General

Inotersen to Treat Polyneuropathy Associated with Hereditary Transthyretin (hATTR) Amyloidosis

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Keywords: siRNA, RNAi, Genetic therapy, protein misfolding, hereditary transthyretin-mediated amylodosis

https://doi.org/10.52965/001c.67910

Health Psychology Research

Vol. 10, Issue 5, 2022

Background

Amyloidosis is a group of diseases with the common pathophysiology of protein misfolding and aberrant deposition in tissue. There are both acquired and hereditary forms of this disease, and this review focuses on the latter hereditary transthyretin-mediated (hATTR). hATTR affects about 50,000 individuals globally and mostly appears as one of three syndromes - cardiac, polyneuropathy, and oculoleptomeningeal. Polyneuropathy is the most common form, and there is usually some overlap in individual patients.

Results

Recently, novel therapeutic options emerged in the form of groundbreaking drugs, Patisiran and Inotersen, small interfering RNA molecules that target TTR and reduce the production of this protein. By targeting TTR mRNA transcripts, Inotersen decreases protein translation and production, reducing the deposition of misfolded proteins. It was shown to be both effective and safe for use and specifically formulated to concentrate in the liver – where protein production takes place.

Conclusion

hATTR is a rare, progressive, and debilitating disease. Its most common presentation is that of polyneuropathy, and it carries a very poor prognosis and a natural history conveying a median survival of < 12 years. Novel therapeutic options are groundbreaking by providing disease-modifying specific, targeted therapies against TTR production and deposition. The use of RNA interference (RNAi) opens the door to the treatment of hereditary diseases by targeting them at the genetic level.

INTRODUCTION

Amyloidoses are a heterogeneous group of human diseases characterized overall by deposition of insoluble proteins, resulting in disruption of the normal structure and function of tissues and organs.[1–4] Hereditary transthyretin-mediated (hATTR) amyloidosis is a debilitating, multisystemic, progressive, and ultimately fatal disorder with variable phenotypic presentations.[1,3–6] Transthyretin (TTR) gene mutations result in misfolded TTR proteins, leading to ac-

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cumulation. The three main phenotypes of hATTR amyloidosis include polyneuropathy, cardiomyopathy, and oculoleptomeningeal involvement – of which polyneuropathy is the most common.[7,8]

The burden of hATTR amyloidosis is significant and well-established. Symptomatic individuals experience decreased quality of life and increase healthcare costs when contrasted with nonaffected individuals from the general population.[1,5,8] The purpose of this review is to highlight hATTR amyloidosis and its current therapies before discussing Inotersen (TEGSEDI), an antisense oligonucleotide, for the treatment of associated polyneuropathy.

PATHOPHYSIOLOGY

Acquired (wild-type) TTR amyloidosis results from a non-mutated form of TTR depositing in tissues, as seen in senile systemic amyloidosis.[2] On the other hand, hATTR amyloidosis results from a mutated form of TTR depositing in tissues. TTR is a tetrameric plasma transport protein synthesized primarily in the liver, with small amounts also made in the retinal pigment epithelium of the eye and choroid plexus.[2,3,7] Each monomer contains 127 amino acid residues arranged in beta-pleated sheets, with binding sites for thyroxine and a retinol-complex.[1–3,8,9] At a physiologic baseline, < 1% of thyroxine sites are bound, and < 50% of retinol-complex sites are bound.[7] hATTR amyloidosis results from the dissociation of the tetrameric quaternary structure into component monomers, followed by monomer misfolding, eventually leading to a conglomeration of monomers in the formation of insoluble fibrils. These fibrils deposit in various tissues, altering their structure and function, in addition to causing local damage via direct mechanical compression of surrounding structures.[2,3,7]

