and unanswered questions. Given that TfR1 is expressed in both endothelial and muscle cells, the Bicycle LICA system potentially offers the opportunity to enhance the drug in two ways: helping it cross from the blood into the muscle, and helping muscle cells absorb the drug more easily. However, they haven't yet quantified the extent to which these two effects contribute to the drug's overall success (1).

"Furthering the understanding of when the ON [oligonucleotide] cargo dissociates from the Bicycle component of the LICA molecule in the different cell types (e.g. endothelial cells,

myofibers, and cardiomyocytes) and in different intracellular compartments will also be instrumental," the authors state (1).

Also noteworthy and a point of caution, according to the authors, is the difference between the human and mouse heart. In mice, some heart cells may contain the Dmpk gene but not TfR1 (1), "therefore, potentially reducing the efficacy of a TfR1 LICA approach in the mouse cardiac tissue" (1), making the treatment appear less effective in mice than it would hypothetically be in humans.

Future of Bicycle Technology

The study demonstrates that this TfR1-binding Bicycle platform unlocks potent, muscle-specific oligonucleotide delivery, and has the potential to achieve therapeutic-level knockdown at clinically feasible doses, and builds on Ionis's work to conjugate molecules to ASOs to deliver their drugs to more targets. Going forward, Carrer believes the bicycle conjugates will fit nicely into Ionis' pipeline, noting that their versatility allows them to work with a wide range of targets.

"The bicycle technology really now opens the door to potentially new medicine that could treat these devastating diseases that affect the skeletal muscle and heart," Carrer said.

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