The activity of disease allele-selective zinc finger proteins in preclinical models of Huntington's disease

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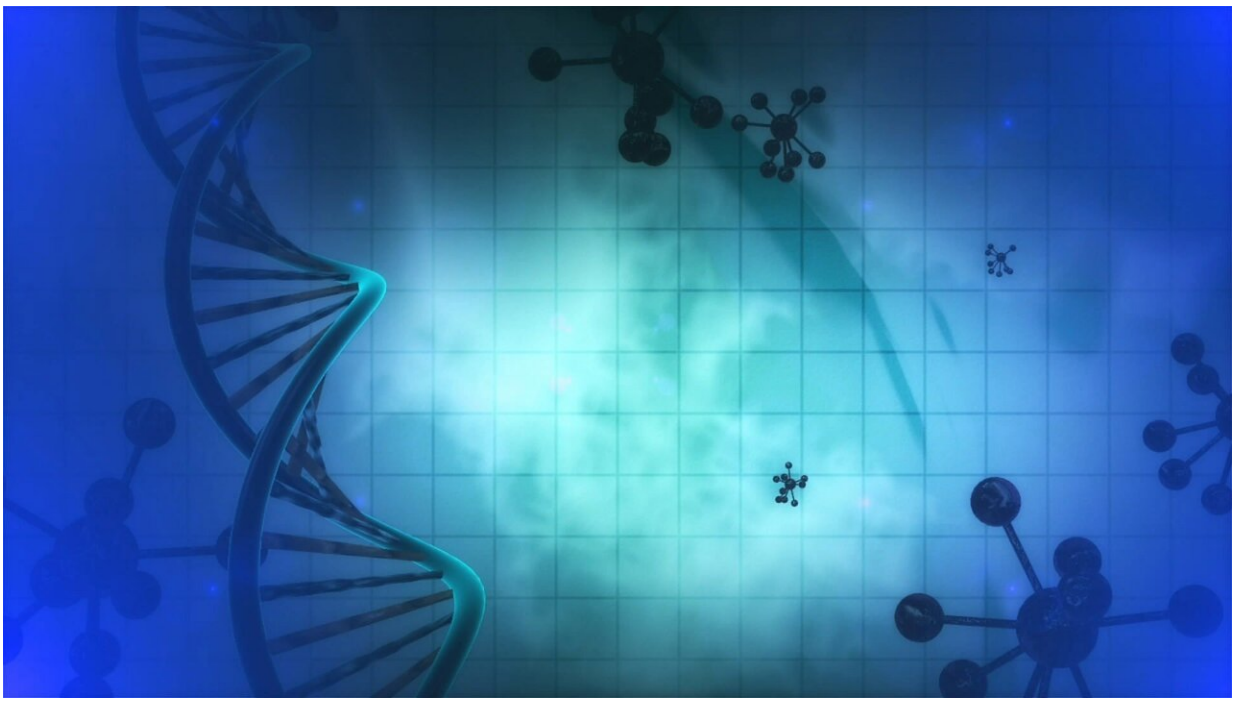

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Sangamo Therapeutics, Inc., a genomic medicine company, today announced the publication of a manuscript describing the activity of allele-selective zinc finger protein transcription-factors (ZFP-TFs) in preclinical models of Huntington's disease (HD). The data were

published online on July 1 and will appear in the July 2019 issue of *Nature Medicine*.

The publication describes research by Sangamo and collaborators at the CHDI Foundation, in which ZFP-TFs were engineered to selectively target the mutant form of the huntingtin gene (HTT) and repress its transcription, selectively lowering production of the mutant Huntingtin protein (mHTT). Preclinical data from HD patient-derived fibroblasts and neurons demonstrated that a single administration of ZFP-TFs resulted in the selective repression of over 99 percent of HD-causing HTT disease alleles over a wide dose range, while preserving the expression of at least 86 percent of healthy wild-type HTT alleles.

Huntington's disease is a progressive, fatal, neurodegenerative disorder caused by a dominant mutation involving the expansion of a CAG trinucleotide repeat in exon 1 of the HTT gene. Fully penetrant disease alleles of mutant HTT have more than 39 CAG repeats, but most HD patients have one healthy wild-type copy of HTT with less than 22 CAG repeats. Led by first author Bryan Zeitler, Ph.D., Sangamo scientists engineered ZFP-TFs capable of preferentially binding longer CAG repeat arrays on the disease allele while avoiding the shorter repeat array on the healthy allele. These ZFP-TFs exhibited disease-allele selectivity and also demonstrated a high level of specificity for the mutant HTT repeat as compared to other CAG-containing genes in the human genome.

