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Advancements in the Treatment of Transthyretin Amyloidosis

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ABSTRACT

Transthyretin (TTR) amyloidosis is a protein misfolding disorder where tetramers of TTR dissociate, causing the formation of insoluble amyloids leading to organ dysfunction and damage. Worldwide, the disease currently affects between 5,000-10,000 people and many novel therapeutic techniques are appearing with the potential to treat TTR amyloidosis. Of the non-CRISPR treatments, they can be broken down into three categories: TTR stabilizers, human monoclonal antibodies, and gene silencers. In this paper, I will discuss both the current state of research and treatment of TTR amyloidosis treatments, as well as the role of CRISPR's potential future therapeutic applications.

Introduction

TTR amyloidosis is caused by a misfolding of the TTR protein in tissues—primarily the nerves and the heart. When functioning normally, TTR acts as a transport protein for the hormones thyroxine and retinol. Consisting of 127 amino acids, the protein forms a tetramer: it contains 4 identical subunits, which form two homodimers that create the binding site for these hormones. Mutations in the protein, which are typically autosomal dominant, lead to a decrease in the amount of wild-type TTR tetramers; instead, the misfolded monomers form an insoluble amyloid fibril, which builds up in the cell. Though the TTR protein is primarily produced in the liver, its monomers may enter different tissues, causing organ damage. In the United States, the most common TTR mutation is a change from Val to Ile at the 122nd amino acid (as shown in figure 1), although other mutant isoforms can be identified.[1] Figure 2 shows the differences in the structure at the protein level between the wild-type and mutated forms.

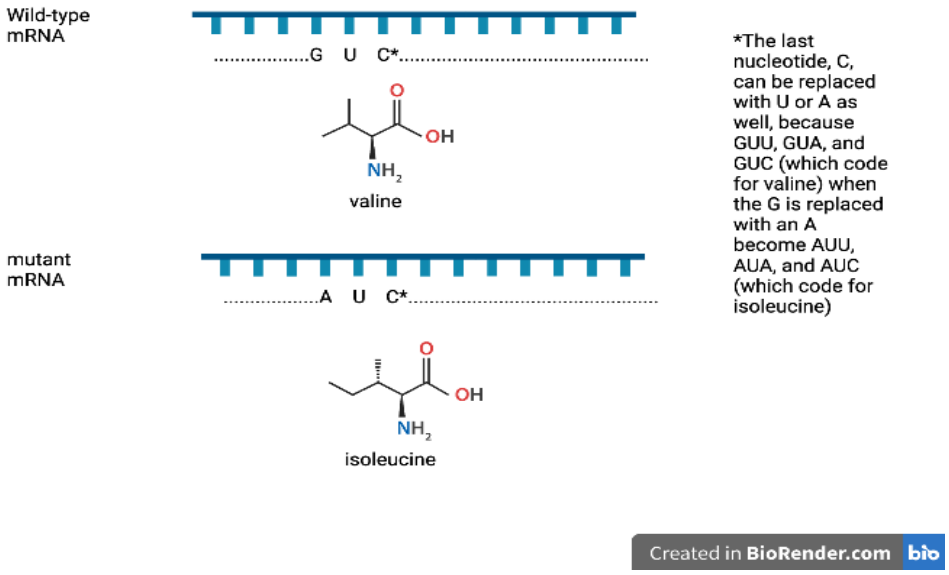

Figure 1. val122ile mutation. Shown above is the mRNA sequence of both the wild-type and the most common mutation at the 122nd amino acid. Created with BioRender.com.

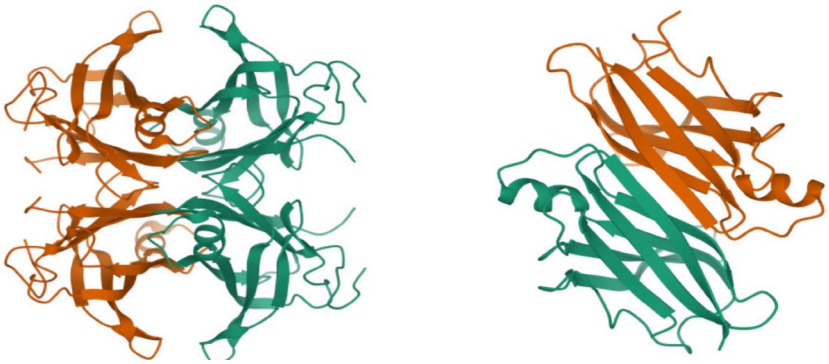

Figure 2. Wild-type vs mutated form of the protein.[29] RCSB PDB IDs: 1TTB and 1TTR, respectively. The image on the left shows a wild-type TTR protein, whereas the image on the right shows a TTR protein with the Val122Ile mutation (most common TTR mutation).[30] The mutated protein has a more barrel-like shape, whereas in the wild-type protein, the tetrameric formation is clearly visible.