In early-onset disease with polyneuropathy, there is abundant amyloid fibril deposition, which mainly affects small myelinated and unmyelinated fibers, leading to superficial sensory and autonomic dysfunction.[10] Deposition directly damages Schwann cells by breaking down the basement membrane and distorting Schwann cell processes, resulting in Schwann cell atrophy.[10] In late-onset disease with polyneuropathy, large nerve fibers are predominantly affected with relatively low deposition quantities of mutant TTR amyloid. Instead of depositing in tissues, the mutant TTR amyloid circulating in plasma enters the endoneurium of Schwann cells through the blood-nerve barrier, indirectly damaging Schwann cells by disrupting the structure of endoneurial microvessels.[10]

Interestingly, damage to Schwann cells in late-onset disease is unrelated to the amount of surrounding amyloid deposition.[10] The genetic mutations, seen in hATTR amyloidosis, allow for more inherently unstable tetrameric structures, predisposing them to break down into monomeric components, explaining the overall earlier onset of disease in either form of hATTR amyloidosis with polyneuropathy compared to senile systemic amyloidosis.[2] Furthermore, whereas wild-type TTR amyloid monomers require proper folding with each additional monomer added to the growing fibril, mutated forms of TTR do not require any post-transcriptional folding for monomers to be added, further contributing to why fibrils form at faster rates in hATTR amyloidosis.[7]

DIAGNOSIS AND CLINICAL PRESENTATION

CLINICAL PRESENTATION

hATTR amyloidosis is a debilitating, progressive, and multisystemic disorder with a heterogenous clinical presentation.[1–4,7] Of the three main phenotypes (polyneuropathy, cardiomyopathy, and oculoleptomeningeal involvement), polyneuropathy is the most common globally and is further classified by early and late-onset disease.[7] The early-onset disease usually manifests before age 50 and is associated with significant autonomic dysfunction, while the late-onset disease is related to mild autonomic dysfunction. Polyneuropathy can be focal, sensorimotor, or autonomic.[1,4,11,12] Focal neuropathy most commonly involves the median nerve, which presents as carpal tunnel syndrome and rarely can manifest as vocal cord paresis.[3] Autonomic dysfunction can manifest as orthostatic hypotension, alternating postprandial diarrhea and constipation, postprandial vomiting secondary to gastroparesis, recurrent urinary tract infections secondary to chronic retention, and sexual dysfunction. Autonomic dysfunction is a significant manifestation of early-onset disease, but less prevalent in late-onset disease.[1,3] Sensorimotor polyneuropathy is length-dependent, affecting small unmyelinated and myelinated fibers first before progressing to affect larger fibers and nerve bundles. It generally begins in the lower extremities as superficial numbness, paresthesias, and allodynia, eventually progressing to loss of proprioception and deep sensation, accompanied by muscle weakness, difficulty walking, and secondary Charcot joint abnormalities, in the background of intensifying neuropathic pain.[1,3] There are three stages of clinical progression – ambulatory, ambulatory with assistance, and wheelchair-bound. Patients generally reach the second stage 5-6 years after symptoms begin, and the third stage 7-9 years after symptoms begin with death ensuing 10-15 years after initial presentation.[7] Death is often hastened by cachexia and recurrent infections seen with compromised autonomic and sensory function.[13]

DIAGNOSIS

Diagnostic studies for patients with superficial (early) polyneuropathy involve autonomic function testing (AFT) and quantitative sudomotor axon reflex testing (QSART). In later stages, when nerve involvement is greater, EMG can show fibrillation potentials, positive sharp waves, and large motor unit potentials with reduced recruitment, while nerve conduction studies show reduced axonal sensory and motor amplitudes with mild conduction velocity slowing.[3] The gold standard for diagnosis is tissue biopsy. The location of the biopsy may be related to the specific clinical presentation of the patient (*i.e.,* sural nerve for lower extremity neuropathy or tenosynovial tissues for median nerve involvement during carpal tunnel release) or obtained from

more generalized locations (*i.e.*, salivary gland, gastric mucosa via endoscopy, or subcutaneous fat aspiration). Concurrent with biopsy analysis, patients also often receive genetic testing for specific genotypic mutations.[1,3] It is important to note that due to the patchy distribution of amyloid deposits, negative biopsy results do not exclude the presence of mutated amyloid deposits. In the event of negative biopsy results but a high clinical suspicion, nuclear tracing with Tc-pyrophosphate can show the extent and distribution of mutated TTR.[1,5,14,15]

