Protein Replacement Therapy for Friedreich's Ataxia: A Review

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Friedreich's ataxia is a devastating hereditary disease caused by deficiency of frataxin, a mitochondrial protein. Patients experience progressive neurodegeneration and heart problems, and have a life expectancy of 30 to 50 years. There is no approved cure. Current treatment revolves around symptom management, but a number of exciting therapies in the pipeline have the potential to slow or even halt disease progression. TAT-Frataxin (CTI-1601), a protein replacement therapy which takes advantage of a cell-penetrating peptide sequence to deliver the underexpressed protein directly to mitochondria, has shown promise in animal studies. Pending results from Phase I clinical trials, this therapeutic has important advantages that differentiate it from other molecules currently under investigation.

1 Introduction

Friedreich's ataxia (FA) is the most prevalent of the hereditary cerebellar ataxias, with an incidence of approximately 1 in 40,000 people.[1] This condition is inherited in an autosomal recessive pattern and primarily affects individuals of European, Middle Eastern, or North African ancestry. FA is most often identified between ages 10 and 16 and progressively worsens over time, with most patients requiring full-time use of a wheelchair by the third decade.[2] Though patients invariably present with neurological symptoms, FA is a multisystem disease which also affects the musculoskeletal system, heart, and pancreas. Life expectancy is typically around 30 to 50 years and the majority of deaths are due to heart disease. There is no approved cure for FA and current treatment options are based on symptom management. However, recent disease-modifying approaches leveraging our understanding of the genetic and biochemical underpinnings of this condition have shown promising preclinical and early clinical results. This paper examines one of the most exciting prospects, a protein replacement therapy developed by Larimar Therapeutics, and contrasts it with other agents in the treatment pipeline.

2 Genetics, Pathophysiology, and Clinical Presentation

As an autosomal recessive disorder, it is essential to understand the genetic basis of FA when evaluating potential disease-modifying treatments. The condition is caused by a GAA trinucleotide repeat expansion in the first intron of the frataxin (*FXN*) gene, which encodes for the frataxin protein. In the majority of cases this repeat expansion occurs in both alleles, though 1-3% of patients are observed to have an expansion in one allele and point mutation or deletion in the other.[2] In normal chromosomes, trinucleotide tracts typically contain about 40 repeats. The pathological threshold for FA appears to be 70, and patients most commonly have between 600 and 900 triplets.

The functional consequence of repeat expansions in the *FXN* gene is silencing of transcription and a reduced level of frataxin protein compared to unaffected populations. A number of different mechanisms mediate this silencing effect. DNA at the pathological expansions can self-associate, forming structures which physically block the progress of RNA polymerase II, which in turn leads to stalled or aborted transcription.[3] Furthermore, this repeat expansion is associated with heterochromatinization, or the dense packing of DNA by various enzymes, which inhibits any further molecular interactions with the underlying gene sequence and effectively inactivates the gene.[2] In addition, epigenetic markers such as abnormally extensive DNA methylation upstream of the repeat expansion are suggestive of transcriptional suppression and correlate with disease severity.[4]

As a result of impaired *FXN* transcription, frataxin production in FA patients is reduced to 5-20% of normal levels.[5] Frataxin is a mitochondrial protein with important roles involving the electron transport chain, iron homeostasis, and protection against

oxidative stress.[6] Crucially, it activates the formation of iron-sulfur clusters which subsequently act as cofactors to enzymes catalyzing oxidation-reduction reactions in mitochondrial electron transport. An absence of frataxin then results in a profound reduction in the cell's ability to generate adenosine triphosphate,[7] the energy molecule necessary to carry out metabolic functions.

Because iron is no longer being utilized to assemble iron-sulfur clusters, it accumulates in the cell and becomes oxidized, leading to an increase in the production of reactive oxygen species. At the same time, frataxin appears to promote detoxifying enzymatic reactions within the cell and directly reduce the formation of free radicals by chelating metal ions.[8] Its absence then tips the balance greatly toward oxidative stress beyond what the cell's antioxidant machinery can handle. All these processes converge to promote cell injury and ultimately, apoptosis and progressive end-organ damage.

Cell types which are especially vulnerable include neurons, cardiomyocytes, and pancreatic beta cells, contributing to the clinical phenotype of FA.[2] Within the central and peripheral nervous systems, atrophy is most prominent in the dentate nuclei, spinocerebellar tracts, dorsal root ganglia, and posterior columns. This results in a mixed ataxia involving both cerebellar pathology and peripheral sensory neuropathy. Patients consistently demonstrate an unsteady gait, loss of balance, and limb ataxia with absent reflexes and loss of vibration and proprioceptive sensation. Dysarthria presenting as slow, slurred speech progresses toward near-unintelligibility in the advanced stages. Other neurological symptoms, though variable in presentation, can include difficulties swallowing, vision and hearing loss, and cognitive deficits.

