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(54) METHODS OF TREATING TRANSTHYRETIN (TTR) MEDIATED AMYLOIDOSIS

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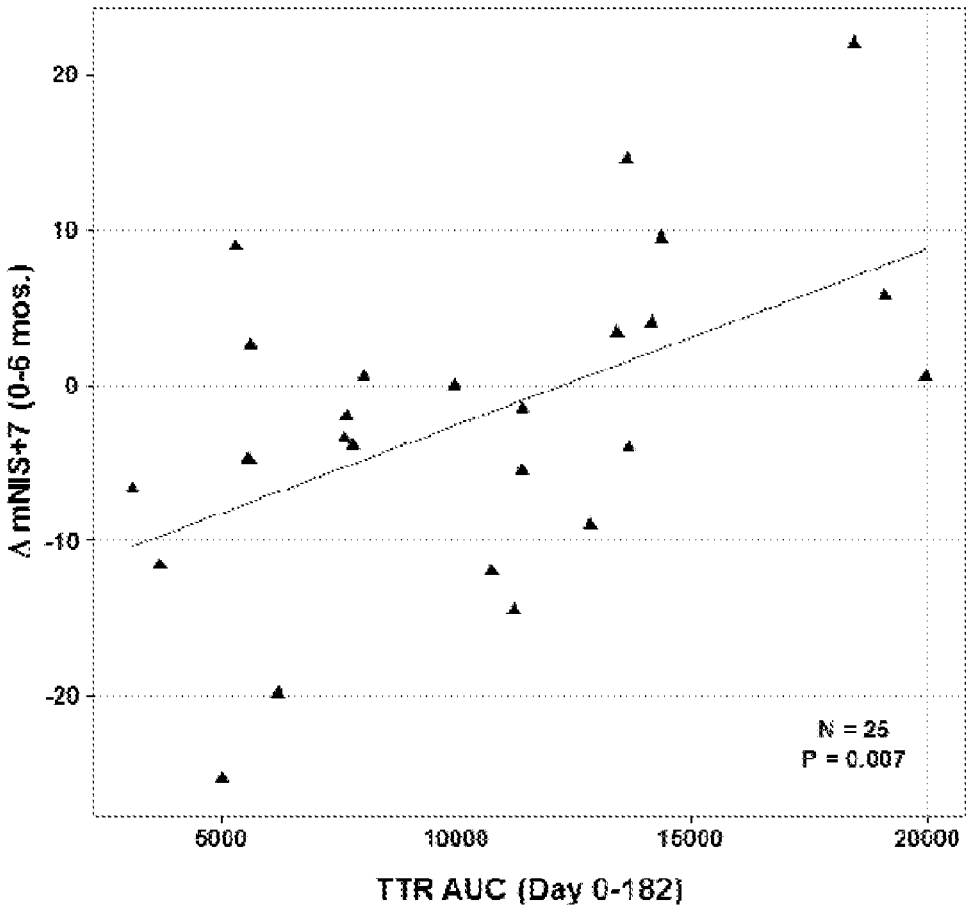
(57) **ABSTRACT**

Disclosed herein are methods for reducing or arresting an increase in a Neuropathy Impairment Score (NIS) or a modified NIS (mNIS+7) in a human subject by administering an effective amount of a transthyretin (TTR)-inhibiting composition.