Two of the most common phenotypic manifestations of this disease are cardiomyopathy and polyneuropathy. Cardiomyopathy is a degenerative heart disease that makes it difficult for the heart to pump blood to parts of the body, possibly resulting in heart failure (HF). Some symptoms include shortness of breath, irregular heartbeats, and thickening of the heart muscle.[2] In polyneuropathy, many of the body's peripheral nerves stop working simultaneously. Its symptoms include muscle spasms, loss of sensation, weakness, and pain. Table 1 indicates the specific phenotypic manifestations of the disease and in which organ system they are located.

Table 1. Extracardiovascular Manifestations of Cardiac Amyloidosis[3]

Organ System	Manifestation
Autonomic	Orthostatic hypotension Sweating abnormalities Gastroparesis

Musculoskeletal	Muscle weakness Arthropathy Fatigue Cachexia/weight loss Biceps tendon rupture
Renal	Milder renal insufficiency (mainly as a result of heart failure)
Gastrointestinal	Elevated liver enzymes Nausea Constipation Early satiety, abdominal bloating (gastroparesis might be secondary to dysautonomia and/or gastrointestinal involvement)
Ocular	Vitreous opacities
Neurological	Peripheral sensorimotor neuropathy Carpal tunnel syndrome (bilateral)

Diagnosis

The diagnosis process for ATTR amyloidosis is very complex. Traditionally, it requires cardiac biopsy with amyloid speciation using mass spectrometry or immunofixation, which can only be performed at a small number of labs. However, new technology has facilitated the diagnosis of a subset of patients with the disease using noninvasive techniques. Using cardiac scintigraphy, researchers use technetium(Tc)-labeled radiotracers to conduct SPECT imaging: if uptake of one specific radiotracer, ^{99m}Tc-pyrophosphate, is equal to or greater than bone uptake, and gammopathy is ruled out as a potential diagnosis, researchers can diagnose a patient with ATTR amyloidosis with high confidence.[1]

Diagnosis Rate

Hereditary transthyretin (ATTRv) amyloidosis affects about 5000-10,000 persons across the world and is categorized as a rare disease. In addition, as well as being reported in 36 countries, this disease is endemic to Portugal, Sweden, and parts of Japan. These three countries contributed to about 43% of those affected by TTR amyloidosis, despite their general populations only making up 3% of the entire population surveyed.[4]

Emotional Aspect of the Disease On Patients

The patients identified three common themes related to quality of life and symptoms: a “diagnostic odyssey,” symptoms and impact, and family reaction/dynamics. As TTR amyloidosis has been a relatively unknown disease, patients reported long diagnostic processes in which their primary care physicians and doctors misdiagnosed them or prescribed them ineffective medication. This led to long periods of time that patients spent searching for answers. To this day, patients are still misdiagnosed and have to endure this diagnostic odyssey–oftentimes, it is not doctors or physicians that give accurate diagnoses but rather family members who are able to find information about TTR amyloidosis on the internet. Furthermore, TTR amyloidosis comes with a host of different symptoms that affect actions in everyday life. Patients reported a wide array of symptoms, such as

a dramatic loss of strength and stamina, insomnia, and difficulty walking. One patient reported that he was forced to retire early because he worked with his hands and the disease caused numbness and he would drop tools constantly. Thirdly, spouses of affected patients experienced stress from both the illness's physical effects and watching their loved ones deal with it. Spouses often took responsibility for the administration and management of medication, among many other tasks, but they often felt guilty that they could not help their spouses more. Family members were an incredible support system. Some patients felt guilty about having children, yet family became their motivation to continue fighting the disease.[5]

In recent years, research into potential treatments has yielded significant results. Aside from CRISPR, the treatments can be broken down into three categories: TTR stabilizers, gene silencers, and human monoclonal antibodies. CRISPR, on the other hand, uses a Lipid Nanoparticle (LNP) delivery system to edit DNA directly, correcting the aforementioned mutations responsible for the disease.