Cardiomyopathy is a common feature of FA, with most patients showing cardiac wall abnormalities at time of diagnosis. Left ventricular hypertrophy tends to slowly regress over time concurrent with a progressive increase in left ventricular dilatation. In the advanced stages this results in supraventricular tachyarrhythmias, most commonly atrial fibrillation, which contribute to worsening systolic function and eventual heart failure, the cause of death in a majority of patients.[2] Diabetes mellitus is also highly prevalent in FA patients, as insulin-producing beta cells are affected by the loss of frataxin.

3 TAT-Frataxin

Many of the therapeutics currently under investigation for the treatment of FA target different steps in pro-degenerative pathways characterizing this disorder, showing mixed results in preclinical and clinical trials. Due to the complexity of the disease, a "cocktail approach" of numerous drugs encompassing disease-modifying therapies, antioxidants, and neuroprotective agents is likely to represent the best treatment strategy moving forward.[9] However, the investigational therapies that address the upstream cause of disease, i.e. underexpression of frataxin, appear to have the greatest potential benefit out of any individual treatments.[10]

Among these is the protein replacement therapy TAT-Frataxin (CTI-1601), which is a fusion protein consisting of mature frataxin attached to transactivator of transcription (TAT) peptide along with a mitochondrial targeting sequence. TAT is derived from human immunodeficiency virus type 1 (HIV-1) and acts as a cell-penetrating peptide allowing for efficient delivery of the fusion protein into all tissues, including the brain. Though TAT is a relatively simple molecule, its biochemical properties allow it to either directly translocate with cargo through the cell membrane or mediate endocytosis in a receptor-dependent or -independent manner under varying conditions.[11] Once internalized into the cell, the targeting sequence enables translocation of TAT-Frataxin through the outer and inner mitochondrial membranes into the matrix space, where proteases cleave the fusion protein to remove the targeting sequence and TAT, leaving only frataxin.

Preclinical data in FA animal models is encouraging and supports further clinical investigation of this molecule. TAT-Frataxin has been shown to penetrate tissues in sufficient amounts such as to confer survival benefits and improve cardiac function in animals.[7]

In a study[12] using a knockout mouse model with loss of *FXN* gene in heart and neural crest-derived tissue, animals received intraperitoneal (IP) injections of the fusion protein beginning at either 3 or 12 days of life, the latter of which is analogous to the typical age at diagnosis in humans. The treatment groups lived substantially longer and had improved growth velocity when compared to controls. They also showed increased diastolic filling of the left ventricle and higher cardiac output. Histological evidence supported these findings, showing fewer signs of cell death in heart tissue of treated animals. Treatment was also able to reconstitute iron-sulfur cluster-dependent enzymes affected by the loss of frataxin.

It is important to note that though TAT-Frataxin improved survival and other outcomes to a similar extent regardless of when treatment began, it did not achieve complete rescue when compared to wild-type mice. Patients with FA typically have 5-20% of normal frataxin levels in affected tissues but not complete loss. As such, the knockout mouse model used in this study represents a very severe disease phenotype.

A subsequent study[13] showed more modest benefits which nonetheless provides valuable insights to inform our interpretation of future clinical trials. Transgenic mice with pathological human *FXN* gene receiving biweekly intravenous (IV) injections of TAT-Frataxin expressed a threefold increase of frataxin in the mitochondria compared to controls, amounting to around 15% of normal values. These levels persisted for at least two weeks following treatment discontinuation, and were sufficient to restore activity of enzymes involved in the electron transport chain. Different routes of administration and doses were then evaluated for their effects on survival. IV delivery did not result in significant changes to lifespan. IP injections with 4 mg/kg TAT-Frataxin also did not improve survival, though 10 mg/kg showed a slight, but significant increase in lifespan.

These results suggest a dose-dependent response and it is unclear whether higher doses than tested would confer proportionally higher benefits. The route of administration also impacts delivery to affected tissues, and it is possible that the relatively modest increase in mitochondrial frataxin levels can be attributed to limitations of IV administration used in this study, which was unable to effect a significant change in mean survival. In addition, although cell studies have shown penetration into and improved survival of dorsal root ganglion neurons, it remains unclear how protein replacement impacts neurological outcomes in animal models.

Internal data provided by Larimar Therapeutics[14] seek to address some of these questions. When injected subcutaneously (SC), TAT-Frataxin was shown to significantly improve mean survival and left ventricular function, prevent left ventricular dilatation, and increase gene expression in cardiac tissue in knockout mice to levels comparable with wild-type controls. They also found that activity of affected mitochondrial enzymes plateaued at a dose of 30 mg/kg and was equivalent to activity in wild-type animals. Finally, protein replacement prevented the development of ataxic gait in knockout mice, and human frataxin was shown to penetrate the central nervous system. These results warrant further investigation to evaluate TAT-Frataxin efficacy and safety in humans.