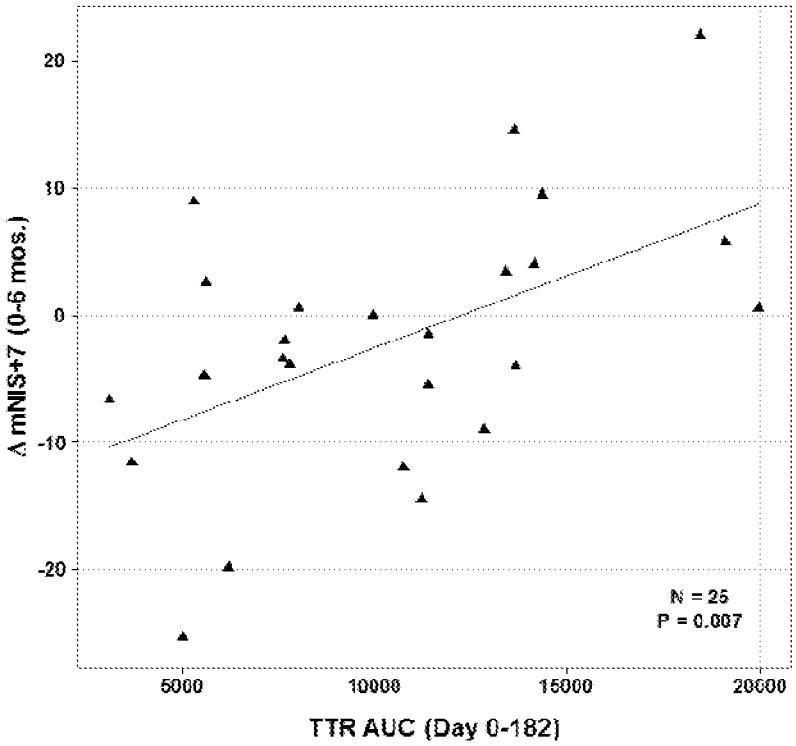


FIG. 1

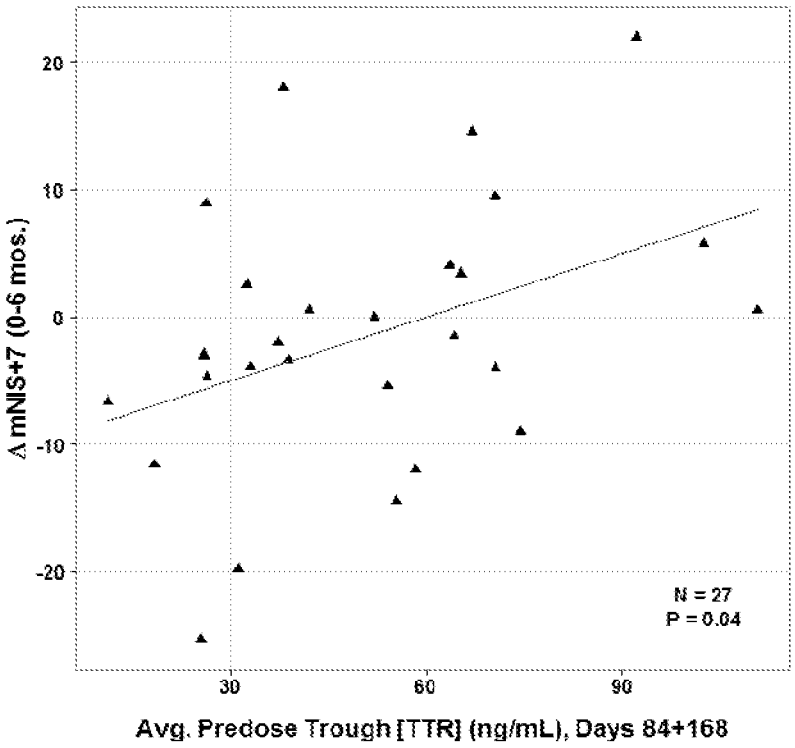


FIG. 2

METHODS OF TREATING TRANSTHYRETIN (TTR) MEDIATED AMYLOIDOSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 62/044,100, filed on Aug. 29, 2014 and to U.S. Provisional Patent Application No. 62/150,596, filed on Apr. 24, 2015, both which are incorporated by reference in their entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] Transthyretin (TTR) is a tetrameric protein produced primarily in the liver. Mutations in the TTR gene destabilize the protein tetramer, leading to misfolding of monomers and aggregation into TTR amyloid fibrils (ATTR). Tissue deposition results in systemic ATTR amyloidosis (Coutinho et al., Forty years of experience with type I amyloid neuropathy. Review of 483 cases. In: Glenner et al., Amyloid and Amyloidosis, Amsterdam: Excerpta Media, 1980 pg. 88-93; Hou et al., Transthyretin and familial amyloidotic polyneuropathy. Recent progress in understanding the molecular mechanism of neurodegeneration. FEBS J 2007, 274: 1637-1650; Westermark et al., Fibril in senile systemic amyloidosis is derived from normal transthyretin. Proc Natl Acad Sci USA 1990, 87: 2843-2845). Over 100 reported TTR mutations exhibit a spectrum of disease symptoms.

[0003] TTR amyloidosis manifests in various forms. When the peripheral nervous system is affected more prominently, the disease is termed familial amyloidotic polyneuropathy (FAP). When the heart is primarily involved but the nervous system is not, the disease is called familial amyloidotic cardiomyopathy (FAC). A third major type of TTR amyloidosis is called leptomeningeal/CNS (Central Nervous System) amyloidosis.