Treatments Used in ATTR Amyloidosis

Table 2. List of non-CRISPR Treatments used in ATTR Amyloidosis.[1] This table indicates the FDA-approved and investigational TTR amyloidosis treatments, their mechanism of action, and their recommended dosages. There are three main classes of treatments: TTR stabilizers, gene silencers, and human monoclonal antibodies.

Type of FDA-Approved Pharmacological Treatment	**Name of Treatment**	**Mechanism of Action**	**Dosage**
TTR Stabilizer	Tafamidis	Binds to TTR at the thyroxine binding sites, stabilizing the tetramer and slowing dissociation into monomers, the rate-limiting step in the amyloidogenic process	80 mg once a day
TTR Stabilizer	Tolcapone	Inhibitor that binds both thyroxine-binding pockets	100 mg every 8 hours
Gene Silencer	Patisiran	Small-interfering RNA that causes degradation of TTR mRNA	0.3 mg/kg of body weight
Gene Silencer	Inotersen	Single-Stranded antisense oligonucleotide that silences TTR production	284 mg injected subcutaneously once weekly
Gene Silencer	Vutrisiran	RNA interference therapeutic that inhibits production of TTR	25 mg administered subcutaneously once every 3 months
Type of Investigational Treatment	**Name of Treatment**	**Mechanism of action**	**Dosage**

TTR Stabilizer	AG10	Highly selective and potent inhibitor of the amyloidogenesis cascade	TBD (researchers are testing 800 mg administered twice a day)
Human Monoclonal Antibody	PRX004	Inhibit amyloid fibril formation, neutralize soluble aggregate forms of TTR, and promote clearance of insoluble amyloid fibrils through antibody-mediated phagocytosis	TBD
Human Monoclonal Antibody	N1006	Human monoclonal antibody that targets TTR amyloid	TBD
Gene Silencer	AKCEA-TTR-LRx	Single-stranded antisense oligonucleotide that silences TTR production and a receptor-mediated delivery system	TBD
Gene Silencer	NTLA-2001	Inactivates the disease-causing gene in the liber using gene editing via CRISPR/Cas9	0.3 mg/kg of body weight

TTR Stabilizers

As the name suggests, these compounds stabilize the TTR tetramer, preventing it from dissociating. Tafamidis inhibits the TTR amyloidogenesis cascade.[6] It works by selectively binding to thyroxine binding sites on the TTR tetramer, thereby stabilizing it.[22] There are two oral formulations of tafamidis, Vyndaqel and Vyndamax, that have been approved by the FDA to treat ATTR-CM. The FDA approved an 80-mg dose of tafamidis in May 2019, and further results indicated stronger stabilization of the TTR tetramer with higher doses of tafamidis.[7,8]

Currently, there is another stabilizer, AG10, that is in development. This treatment reduces the dissociation rate of the tetramer by forming hydrogen bonds between close-by serine residues at position 117 of each monomer, and this mutation is known as T119M.[9] Currently, there is a phase 3 clinical trial evaluating the safety and efficacy of an 800 mg dosage twice a day in comparison with a placebo.[10]

Gene Silencers

The FDA has approved three treatments that act as gene-silencers: patisiran and inotersen. These treatments do not directly target ATTR-CM, but they are still considered treatment for patients with polyneuropathy.[2]

Patisiran is an RNAi where the interfering RNA "knocks out" the gene responsible for TTR production in the liver.[11] It targets a sequence within the untranslated region (UTR) of the TTR mRNA: once it enters the cytoplasm, it is loaded into a RNA-induced silencing complex (RISC) that binds to a complementary sequence on the 3' UTR of both the mutant and wild-type TTR mRNA. Within the RISC, there is an endonuclease that facilitates cleavage of the mRNA sequence, thereby "knocking out" the mutant and wild-type TTR mRNA, suppressing production of the TTR protein. The recommended dose, which is administered once every three weeks via intravenous (IV) infusion, is 0.3mg/kg of body weight, and patients weighing 100 kg or more are recommended to take 30mg.[23]A relatively recent study investigated patisiran's effect on cardiac amyloid load, and TTR gene knockdown in treated patients averaged about 86%, and most patients had >80% gene knockdown. There were few adverse side effects and patisiran was associated with a reduction in extracellular volume.[12]