INOTERSEN

Inotersen is an RNA-targeted therapy that was recently approved by the United States Food and Drug Administration (FDA) for treating polyneuropathy associated with hATTR amyloidosis for adults in October 2018.[16,17] Two months prior, the Europe Union approved Inotersen for treating adults with stage 1 or 2 hATTR polyneuropathy.[18]

Inotersen has a molecular formula of $C_{230}H_{299}N_{69}Na_{19}O_{121}P_{19}S_{19}$ with a molecular weight of 7600.73 g/mole. It is classified as a 2'-O-methoxyethyl-modified second-generation antisense oligonucleotide inhibitor that comes in the form of a white to pale-yellow solid soluble in solutions of water and phosphate buffer.[19] Based on the results and study design from the NEURO-TTR phase 3 clinical trial, inotersen was found to be effective when given subcutaneously in 300 mg doses once weekly. When administered in this fashion, the drug reaches steady-state concentrations in the liver after approximately 13 weeks.[20] The results and data of the NEURO-TTR study will be discussed in detail in a later section.

In regards to pharmacokinetics, the drug exhibits dose-dependent properties. Following subcutaneous administration, it rapidly enters the systemic circulation and achieves peak plasma concentrations after a median of 2-4 hours. About 94% of the drug remains bound to plasma protein and has a mean reported volume of distribution of 293 L. Tissue endonucleases metabolize the drug into smaller inactive nucleotides that are ultimately metabolized by exonucleases and excreted by the kidney with a half-life of about one month.[21,22]

Throughout the phase 2 and 3 clinical trials, several side effects and adverse events have been documented. Side effects included erythema, induration, and itching associated with the drug injection site but did not result in discontinuation of the drug.[20,23] The phase 3 NEURO-TTR study, however, did report adverse events requiring treatment or management. These adverse events included fatigue, nausea, vomiting, diarrhea, constipation, headache, pyrexia, chills, myalgia, and arthralgia; lastly, of all adverse events, the most serious are thrombocytopenia and glomerulonephritis.[19,20] Thus, providers should monitor platelet counts and kidney function to prevent life-threatening hemorrhagic events and renal failure requiring hemodialysis, respectively.[19,20]

MECHANISM OF ACTION

Since TTR is a protein derived from the translation of mRNA, theoretically, one could target the mRNA responsible for producing TTR as a potential site of action for a drug. Inotersen, an antisense oligonucleotide (ASO), was designed to exploit this principle. Like all other ASOs, inotersen follows Watson-Crick hybridization, yielding much more specificity than other small molecules.[24] Since the idea of ASOs was proposed back in 1978, much research has gone into producing ASOs with increased affinity for their target sequence and resisting degradation by nucleases.[24–26] Structural modifications such as adding phosphorothioate substitutions aid in systemic distribution, allowing for various routes of administration.[24,27,28] ASOs are organized into different chemical classes, and although the classes share similar biological properties and physicochemical characteristics, their specific analog or modification has profound effects on potency, pharmacokinetics, and efficacy.[29] Inotersen has a chemical structure that contains 2'-O-methoxyethyl (2'-MOE) nucleic acid analogs, which have been associated with improved pharmacokinetics, improved binding affinity to RNA, increased potency, and reduction in toxicity due to nonspecific protein binding.[24] Once bound to RNA, ASOs have two ways in which they alter RNA metabolism/processing. The first way is termed occupancy-only-mediated and leads to inhibition or enhancement of translation and interferes with interactions between the target RNA and essential proteins. The second way is occupancy-mediated-degradation which describes the way in which inotersen works. Specifically, inotersen triggers RNA cleavage by RNase H1 found in the nucleus and cytoplasm.[24]