[0004] The most common mutations associated with familial amyloid polyneuropathy (FAP) and ATTR-associated cardiomyopathy, respectively, are Val30Met (Coelho et al., Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. Neurology 2012, 79: 785-792) and Val122Ile (Connors et al., Cardiac amyloidosis in African Americans: comparison of clinical and laboratory features of transthyretin V122I amyloidosis and immunoglobulin light chain amyloidosis. Am Heart J 2009, 158: 607-614).

[0005] Current treatment options for FAP focus on stabilizing or decreasing the amount of circulating amyloidogenic protein. Orthotopic liver transplantation reduces mutant TTR levels (Holmgren et al., Biochemical effect of liver transplantation in two Swedish patients with familial amyloidotic polyneuropathy (FAP-met30). Clin Genet 1991, 40: 242-246), with improved survival reported in patients with early-stage FAP, although deposition of wild-type TTR may continue (Yazaki et al., Progressive wild-type transthyretin deposition after liver transplantation preferentially occurs into myocardium in FAP patients. Am J Transplant 2007, 7:235-242; Adams et al., Rapid progression of familial amyloid polyneuropathy: a multinational natural history study Neurology 2015 Aug. 25; 85(8) 675-82; Yamashita et al., Long-term survival after liver transplantation in patients with familial amyloidotic polyneuropathy. Neurology 2012, 78: 637-643; Okamoto et al., Liver transplantation for familial amyloidotic polyneuropathy: impact on Swedish patients' survival. Liver Transpl 2009, 15:1229-1235; Stangou et al., Progressive cardiac amyloidosis following liver transplantation for familial amyloid polyneuropathy: implications for amyloid fibrillogenesis. Transplantation 1998, 66:229-233; Fosby et al., Liver transplantation in the Nordic countries—An intention to treat and post-transplant analysis from The Nordic Liver Transplant Registry 1982-2013. Scand J Gastroenterol. 2015 June; 50(6):797-808. Transplantation, in press).

[0006] Tafamidis and diflunisal stabilize circulating TTR tetramers, which can slow the rate of disease progression (Berk et al., Repurposing diflunisal for familial amyloid polyneuropathy: a randomized clinical trial. JAMA 2013, 310: 2658-2667; Coelho et al., 2012; Coelho et al., Long-term effects of tafamidis for the treatment of transthyretin familial amyloid polyneuropathy. J Neurol 2013, 260: 2802-2814; Lozeron et al., Effect on disability and safety of Tafamidis in late onset of Met30 transthyretin familial amyloid polyneuropathy. Eur J Neurol 2013, 20: 1539-1545). However, symptoms continue to worsen on treatment in a large proportion of patients, highlighting the need for new, disease-modifying treatment options for FAP.

[0007] Description of dsRNA targeting TTR can be found in, for example, International patent application no. PCT/US2009/061381 (WO2010/048228) and International patent application no. PCT/US2010/055311 (WO2011/056883).

SUMMARY

[0008] Described herein are methods for reducing or arresting an increase in a Neuropathy Impairment Score (NIS) or a modified NIS (mNIS+7) in a human subject by administering an effective amount of a transthyretin (TTR)-inhibiting composition, wherein the effective amount reduces a concentration of TTR protein in serum of the human subject to below 50 μg/ml or by at least 80%. Also described herein are methods for adjusting a dosage of a TTR-inhibiting composition for treatment of increasing NIS or Familial Amyloidotic Polyneuropathy (FAP) by administering the TTR-inhibiting composition to a subject having the increasing NIS or FAP, and determining a level of TTR protein in the subject having the increasing NIS or FAP. In some embodiments, the amount of the TTR-inhibiting composition subsequently administered to the subject is increased if the level of TTR protein is greater than 50 μg/ml, and the amount of the TTR-inhibiting composition subsequently administered to the subject is decreased if the level of TTR protein is below 50 μg/ml. Also described herein are formulated versions of a TTR inhibiting siRNA.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIG. **1** is a graph illustrating the relationship between progression in ΔNIS or ΔmNIS+7 and TTR concentration.

[0010] FIG. **2** is a graph illustrating the relationship between progression in ΔNIS or ΔmNIS+7 and TTR concentration.

DETAILED DESCRIPTION

[0011] As described in more detail below, disclosed herein

(mNIS+7) in a human subject by administering an effective amount of a transthyretin (TTR)-inhibiting composition, such that the effective amount reduces a concentration of TTR protein in serum to below 50 μg/ml or by at least 80%. In one embodiment the TTR-inhibiting composition is patisiran. Patisiran is a small interfering ribonucleic acid (siRNA) which is specific for TTR, formulated in a hepatotropic lipid nanoparticle (LNP) for intravenous (IV) administration.