Inotersen, also known as Tegsedi, is an FDA-approved single-stranded ASO (antisense oligonucleotide) that silences TTR production in patients with hereditary ATTR polyneuropathy by binding to and degrading the TTR transcripts via the RNAseH pathway, thus stopping TTR production.[13] It is a 2'-O-methoxyethyl-modified RNA (2'-MOE), meaning that on the 5' and 3' ends of the oligonucleotide, there are five 2'-MOE nucleotides, in addition to ten DNA nucleotides that support the RNAseH pathway. Because Inotersen does not distinguish between wild-type and mutated TTR mRNA, it decreases the total concentration of both wild-type and mutated TTR proteins in circulation.[26,27,28] In clinical trials, it was shown to be efficacious, although some of its adverse side effects included thrombocytopenia and renal dysfunction.[14]

Vutrisiran is a 2nd-generation RNAi that prevents TTR formation, and it has shown efficacy in TTR patients with cardiomyopathy and those with polyneuropathy.[16] This treatment is administered subcutaneously (whereas Patisiran is administered intravenously). In clinical trials, Vutrisiran showed evidence of reduced amyloid burden in the heart, as well as improvements in cardiac health measured by the cardiac stress biomarker N-terminal-prohormone BNP (NT-proBNP).[17]

As for treatments still in testing, AKCEA-TTR-LRx is a single-stranded antisense oligonucleotide with a receptor ligand protein, which facilitates direct binding to hepatocyte cells where most circulating TTR is produced.[18] It is currently recruiting for a phase 3 trial that decreases dose frequency in an analysis of patients with ATTR-CM whose projected completion will be in June 2024.[19]

Human Monoclonal Antibodies

In addition, there are two human monoclonal antibody treatments that are still currently undergoing clinical trial testing. These treatments bind to the TTR monomers—to prevent them from destabilizing, nonnative oligomers and aggregates prevent amyloid fibril accumulation.[2]

PRX004 inhibits fibril formation by targeting and clearing misfolded variants of the TTR protein in patients with cardiomyopathy.[20]

NI006 is another antibody that specifically targets the TTR amyloid, and it is being tested in patients with both hereditary and wild-type ATTR-CM.[21]

CRISPR in the Treatment of ATTR Amyloidosis

In addition to the three classes of treatments stated above, CRISPR is a new treatment for amyloidosis. By reducing the amount of TTR protein accumulated in the hepatocytes, CRISPR offers a promising new solution for patients with the disease.

How CRISPR Normally Works

CRISPR-Cas9 is a gene editing technology derived from a bacterial mechanism of sequence-specific protection against viruses in prokaryotes. CRISPR stands for "clustered regularly interspaced short palindromic repeats." When infected with viruses, bacteria insert small segments of viral DNA to their own DNA in a pattern such that CRISPR arrays are produced, enabling the bacteria to "remember" the virus. Upon a second viral attack, the bacteria uses the CRISPR arrays to create RNA segments that recognize and bind to specific regions of the viral DNA. Cas9 nuclease then cleaves the DNA, disabling the virus.

Researchers have manipulated this technology to edit DNA. Like the RNA from the bacteria-produced CRISPR array, small pieces of RNA with short "guide" sequences bind to a specific target sequence of DNA. The Cas9 enzyme attaches to the RNA, and it cuts the DNA at the desired site. After the DNA is cut, researchers use the cell's own repair process to either add, delete, or replace existing pieces of DNA with a custom sequence.[24]