Additionally, RNase H1 is highly selective for cleaving RNA when it is present in the form of a DNA-RNA complex, and its activity is optimized when ASOs are designed to have a gap of 2'-deoxy nucleotides flanked by 2'-MOE analogs.[30] Inotersen utilizes all of these factors just described and has been explicitly designed with ten DNA nucleotides flanked by five 2'-MOE analogs on the 5' and 3' ends of the oligonucleotide.[31–33] Thus, by binding to TTR mRNA within the nucleus of hepatocytes and causing degradation via RNase H1, inotersen ultimately prevents the production of TTR protein.[19,31] Of note, since the binding of inotersen occurs in a 3'-untranslated region of the TTR mRNA where no known mutations exist, it is effective at preventing the production of both mutant and wild-type TTR by the liver.[34] The ability to reduce wild-type TTR in addition to mutant TTR has desired implications when considering transthyretin amyloid cardiomyopathy patients as the initial treatment involving liver transplant could not treat the deposition of wild-type TTR and progression of already existing amyloid deposits stated previously.[31]

ANIMAL STUDIES

Preclinical animal studies demonstrated promising results that paved the way for inotersen's use in humans and revealed its ability to reduce TTR levels. A study with trans-

genic mice, cynomolgus monkeys, and humans showed robust reductions of plasma TTR protein obtained in all three species treated with IONIS-TTRRx (Inotersen), which in mice and monkeys was also associated with substantial decreases in hepatic TTR mRNA levels.[33] Specifically, higher doses of the subcutaneously administered drug resulted in reductions exceeding 90% for both plasma TTR and hepatic TTR mRNA in mice when compared to controls. Results for the monkeys were similar and showed an approximate 90% and 80% reduction in hepatic TTR mRNA and plasma TTR protein levels, respectively. These effects were dose-dependent and lasted for weeks post-dosing.[19,33]

CLINICAL TRIALS

In recent studies, TTR silencers have demonstrated promising outcomes in the treatment of hATTR. Historically, hATTR has been considered a progressively fatal disease characterized by the deposition of mutant and wild-type TTR protein in the heart, peripheral nerves, gastrointestinal tracts, and kidneys.[5,35] In patients who have received the current standard of care, TTR stabilizers (tamfamidis and diflunisal), and orthoptic liver transplant, symptoms continue to progress, and the prognosis is reduced at 2 to 15 years following the onset of neuropathy, and 2 to 5 years following cardiomyopathy.[36–47]

With the completion of phase III trials for inotersen (NEURO-TTR) and patisiran (APOLLO), a short interfering RNA (siRNA), patients with hATTR including those with cardiac involvement or polyneuropathy may have new hope for improved prognosis, and symptom relief. Both classes of drugs are considered TTR silencers, and work by decreasing synthesis of both mutant and wild-type TTR, an effect previously unachieved by prior treatment modalities as wild-type TTR production is unaffected and continues to precipitate as amyloid.[20,48]

In 2002, ISIS 104838, a modified ASO belonging to the same class as inotersen (ISIS 420915), demonstrated proof of concept for ASO inhibition of protein synthesis in its phase I trial. The ASO was able to target TNF-alpha mRNA produced by lipopolysaccharide (LPS)-stimulated monocytes and subsequently suppress keratinocyte expression of TNF-alpha protein by 85%. The plasma-terminal half-life was approximately 25 days, and the participants demonstrated overall good tolerance to the administration of the drug. Moreover, intravenous infusions were associated with transient prolongation of partial thromboplastin time. However, those receiving subcutaneous injections had minimal changes to such values. Otherwise, the trial had only two accounts of rashes, one secondary to reversible thrombocytopenia and another injection site tenderness.[49]

