A New Conjugated siRNA provides broad delivery in the CNS, Lung, and Eye

Ever since the groundbreaking discovery of RNA interference and its role as a gene-silencing mechanism in mammalian cells, an ongoing process of discovery has been underway to harness its potential in treating disease. A key mechanism that induces gene-silencing in the RNA pathway is small interfering RNA (siRNA). Although a few innovative siRNA medications have been approved to treat genetic disorders, intense development efforts are still underway to create safe, effective siRNA medications that can be delivered to a range of cell and tissue types.

However, there are difficulties involved in engineering siRNAs, as modifications will affect the distribution and accumulation of siRNAs in tissues, safety profile, and clearance (1), just to name a few.

Great progress has been made in developing therapeutic siRNAs for targets beyond the liver. For example, “Conjugate-mediated delivery has established itself as the most promising platform for safe and targeted small interfering RNA (siRNA) delivery. Lipophilic conjugates represent a major class of modifications that improve siRNA pharmacokinetics and enable efficacy in a broad range of tissues” (1).

Many promising solutions are in development to engineer effective conjugations that deliver oligonucleotides to targets beyond the liver, and siRNAs are being created that treat diseases of the brain and spinal cord.

RNAi therapeutics are both potent and durable and, as many different central nervous system diseases have been associated with dominant gain of function mutations, siRNA provides an opportunity to treat these diseases by silencing the pertinent genes (2).

With the rapid pace that our field is moving forward, it is no wonder that another possible solution has been engineered, this time a lipophilic conjugate that not only has the ability to distribute siRNA to the CNS, but also to the eye and lung.

In a Behind the Paper, Vasant Jadhav SVP and Head of the RNAi Platform at Alnylam explained that they wanted to "take advantage of the broad cell type specificity of lipophilic conjugates, combined with careful optimization of the lipophilicity to maximize efficiency of delivery without compromising safety." The team comprehensively and systematically evaluated lipophilic siRNA conjugates in an effort to realize this goal.

Finally, they reached a design that allows for broad distribution to tissues in the CNS, eye, or lung after local administration.

This is made possible by conjugating 2'-O-hexadecyl (C16) to siRNAs. To enhance intracellular activity without compromising the safety, potency, and broad bioavailability of siRNAs, the team at Alnylam carefully optimized the lipophilicity of chemically modified siRNAs (2).

They evaluated the effect of different chain lengths of lipophiles introduced to fully chemically modified, metabolically stable siRNAs and discovered that 2'-O-hexadecyl (C16), along with other design features, provided optimal activity in the brain and spinal cord of rats (2).

To test uptake and RNAi activity, siRNAs were designed for cell-type-specific targets in the CNS, and both cell specific uptake and robust knockdown were seen in rodent neurons, astrocytes, and microglia. These encouraging results indicate that C16-siRNAs are distributed widely and taken up productively by most of the therapeutically relevant CNS cell types (2).

A 6-month study in rats tested various doses and the duration of knockdown of *Sod1* mRNA in the spinal cord and regions of the brain. The effects were dose dependent, with more than 75 percent knockdown at the highest dose which was sustained for at least three months in most brain regions (2).

This was followed by a study in NHP central nervous system which also showed potent, durable knockdown, with a 70 percent reduction in the spinal cord and 80 percent in the brain three months after a single, 60 mg intrathecal dose. In addition, the conjugated siRNAs were well-tolerated (2).

A C16 conjugated siRNA (ALN-APP) was then tested in a mouse model of Alzheimer's disease, targeting the amyloid beta precursor protein (APP) gene transcript. It was discovered that

ALN-APP produced a potent and durable knockdown of APP in the CNS. This knockdown resulted in altering the physiological deficits, such as Aβ deposition and inflammation, and ameliorating behavioral deficits (2).

In contrast to intracerebroventricular administration, an invasive method that bypasses the blood-brain barrier by delivering the drug directly to the brain, ALN-APP is administered via intrathecal injection and is still effectively delivered throughout the brain. This, combined with long durability, is a clear advantage when treating people.

ALN-APP is now in clinical development to treat both early onset Alzheimer's disease and cerebral amyloid angiopathy (a disease that increases the risk of stroke and dementia). In fact, a phase 1 study is already underway to evaluate the safety, tolerability, pharmacodynamics, and pharmacokinetics in patients with early-onset Alzheimer's Disease.

Kevin Fitzgerald, Ph.D., EVP, Chief Scientific Officer shared "Our CNS delivery platform provides long durability, which is particularly advantageous in settings of intrathecal administration, where infrequent dosing is desirable. Leveraging this platform, we have initiated dosing in a Phase 1 study in patients with early-onset Alzheimer's disease and expect to report initial human data at or around year-end 2022."

Beyond the CNS

As the goal was to take advantage of the broad cell type specificity of lipophilic conjugates, the team did not focus solely on the CNS. C16-siRNA was also tested in the eye and lung.

The potency and durability of C16-siRNA were tested in NHP eyes. Dose dependent knockdown of *TTR* was observed after a single intravitreal dose with sustained, potent knockdown resulting from higher doses (2).

C16-siRNAs were also delivered to mouse lung tissue. When distributed via intranasal administration, C16-siRNA was distributed through the entire lung, with robust bronchiolar and alveolar uptake. A single 3 or 10 mg kg^{-1} dose administered intranasally provided greater reductions in *Sod1* mRNA than a single 30 mg kg^{-1} dose given through an IV (2).

As C16 conjugates provide "enhanced delivery and siRNA uptake into the alveolar and bronchiolar epithelium" (2) they are an excellent candidate for treating viral respiratory diseases.

It is exciting that local delivery of C16-siRNAs showed potential for silencing target genes in tissues beyond the liver with the benefit of infrequent dosing. As with any other breakthrough, creating therapeutic oligonucleotides requires diligent effort and dedication. Building on decades of foundational work, with further optimization and refinement C16 conjugated siRNAs may provide a way to treat a broad range of diseases, from glaucoma to Alzheimer's disease to viral respiratory diseases.

References:

1. Osborn MF, Khvorova A. Improving siRNA Delivery In Vivo Through Lipid Conjugation. Nucleic Acid Ther. 2018 Jun;28(3):128-136. doi: 10.1089/nat.2018.0725. Epub 2018 May 10. PMID: 29746209; PMCID: PMC5994667.
2. Brown, K.M., Nair, J.K., Janas, M.M. *et al.* Expanding RNAi therapeutics to extrahepatic tissues with lipophilic conjugates. *Nat Biotechnol* (2022).