[0012] TTR-Inhibiting Compositions

[0013] The methods described herein include administration of TTR-inhibiting composition. A TTR-inhibiting composition can be any compound that reduces a concentration of TTR protein in the serum of a human subject. Examples include but are not limited to RNAi, e.g., siRNA. Examples of siRNA include siRNA targeting a TTR gene, e.g., patisirin (described in more detail) below and revusiran. Examples also include antisense RNA. Examples of antisense RNA targeting a TTR gene can be found in U.S. Pat. No. 8,697,860.

[0014] The TTR-inhibiting composition inhibits expression of a TTR gene. As used herein, "transthyretin" ("TTR") refers to a gene in a cell. TTR is also known as ATTR, HsT2651, PALB, prealbumin, TBPA, and transthyretin (prealbumin, amyloidosis type I). The sequence of a human TTR mRNA transcript can be found at NM_000371. The sequence of mouse TTR mRNA can be found at NM_013697.2, and the sequence of rat TTR mRNA can be found at NM_012681.1.

[0015] The terms "silence," "inhibit the expression of," "down-regulate the expression of," "suppress the expression of" and the like in as far as they refer to a TTR gene, herein refer to the at least partial suppression of the expression of a TTR gene, as manifested by a reduction of the amount of mRNA which may be isolated from a first cell or group of cells in which a TTR gene is transcribed and which has or have been treated such that the expression of a TTR gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). The degree of inhibition is usually expressed in terms of

$$\frac{(mRNA \text{ in control cells}) - (mRNA \text{ in treated cells})}{(mRNA \text{ in control cells})} \cdot 100\%$$

[0016] Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to TTR gene expression, e.g., the amount of protein encoded by a TTR gene which is secreted by a cell, or the number of cells displaying a certain phenotype, e.g., apoptosis. In principle, TTR gene silencing may be determined in any cell expressing the target, either constitutively or by genomic engineering, and by any appropriate assay. However, when a reference is needed in order to determine whether a given dsRNA inhibits the expression of a TTR gene by a certain degree and therefore is encompassed by the instant invention, the assays provided in the Examples below shall serve as such reference.

[0017] RNAi

[0018] In some embodiments, the methods described herein use a TTR-inhibiting composition that is an RNAi, [illegible] that targets a TTR gene. The dsRNA includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of a TTR gene, and where the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in length. The dsRNA of the invention can further include one or more single-stranded nucleotide overhangs. TTR-inhibiting siRNAs are described in International patent application no. PCT/US2009/061381 (WO2010/048228) and International patent application no. PCT/US2010/055311 (WO2011/056883), both incorporated by reference herein in their entireties.

[0019] In one embodiment, the TTR-inhibiting composition is patisiran, described in more detail below. In another embodiment, the TTR-inhibiting composition is revusiran, an siRNA specific for TTR conjugated to a Trivalent GalNAc carbohydrate cluster. A complete description of revusiran can be found in international application number PCT/US2012/065691 and US Patent Publication No. US20140315835, the contents of which are incorporated by reference in their entirety.

[0020] A dsRNA includes two RNA strands that are sufficiently complementary to hybridize to form a duplex structure. One strand of the dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence, derived from the sequence of an mRNA formed during the expression of a TTR gene, the other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. The term "antisense strand" refers to the strand of a dsRNA which includes a region that is substantially complementary to a target sequence. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches are most tolerated in the terminal regions and, if present, are generally in a terminal region or regions, e.g., within 6, 5, 4, 3, or 2 nucleotides of the 5' and/or 3' terminus. The term "sense strand," as used herein, refers to the strand of a dsRNA that includes a region that is substantially complementary to a region of the antisense strand. Generally, the duplex structure is between 15 and 80, or 15 and 60 or 15 and 30 or between 25 and 30, or between 18 and 25, or between 19 and 24, or between 19 and 21, or 19, 20, or 21 base pairs in length. In one embodiment the duplex is 19 base pairs in length. In another embodiment the duplex is 21 base pairs in length.

[0021] Each strand of a dsRNA is generally between 15 and 80 or 15 and 60 or 15 and 30, or between 18 and 25, or 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In other embodiments, each is strand is 25-30 nucleotides in length. Each strand of the duplex can be the same length or of different lengths. When two different siRNAs are used in combination, the lengths of each strand of each siRNA can be identical or can differ.

[0022] A dsRNA can include one or more single-stranded overhang(s) of one or more nucleotides. In one embodiment, at least one end of the dsRNA has a single-stranded nucleo[illegible]

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