# Clinical trial shows antisense oligonucleotide safely suppresses mutant ALS gene in pilot human study

December 23 2021

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Using a short, synthetic chain of chemically modified nucleotides engineered in the RNA Therapeutics Institute at UMass Chan Medical School, Robert H. Brown Jr., DPhil, MD, Jonathan Watts, Ph.D., and colleagues have shown the ability to suppress mutant forms of an ALS gene known as C9ORF72 in a single-patient pilot study. C9ORF72 is the most common cause of familial amyotrophic lateral sclerosis (ALS) and familial frontotemporal dementia (FTD). The results, published in *Nature Medicine*, have the potential to catalyze research into treatments for ALS, FTD and other neurodegenerative diseases.

The therapeutic, using an antisense oligonucleotide (ASO) injected into the spinal canal, led to a significant reduction of ALS-related neurotoxins known as dipeptide repeat proteins (DPRs) in the trial subject's spinal fluid. During the course of the trial, the subject's ALS functional score rating and other measures of impact were largely stable or slightly improved. The patient, who had been experiencing weakness in the legs and feet before treatment, had no neurological or medically adverse effects from the treatment.

"While other teams have documented that this gene can be suppressed in cells in culture, this is the first time this type of antisense oligonucleotide treatment for C9ORF72 ALS has been demonstrated in a person with ALS," said Dr. Brown, the Leo P. and Theresa M. LaChance Chair in Medical Research, and professor of neurology at UMass Chan Medical School and lead author of the *Nature Medicine* study. "The results are very encouraging. It means this is a viable approach to suppressing the mutant C9ORF72 protein that causes most cases of familial ALS. The

next step is to launch a multi-person clinical trial to see if this treatment can slow progression of the disease."

Antisense oligonucleotides are short, synthetic, single-stranded oligonucleotides that can alter RNA and reduce, restore or modify protein expression. The type of ASO used in this research prevents gene expression by binding to messenger RNA (mRNA) strands. Once this binding takes place, this hybrid sequence is targeted and naturally degraded by enzymes in the cell.

The appeal of developing an antisense oligonucleotide to treat ALS and other neurodegenerative diseases is its simplicity, according to Dr. Watts, associate professor of RNA therapeutics and co-lead-author of the *Nature Medicine* study. "ASOs are essentially anti-messenger RNA agents. Using the genetic sequence you want to target, you can design an antisense oligonucleotide sequence that binds to that mRNA so the mutant protein never gets made," explained Watts. "Once you've established how to deliver an ASO to a certain type of cell, theoretically it should be possible to repeat for other neurodegenerative diseases. All you would have to change is the nucleotide sequence."

By eliminating the disease-causing protein from the cell, scientists believe this strategy could potentially stop and even reverse disease progression. So far, four ASO-mediated therapies have received approval from the U.S. Food and Drug Administration. Three are for the treatment of Duchenne muscular dystrophy and one is for spinal muscular atrophy.

ALS is a progressive, neurodegenerative disorder that involves the loss of motor neurons that control voluntary muscles. Roughly 10 percent of ALS is familial—inherited from a person's parents—and caused by a genetic mutation in a person's DNA. The remaining 90 percent of cases are classified as sporadic and occur in cases with no family history of

disease. An estimated 6,000 people in the United States are diagnosed with ALS each year. It is not fully understood why motor neurons die in ALS, but this neurodegeneration is thought to involve a complex array of cellular and molecular processes.

Mutations in the C9ORF72 gene, the target of the *Nature Medicine* study, account for 40 percent of familial cases of ALS, as well as about 10 percent of non-familial cases. These mutations also cause about 25 percent of familial cases of FTD. This overlap is significant because it is among the first pathogenic mechanisms identified to link ALS and FTD. It also suggests that a similar therapeutic strategy may potentially treat both diseases.

ALS patients with the C9ORF72 mutation have an abnormally long repeating pattern of a six-letter string of nucleotides—GGGGCC—in their C9ORF72 genetic sequence. In a person without the mutation, there are typically fewer than 20–30 of these repeats. But in people with the mutation, the repeat can occur hundreds of times. This repeated sequence interferes with normal expression of the protein made by C9ORF72, and additionally produces the neurotoxins known as dipeptide repat proteins.

While scientists have long believed that knocking down the single-gene mutations that give rise to neurodegenerative diseases could have a therapeutic benefit, it has proven difficult to deliver oligonucleotides agents to neurons safely and efficiently. Therapeutic development of treatments has also been hindered, in some cases, by the need to eliminate mutant proteins while leaving enough functional proteins behind for cells to thrive.

"We can't just eliminate all the C9ORF72 protein from neurons because that runs a risk of harming the cells," said Watts. "Any potential treatment needs to be more selective in its targeting."

To do this, Watts and Brown targeted two specific isoforms of the C9ORF72 gene that generate the toxic DPRs and identified several ASOs that knock down DPR levels. Once the ASOs were identified, Watts modified the ASO backbone to improve its safety, distribution and stability in the brain and spinal cord. Watts defined combinations of various phosphate and sugar backbones that permit the ASOs to be taken up effectively and safely by the cells. "We used a 'naked' oligonucleotide," said Watts. "Since the chemical modification pattern we used doesn't require a delivery vehicle, we could just inject the ASO into the spinal fluid."

"This study provides a proof-of-concept that ASO therapy in a human can effectively and safely suppress levels of the expansion harboring C9ORF72 protein," said Brown. "That is, this intervention targets not only the mutant allele but the miscreant transcripts and DPRs generated by that allele. This is the first report of C9ORF72 DPR suppression in humans. Our findings strongly encourage the view that suppressing the expression of mutant C9ORF72 is possible and should be explored further for clinical benefit."

Besides Brown and Watts, other study authors included Helene Tran, Ph.D., instructor of neurology at UMass Chan and Michael Moazami Ph.D., former postdoctoral associate in the Watts Lab and currently a medical student at Oxford University Medical School, Oxford, United Kingdom, as well as an extensive clinical trials team.

**More information:** Jonathan Watts, Suppression of mutant C9orf72 expression by a potent mixed backbone antisense oligonucleotide, *Nature Medicine* (2021). DOI: 10.1038/s41591-021-01557-6. www.nature.com/articles/s41591-021-01557-6