Zinc Finger Transcription Factors: The On/Off Switch for Genes Inspired by Frogs

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Have you ever noticed how effortlessly frogs cling to nearly every surface? Their sticky little fingers easily grab and hold onto just about anything they want. It turns out that the frogs have protein structures that do the same thing, and these structures could be the key to unlocking therapies for amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), two of the most debilitating neurological conditions affecting millions of people today.

While studying the African clawed frog in 1985, researchers discovered the frog's proteins had finger-like protrusions, bound by zinc ions, that tightly gripped tiny gene segments. Each protrusion, aptly named "zinc finger," worked like a homing device: It could recognize and bind to specific DNA sites, activating or silencing them.

Humans have zinc finger proteins, too, that are part of a class of proteins called transcription factors. These protein molecules are, in essence, on/off switches for the

genes held in each cell of our bodies.

Fast-forward nearly 35 years to 2018: Pfizer partnered with genomic medicine company Sangamo Therapeutics to leverage this molecular discovery. And today, that collaboration continues to design human-derived zinc finger technologies that target the genetic mutation that causes the most commonly-inherited form of ALS as well as FTD.

What Are ALS and FTD?

ALS is a rare condition that gradually causes the motor neurons around the brain and spinal cord to deteriorate and die. Over time, patients with the condition slowly lose their ability to talk, walk, and eat; eventually, they may develop full paralysis. Physicist Stephen Hawking, for example, had ALS. So did Lou Gehrig, and ALS is also known as Lou Gehrig’s disease.

According to Amy Pooler, PhD, Vice President and Head of Neuroscience at Sangamo, ALS does not have a singular cause. Instead, several genes are involved, pointing to a need for more targeted, personalized approaches in treating the disease.1

FTD (sometimes still called Picks disease) is an uncommon degenerative disease that commonly causes dementia and personality changes in people who are considered “too young” for dementia.2 Because symptoms often appear in people aged 45-65, affected individuals are frequently misdiagnosed with a psychiatric condition.3 As the disease progresses, people with FTD may lose the ability to use language properly; they may also develop muscle spasms or weakness, rigidity, poor coordination and balance, or difficulty swallowing.4

In the United States, ALS affects approximately 30,000 people.5 Approximately 60,000 people in the United States have FTD, which accounts for 10% to 20% of all dementia diagnoses.6 More than 90% of ALS cases are sporadic, meaning they occur in patients with no family history of the condition. The remaining 10% of ALS cases are due to genetic inheritance.7 Mutations in *C9ORF72* are the most common inherited form of ALS and cause 25% to 40% of all inherited cases of ALS and 25% of FTD cases.8,9

The *C9ORF72* Mutation

Both ALS and FTD relate to a mutation in the *C9ORF72* gene known as a "repeat expansion," in which a segment of nucleotides repeats excessively, much like a broken record.10 The replicated section of the gene is a hexanucleotide (GGGGCC) that may be copied thousands of times and produce a wide variety of abnormal RNA and protein

molecules.

As a next step, Pfizer and Sangamo's partnership aims to decipher how the *C9ORF72* mutation causes disease. That answer could open the door to the first therapies to counteract the impact of these mutations.

"What's interesting about this collaboration is that it's exploring an approach to a class of mutations and, by extension, a group of diseases," says Christine Bulawa, PhD, a Senior Director in the Pfizer Rare Disease Research Unit in Cambridge, Massachusetts. "We're targeting a subset of ALS, but if successful, the approach might be applicable to other diseases that are caused by this type of molecular defect at the DNA level—a little string of nucleotides that tends to expand and get longer. The longer they get, the worse the disease is."

ALS Treatment and Zinc Finger Technology

To combat *C9ORF72* mutations, the zinc finger technology has the potential to bind to the repeating segments of nucleotides and turn the gene off, silencing its harmful effects. However, this ALS treatment works differently than genetic therapies that replace a mutated gene with a healthy version. The genetic inheritance of ALS is dominant, meaning that even when you have a healthy copy of *C9ORF72*, your mutated copy can take over, causing you to develop the disease. That's why it's important to create a therapy that can effectively "knock down" or turn off mutated *C9ORF72*.

"Our strategy is to mitigate the damaging effects of the mutant form of *C9ORF72*, to reduce the abnormal RNA and protein molecules that are produced from the expanded repeat," Bulawa says. "It's a totally different approach to gene editing. We're not making any cuts to or removing any of the repeats. We're basically making a potential therapy that can bind to the mutated nucleotides and then, prevent them from being expressed."

To accomplish this goal—to create a gene on/off switch—the zinc finger technology relies on an engineered protein that has two domains. First, the zinc finger domain is engineered to target GGGGCC and specifically bind to its excessive repeats. Second, the repressor domain turns off the repeat expansion and drives down the replication of abnormal RNA and protein molecules.

As a result, this technology could help push treatment for ALS into the next phase, getting investigators one step closer to stopping this degenerative nerve disease in its tracks.

"Current treatments that are available for ALS are really focusing on symptom management. But there's a critical unmet need for effective, disease-modifying therapeutics, something that is different," Pooler says. "We believe with [this] approach, by targeting the disease at the DNA level—the gene level—this represents a new therapeutic approach that could be transformative for patients who are living with these devastating diseases."11

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