CRISPR's Lipid Nanoparticle Delivery Complex and NTLA-2001

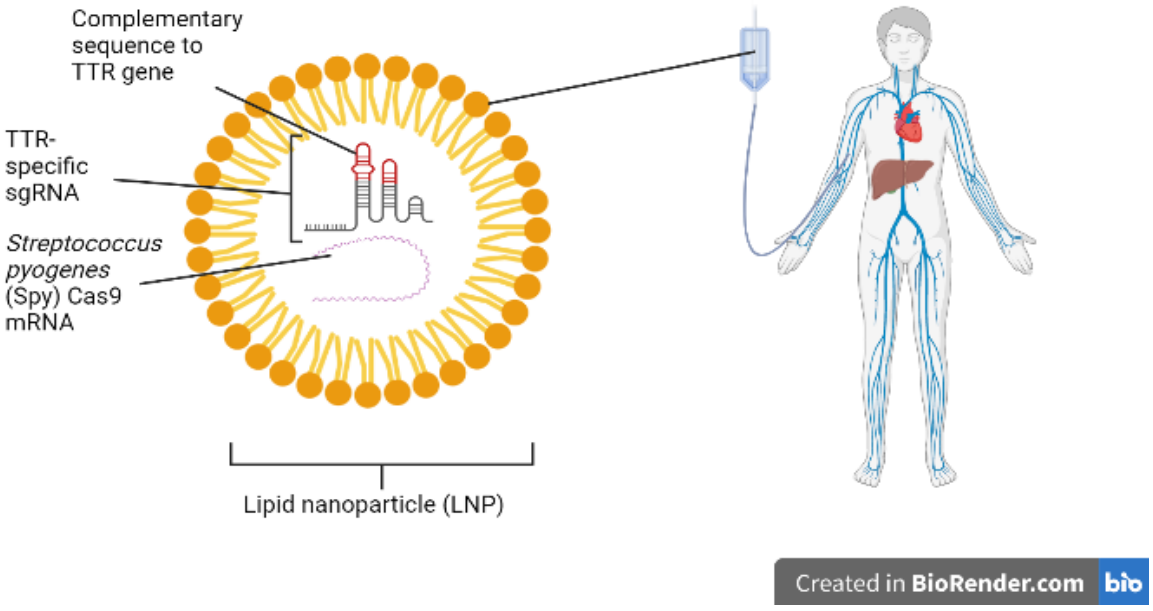

Figure 3. NTLA-2001 Complex[25]. Created with BioRender.com.

The complex, NTLA-2001, is made up of two parts: TTR-specific sgRNA (containing a complementary sequence to the TTR gene) as well as *Streptococcus pyogenes* (Spy) Cas9 mRNA. The sgRNA acts as a highly accurate guide for the Cas9 enzyme to cleave the correct DNA sequence on the TTR gene. These two subparts are grouped together in a lipid nanoparticle (LNP), which is opsonized by Apolipoprotein E. This complex is administered via IV, and it is rapidly sent to the liver via the hepatic artery. Once it reaches the liver, the LNP's ApoE proteins bind to LDL receptors on the surface of the hepatocyte, which leads to an endocytosis of the LNP encapsulated by an endosome. This endosome later breaks down, releasing the Cas9 mRNA and sgRNA into the cytoplasm, where the Cas9 mRNA is translated into the Spy Cas9 endonuclease—an enzyme.[25] Figure 3 shows the components of the NTLA-2001 complex, as well as how it is administered into the body.

Limitations of CRISPR in Treating TTR Amyloidosis

Though CRISPR is undoubtedly a scientific breakthrough in terms of treating TTR amyloidosis, it has several limitations. First, it is difficult to deliver the CRISPR material to large numbers of mature cells. In addition, it is not 100% efficient, so cells that receive the CRISPR-Cas9 complex may not be able to have their genes edited. Finally, using CRISPR runs the risk of creating "off-target" edits, which can have detrimental consequences, such as unintended point mutations, insertions, deletions, inversions, and translocations.[29,30,31,32,33,34] CRISPR has not yet been used in any other types of amyloidosis, but in the future it may be used in AL amyloidosis, a highly similar disease.

Discussion

TTR amyloidosis is a fatal disease caused by misfolded mutated monomers of the TTR protein, resulting in a buildup of amyloid fibrils. There are many different treatments, which can be categorized into three groups: TTR stabilizers, gene silencers, and human monoclonal antibodies. One breakthrough treatment, CRISPR-Cas9, which is packaged in a LNP, has shown to be effective in this disease.

There are many directions in which this scientific breakthrough could lead. For example, because the amyloid fibrils that are formed by interactions between misfolded monomers are very structurally sound and difficult to break, perhaps a LNP system using CRISPR could be delivered to the hepatocytes directly to edit the amyloid fibril mRNA, making the fibrils less stable. In doing so, this process would facilitate the degradation of the accumulated fibrils.

Though the field of treatments for TTR amyloidosis is rapidly changing, there are still several pitfalls: certain parts of the body have not yet been targeted by any therapeutic options. For instance, there are no treatments that pertain to the ocular or central nervous system (CNS) manifestations of the disease, nor are there any approved treatments that have been successful in crossing the blood-brain barrier. More clinical trials are needed to determine the best time to start administering treatments after diagnosis, and the therapeutic value of combining various treatments in a "drug cocktail" has not yet been tested, either.

The NTLA-2001 complex, with its CRISPR-Cas9 approach, may be used to treat other diseases by replacing its sgRNA, and this is currently being investigated. Researchers are taking it one step further by not only "knocking out" expression of harmful protein products but also inserting genes that code for functional proteins which may be used to combat the pathologic deficiencies that mutations may cause.

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