In 2013, another study demonstrated proof of concept for RNA interference of TTR with siRNA. At the time, ALN-TTR01 and ALN-TTR02 were compared in a multicenter, randomized, single-blind, placebo-controlled phase 1 trial. ALN-TTR01 and ALN-TTR02 were two formulations of lipid nanoparticles containing identical anti-TTR siRNA. The lipid nanoparticles were designed to facilitate drug delivery to the liver and were ultimately found to be well tolerated and persistent in effect. At 28 days out from a single dose of siRNA, greater than 50% suppression of TTR was observed for both mutant and wild-type protein.[48]

Of the two, ALN-TTR02 was considered for further studies due to its lower rates of adverse effects. A sole participant (7.7%) in the ALN-TTR02 group (n = 13) experienced mild-to-moderate infusion reactions in comparison to 5 (20.8%) in the ALN-TTR01 (n=24) group. Additionally, the one patient that experienced side effects in the ALN-TTR02 group also received a dose of 0.5mg/kg. This dosing was revealed to be higher than the optimal dose of 0.3 mg/kg, where effects on suppression begin to plateau. For such infusion reactions, symptoms resolved with slowing of the infusion rate.[48] Thyroid functions and symptoms of vitamin A deficiency were also monitored due to TTR's role in binding thyroxine and vitamin A.[9,48,50–52] No evidence of dysfunction was noted in any of the participants; however.[48]

In 2018, phase III trials for patisiran and inotersen were completed. Both were multicentered, international, randomized, double-blind, placebo-controlled studies that enrolled patients with a known diagnosis of hATTR with peripheral neuropathy both with and without cardiac involvement.[20,53] In the APOLLO trial for patisiran, 225 patients were randomized in a 2:1 ratio to obtain a 0.3 mg IV infusion of patisiran or placebo every three weeks for 18 months.[53] In the NEURO-TTR trial for inotersen, 172 patients were randomized in a 2:1 ratio to obtain a 15-month trial of weekly 300 mg subcutaneous injection of inoterson or placebo.[20]

In the APOLLO trial, patisiran demonstrated promising effects with only mild to moderate side effects. By three weeks following the first infusion, serum TTR levels were at their nadir, where they remained at a median of 81% suppression across the different subgroups.[53] Clinical outcomes were monitored through Modified Neuropathy Impairment Score+7 (mNIS+7), which factors in motor strength, sensation, reflexes, and autonomic function, and the Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) questionnaire (both rubrics equate higher numbers with increased impairment or worse quality of life).[53,54] At 18 months, the change in the least-square means of mNIS+7 from baseline for the patisiran group had reduced by 6.0, compared with an increase of 28.0 in the placebo group (difference of -34.0 points, P <0 .001). Norfolk QOL-DN at 18 months also reflected improvements with patisiran group as seen by a decrease of 6.7 from baseline in comparison to the placebo group, which increased by 14.4 (difference of -21.1 point difference, P < 0.001). Other endpoints such as motor function and nutritional status were also assessed and favored patisiran over placebo. Patisiran was associated with higher rates of peripheral edema and infusion-related reactions. These events were, however, mild to moderate, and were found to decrease over time.[53]

Similarly, inotersen also had promising outcomes in terms of slowing the progression of hATTR in the NEURO-TTR trial. Across 15 months, least-square means of mNIS+7 increased by 5.8 in the inotersen group and 25.5 in the

placebo group in comparison to baseline (difference of -19.7, P < 0.001), while Norfolk QOL-DN score increased by 1.0 with inotersen and 12.7 with placebo (difference of -11.7, P<0.001). These results were independent of staging, mutation type, and presence of cardiomyopathy. Even though the change in least-squares means of mNIS+7 with inotersen is favorable as compared to patisiran, it is noteworthy that 36% of patients in the inotersen group had improvements in their mNIS+7 and 50% had improvement in their Norfolk QOL-DN score (change from baseline was less than 0). On an individual level, this meant that some patients were experiencing improvements in their polyneuropathy in contrast to slowed and continued progression of the disease. Serum TTR levels achieved steady-state at week 13, where it remained at a nadir of 79.0% suppression.[20]