4 Evaluating the Clinical Trials

Taken together, findings from the peer-reviewed and internal studies suggest that a successful clinical trial will be able to demonstrate sufficient penetration of TAT-Frataxin into affected tissues in humans, while replicating its beneficial effects on cardiac and neurological functioning in animal models, while also minimizing adverse effects.

Protein replacement to 15% of normal levels appears to at least partially restore cellular metabolic function in animal models of FA.[13] However, there seems to be a dose-dependent response and near-physiological concentrations of protein may be associated with more complete rescue of the disease phenotype. Since heterozygous carriers express around half of normal frataxin levels and are asymptomatic, protein replacement to 50% may be an optimal strategy.[6] An important aim of the clinical trials will be to determine the maximum effective dose while maintaining a favorable safety profile.

Another consideration is whether excess frataxin levels can lead to toxicity, taking into account that TAT-Frataxin is administered systemically. Frataxin overexpression in a human cell model was shown to affect cellular metabolism and increase oxidative stress in a manner similar to gene underexpression, as occurs in FA.[15] In cardiac tissue, frataxin overexpression caused significant toxicity above 20-fold the normal endogenous level, and was safe up to 9-fold.[16] Human studies will need to ensure a delicate balance of frataxin levels is maintained, particularly in off-target tissues on which fewer data are available.

Larimar Therapeutics completed a single ascending dose study[17] for TAT-Frataxin in November 2020 and a multiple ascending dose study is currently underway. Among the primary and secondary endpoints evaluated in this Phase 1 trial, expected to report in Q2 2021, encouraging findings will include a low number of adverse events and significant changes from baseline in frataxin levels, especially if they approach physiological concentrations.

Important metrics to consider in Phase 2 and beyond include clinical assessments such as the Freidreich's Ataxia Rating Scale (FARS), Scale for the Assessment and Rating of Ataxia (SARA), or International Cooperative Ataxia Rating Scale (ICARS), all of which have been validated for use in FA patients and cover aspects of the neurological examination.[18] Cardiac assessments include echocardiographic testing measurements and cardiac function as measured by peak workload on exercise testing and peak oxygen consumption. Adverse effects over the longer term are critical to monitor. TAT-Frataxin has the advantage of delivering an endogenous protein and is likely to be well-tolerated by most patients at physiological levels, but potential side effects may include immune reactivity in response to the attached cell-penetrating peptide sequence.[7]

5 Conclusion

The most promising therapeutic candidates for FA include disease-modifying agents that target the underlying genetic cause of this condition. As

a protein replacement therapy, TAT-Frataxin has shown encouraging results in animal studies, even those modeling very severe disease phenotypes. A successful Phase 1 readout will entail a favorable safety profile in ascending dose trials and a significant increase in frataxin levels in various tissues, preferably nearing physiological levels at sufficient doses.

Within the FA treatment pipeline, the most comparable approach is gene therapy using adeno-associated virus (AAV) vectors, which are able to efficiently deliver copies of normal *FXN* gene into cells. The newly introduced genetic material is then converted into frataxin protein. AAV vectors have attracted considerable investment and research attention as the dominant platform for *in vivo* gene therapy, as they are nonpathogenic and can preferentially target tissues of interest.[19] Of note, AAV gene therapies for the treatment of two other diseases, Leber's congenital amaurosis and spinal muscular atrophy, have already received FDA approval. In the context of FA, preclinical studies have shown complete rescue of sensory neuropathy and cardiomyopathy associated with frataxin deficiency.[10,20]

Though preclinical data are promising, some safety concerns remain. AAVs typically do not integrate into the host genome, making them ideal for treatment of diseases that affect non-dividing cells, like neurons. However, studies have shown that under certain circumstances vector integration can indeed occur, and this has been associated with insertional mutagenesis and tumor formation in animal models, though long-term risk in humans appears to be low.[21] Another concern is the potential for immunotoxicity. Immunomodulation strategies are often required along with systemic administration of AAVs to inhibit the host immune response, such as the use of periprocedural corticosteroids and immunosuppressive agents.[22] Neuroinflammatory responses in particular are a critical concern. Toxicity has been observed in dorsal root ganglion sensory neurons in primate studies following AAV injection.[23] Pathology was more severe in older animals, a finding that needs to be taken into account in diseases like FA which already show signs of neurodegeneration at time of diagnosis.

As TAT-Frataxin involves direct replacement of an endogenous protein without the introduction of genetic material, it presents fewer theoretical safety risks than other methods. It remains to be characterized whether clinical trials can show improved safety, tolerability, and efficacy compared to other approaches such as AAV gene therapy. Because FA is a complex disease, it is likely that optimal treatment strategies will involve the use of disease-modifying therapies along with pharmacological agents such as enhancers of mitochondrial function and antioxidants. Nonetheless, protein replacement with TAT-Frataxin is primed to become a mainstay of treatment if clinical trial results can demonstrate its potential in FA patients.

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