Development of an RNA Therapeutic for the Treatment of Charcot Marie Tooth 1A

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Institution Receiving Award: DTx PHARMA
Program: PRMRP
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This proposal from DTx Pharma addresses the fiscal year 2022 (FY22) Peer Reviewed Medical Research Program (PRMRP) Portfolio Topic of Neuroscience, specifically, the FY22 PRMRP Topic Area of Peripheral Neuropathy. The FY22 PRMRP Strategic Goal addressed in this application will be Treatment, namely, to develop and evaluate novel treatments, strategies or therapeutic targets, including research to repurpose existing drugs, for associated neurological diseases and psychological conditions and develop and test treatment strategies to manage symptoms and improve quality of life for those affected by associated neurological and psychological conditions.

Charcot-Marie-Tooth disease (CMT) encompasses a heterogeneous group of inherited peripheral neuropathies affecting both motor and sensory nerves. The disease is slowly progressive and variable, and those affected may have difficulties with every-day activities and a shorter life expectancy. While the age of disease diagnosis varies, only 12.5% of the population is expected to be diagnosed before 14 years of age. Accordingly, it is expected that military Service Members will be diagnosed as the disease progresses during their military careers.

Charcot-Marie-Tooth type 1A Disease (CMT1A) affects 75,000 patients in the United States and is caused by a duplication of the gene for peripheral myelin protein 22, or PMP22, a transmembrane glycoprotein commonly expressed in the Schwann cells of peripheral nerves. The duplication in the PMP22 gene results in an overproduction of PMP22 protein and, in turn, the excess PMP22 leads to defects in the myelination of peripheral nerves. Defects in myelination impair the transmission of electrical signals needed to drive muscle contraction. These defects eventually lead to muscle atrophy and destruction of neurons which in patients is expressed as weakness, difficulty walking and abnormal hand functioning. There are no approved treatments for CMT1A.

Preliminary Data/Progress Report: The genetic underpinnings of CMT1A make RNA interference an attractive approach for the potential treatment of CMT1A. The National Institutes of Health/ National Institute of Neurological Disorders and Stroke previously funded our Phase 1 SBIR grant to apply our Fatty Acid Ligand Conjugated OligoNucleotide platform, the FALCON, to explore the delivery of siRNA to peripheral nerves and Schwann cells by screening siRNAs designed to reduce the expression PMP22 in a mouse model of CMT1A. We synthesized 60 siRNAs and funneled them through a screening cascade that included in vitro target engagement assays and in vivo target engagement, safety and duration of action studies in C3 CMT1A mice -- a rodent model of CMT1A that faithfully recapitulates most aspects of the disease. The screening activities led to the identification of the fully optimized development candidate siRNA that we call DTx-1252. DTx-1252 represses human PMP22 mRNA expression dose-dependently in C3 CMT1A mice across several distinct peripheral nerves, its effects are maintained maximally for at least 60 days following a single intravenous injection, and it was not shown to result in any adverse signals in clinical chemistry or liver histopathology in C3 CMT1A mice. In wildtype rats, we have not observed any

adverse findings up to at least 100 mg/kg to date. Formal dose-range finding (DRF) toxicology studies in rats and non-human primates (NHPs) are scheduled to dose in May and June of 2022 respectively. In therapeutic efficacy studies with repeated monthly administration of 3 mg/kg over 12 weeks, treatment restored myelination, nerve conduction velocities (NCVs) and compound muscle action potentials (CMAPs) to near wildtype levels. Further, treatment improved performance in grip strength and beam-walking assays to near wildtype mouse levels.

The goal of this project is to better understand the pharmacology of DTx-1252 in disease models, manufacture DTx-1252 to support FIH studies and complete the required GLP toxicology studies necessary for repeat dosing studies in CMT1A patients.

Specific Aim 1: Manufacture of a GMP batch of DTx-1252 to support first-in-human (FIH) studies

Specific Aim 2: Fill in gaps in our knowledge around DTx-1252 in vivo pharmacology, and the impacts of motif modification on duration of benefit and route of administration.

Specific Aim 3: Good Laboratory Practice (GLP) Multi-Dose Rat and NHP toxicity studies.

Project Milestone: At the completion of the project, we anticipate having filed an IND to support a SAD study in humans, having the critical data required for repeat dosing in CMT1A patients and having a more robust understanding of the pharmacology of DTx-1252 in rodent models of disease to support a compelling investigator's brochure. This package of data should be more than sufficient to support both efforts to raise capital to advance DTx-1252 through proof-of-concept studies in man or alternatively, to attract a large pharmaceutical partner to co-develop DTx-1252 with the DTx Pharma team.

Impact: Critically, these data will not only allow us to move DTx-1252 into the clinic to treat CMT1A patients, but also serve as validation of the FALCON platform for delivering RNA therapeutics like siRNA or ASOs to target many tissues, beyond kidney and liver, and including peripheral nerve.