The most severe adverse effects associated with the inotersen group were thrombocytopenia (3%) and glomerulonephritis (3%). The inotersen group had five accounts of death (4%); 4 of them were likely consistent with the natural progression of hATTR, although Benson et al. were unable to conclude whether this difference in deaths compared to the placebo group (n = 0) was secondary to chance, rapid acceleration of the disease, or other cause. One death was secondary to grade 4 thrombocytopenia leading to intracranial hemorrhage. Two other cases of grade 3 thrombocytopenia were identified and reversed with discontinuation of inotersen and treatment with glucocorticoids. Following the implementation of weekly platelet monitoring, no cases of grade 3 or 4 thrombocytopenia were observed. As for patients that presented with renal complications, all 3 cases revealed crescentic glomerulonephritis on biopsy. One patient had a return of estimated glomerular filtration rate following glucocorticoids and cyclophosphamide, while another went undiagnosed, resulting in permanent hemodialysis. Monitoring of renal function was subsequently scheduled more frequently at every 2-3 weeks. A third patient with renal dysfunction was later discovered in its early stages. The patient presented with only proteinuria without a decline in renal function. Proteinuria resolved following glucocorticoid treatment.[20] After comparison with clinical data from other patients receiving ASO systematic treatment from the same 2'-O-methoxyethyl-modified chemical class, adverse events of thrombocytopenia and glomerulonephritis were likely a drug-disease interaction.[55–57]

Ienotersen and patisiran have demonstrated excellent outcomes in patients with hATTR with polyneuropathy. Inotersen and patisiran both slowed the progression of the disease and even potentially reversed progression as reflected by decreasing mNIS+7 and Norfolk QOL-DN scores.[20,53] It is important to keep in mind that the NEURO-TTR trial for inotersen was three months shorter than the APOLLO trial for patisiran, and the changes in mNIS+7 observed in patisiran were gradual, indicating slow time-dependent improvement. Additionally, inotersen took 13 weeks for suppression of TTR to reach its maximal effect, in comparison to less than three weeks with patisiran.[20,53] It is possible that the four deaths observed within the inotersen group may have potentially skewed results for the inotersen group as the deaths may have been due to a more aggressive form of the disease.

DISCUSSION

Polyneuropathy is the most common phenotypic presentation of hATTR amyloidosis resulting in significant disability. Traditionally, definitive management included liver transplantation, but this is not an option for some patients. In recent years, RNA-targeted therapies started receiving attention, specifically regarding alleviating symptoms of neuropathies as these represent the hallmark of early-onset hATTR amyloidosis. Inotersen is a disease-modifying agent that continues to demonstrate promising results, giving hope that it will improve the disease course and quality of life for patients.

CONCLUSION

hATTR is a rare, progressive, and debilitating disease, commonly presenting with polyneuropathy and affecting multiple organs due to a mutated TTR amyloid deposition. It carries a very poor prognosis with a median survival of fewer than 12 years. Novel therapeutic options with targeted therapies against TTR production and deposition are groundbreaking. The use of RNAi opens the door to the treatment of hereditary diseases by targeting them at a genetic level.

ETHICAL CONSIDERATIONS

HCA Centralized Algorithms for Research Rules on IRB Exemptions (CARRIE)/ IRB manager issued approval [2022-]. Based on the information provided and attested as true, the research plan described does not require IRB oversight. This is because the investigators are not engaging in research with human subjects as defined by federal regulations.

AUTHOR CONTRIBUTIONS

All authors were involved in the writing and editing of the manuscript.

DISCLOSURES

There are no conflict of interests with the authors.

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