"Ever since the mutation that causes Huntington's Disease was identified in 1993, the ultimate goal for HD research has been to develop a therapy that could directly target the mutant CAG repeat while avoiding the wild-type form given its important role in many cellular functions," said Gillian Bates, Ph.D., Professor of Molecular Neuroscience, Queen Square Institute of Neurology, UCL, London, who played a key role in the international effort to clone the HTT gene and disease causing

mutation and is not involved in the study. "Sangamo's ZFP-TF approach is particularly compelling because it represents a potentially universal allele-selective treatment that could possibly require a one-time administration. If successfully translated into the clinic, this could be transformative for patients and their families."

Data from preclinical in vivo studies using different HD mouse models demonstrated improvements in a range of molecular, histopathological, electrophysiological, and other functional endpoints following treatment with Sangamo's ZFP-TFs. In neurons cultured from the zQ175 mouse model (~188 CAG repeats) of HD, recombinant AAV delivery of ZFP-TFs to primary neurons resulted in reduction of mutant HTT mRNA and HTT protein by more than 98 percent with no reduction of wild-type HTT. In vivo, toxic aggregates of the mutant HTT protein were reduced by greater than 99 percent. Moreover, the well-characterized zQ175 electrophysiological deficits in the brain were fully reversed following ZFP treatment. Functional restoration of neuronal biomarkers was also demonstrated by several measures, including the use of PET imaging ligands in living mice. This outcome has the potential to be translated for use as an efficacy marker in clinical trials. The results were confirmed and extended in an additional mouse model of HD, in which treatment with ZFP-TFs led to the repression of mutant HTT protein and significant improvement in motor function.

Finally, extensive in vivo tolerability assessments showed no evidence of a neuroinflammatory response or changes in behavior or locomotor function in mice treated with ZFP-TFs out to 15 months of age. This suggests that the long-term striatal expression of ZFP-TFs is generally well-tolerated in mice.

"These studies present the first direct demonstration of disease allele-selective transcriptional repression at the mutated Huntingtin gene locus. While several HTT-lowering therapies are advancing into the clinic, they

all rely on indirect approaches that do not directly target the mutation. Moreover, these strategies either lower both mutant and normal HTT or employ allele-targeting that is limited to a subgroup of patients, in some cases requiring multiple intrathecal injections over a patient's lifetime," said Adrian Woolfson, M.D., Ph.D., Sangamo's Executive Vice President of Research and Development. "Sangamo's engineered ZFP-TFs demonstrated a combination of high selectivity, genome-wide specificity, and long-term tolerability that we believe establishes a new benchmark for engineered transcription factors. Overall, these data provide compelling preclinical evidence for the potential viability of Sangamo's ZFP-TF gene regulation platform as a novel disease modifying therapeutic approach for the treatment of Huntington's disease."

About Huntington's Disease

Huntington's disease (HD) is an inherited neurodegenerative disease that typically presents in adults aged between 30 and 50. HD is caused by a mutation in one of the alleles of the huntingtin gene (HTT), leaving only one functional or healthy copy of HTT in the cell. The mutated HTT produces the mutant HTT protein, leading to profound neuronal loss and progressive deterioration of motor, psychiatric, and cognitive abilities. There are currently no disease-modifying therapies available for HD.

About Sangamo's Gene Regulation Platform

Sangamo's zinc finger protein transcription factor (ZFP-TF) gene regulation technology is designed to either selectively repress (down-regulate) or activate (up-regulate) the expression of a specific gene or gene allele following a single administration. This technology enables targeting of a broad range of diseases requiring regulation of endogenous gene expression and differs from other approaches such as gene therapy

or zinc finger nuclease-mediated (ZFN) genome editing, which are designed to replace or correct a missing or mutated gene or DNA sequence.

Sangamo is developing ZFP-TFs as a novel therapeutic approach for diseases of the central nervous system (CNS). Sangamo has a collaboration with Pfizer, deploying the ZFP-TF gene regulation approach to repress the expression of the mutated C9ORF72 gene allele linked to genetic forms of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Sangamo is also developing ZFP-TFs to down-regulate the expression of tau, a protein associated with Alzheimer's disease and other tauopathies.

Takeda Pharmaceutical Company Limited is working with Sangamo on further engineered ZFP-TFs designed to selectively target the mutant HTT gene and repress its transcription. Takeda intends to evaluate this potential clinical candidate for the treatment of HD in potential preclinical Investigational New Drug (IND)-enabling studies.

More information: Bryan Zeitler et al. Allele-selective transcriptional repression of mutant HTT for the treatment of Huntington's disease, *Nature Medicine* (2019). DOI: 10.1038/s41591-019-0478-3

Provided by Sangamo Therapeutics, Inc.