HOT TOPICS IN NEPHROLOGY

Renal Amyloidosis

Amyloidosis is a group of rare diseases, where a specific protein suffers a pathogenic misfolding in its conformation, with subsequent aggregation, and insoluble fibril formation, acquiring a beta-sheet conformation, that has specific properties. This amyloidogenic protein may accumulate in various organs, including the kidneys, and eventually leads to organ failure. More than 25 different proteins are known to cause systemic or localized amyloidosis in humans.

Treatment is far away from expected and relies on reducing the production of the amyloid precursor protein. So, it is of crucial importance to correctly identify the type of amyloidosis. We can use immunohistochemistry or immunofluorescence on renal tissue. Additionally, mass spectrometry is increasingly used, when the former does not give a definitive answer.

Image

Lambda AL amyloidosis (IF x400).
Courtesy of Dr. Fernanda Carvalho, MD. Renal Morphology Unit – Department of Nephrology, Hospital Curry Cabral, Lisbon.
Do you know that...
... Patients with AL amyloidosis have bleeding diathesis? This is thought to be caused by factor X deficiency, resulting from the binding of factor X to amyloid fibrils. Nevertheless, it seems that patients submitted to renal biopsies don’t have a higher incidence of bleeding complications, compared with patients without amyloidosis.

... Non-mutated TTR protein (wild type) can also lead to amyloidosis? This is a cause of congestive heart failure of unknown etiology, and diagnosis only can be made after detecting TTR amyloid deposits in tissue, in a patient without known mutations.

Bibliography

AA amyloidosis: basic knowledge, unmet needs and future treatments – Laura Obici and Giampaolo Merlini. Swiss Med Wkly, 2012; 142:w13580


Ana Carina Ferreira
Chair of YNP – ERA EDTA

Meet the expert - discussion of a recent paper

Expert – Luisa Lobato, MD, PhD
Safety and Efficacy of RNAi Therapy for Transthyretin Amyloidosis

Teresa Coelho, M.D., David Adams, M.D., Ph.D., Ana Silva, M.D., Pierre Lozeron, M.D., Philip N. Hawkins, Ph.D., F.Med.Sci., Timothy Mant, M.B., Javier Perez, M.D., Joseph Chiesa, M.D., Steve Warrington, M.D., Elizabeth Tranter, M.B., Malathy Munisamy, M.D., Rick Falzone, M.P.H., Jamie Harrop, B.A., Jeffrey Cehelsky, M.B.A., Brian R. Bettencourt, Ph.D., Mary Geissler, M.P.H., James S. Butler, Ph.D., Alifica Sehgal, Ph.D., Rachel E. Meyers, Ph.D., Qingmin Chen, Ph.D., Todd Borland, B.S., Renta M. Hutabarat, Ph.D., Valerie A. Clausen, Ph.D., Rene Alvarez, Ph.D., Kevin Fitzgerald, Ph.D., Christina Gamba-Vitalo, Ph.D., Saraswathy V. Nochur, Ph.D., Akshay K. Vaishnaw, M.D., Ph.D., Dinah W.Y. Sah, Ph.D., Jared A. Gollob, M.D., and Ole B. Suhr, M.D.

Perspective

Coelho et al., reported two Phase I clinical trials in hereditary transthyretin amyloidosis, preceded by nonhuman primates studies.

The perspective of using TTR gene silencing in a sequence-specific manner with small interfering RNAs (siRNA) is innovative, although this type of approach did not show promising results in other types of diseases. So, a stimulating paper will be commented.

Background and rationale of the study

Hereditary transthyretin amyloidosis (ATTR) is an autosomal dominant disease, caused by mutations in the TTR gene. More than 100 pathogenic mutations have been described, the most prevalent being V30M (c.148G.A, p.Val50Met). The precursor protein of circulating transthyretin (TTR) is mainly synthesized and excreted by the liver. Non-mutated TTR also forms amyloid deposits, suggesting that wild-type TTR has an intrinsic amyloidogenic property.

ATTR is the most common hereditary form of amyloidosis and although each TTR variant has a different involvement, peripheral neuropathy and cardiomyopathy are predominant. Kidney deposits are well recognized since the original description of the disease and a renal phenotype is also frequent. Deposition of wild-type TTR characteristically results in the form of cardiomyopathy previously known as senile systemic amyloidosis.

The disorder left untreated progresses to death within 10 years. Twenty-five years ago, liver transplantation was introduced as a treatment, since this suppresses the production of circulating mutant TTR and theoretically stops the amyloid formation.

Stabilizing the properly folded tetrameric protein is regarded to be an efficient strategy for treating ATTR. Several small molecules bind to the T4-binding sites, stabilize the TTR
tetramer and inhibit TTR amyloid fibril formation. Tafamidis, a stabilizer of TTR, is an oral therapy approved for ATTR neuropathy. Diflunisal and other several natural products that inhibit TTR amyloid fibril formation where progressively investigated to stop the disease. Assuming that normal and mutated TTR may be amyloidogenic, a research moved towards the inhibition of TTR synthesis by the liver, the rationale for small interfering RNAs (siRNA) therapy in transthyretin amyloidosis.

The Central Research
Innovation of RNA interference therapy targeting the TTR gene
RNA interference is a cellular mechanism for gene silencing in a sequence-specific manner in which siRNA that are bound to the RNA-induced silencing complex mediate the cleavage of target messenger RNA. Typically, most siRNA enter cells via endocytosis, suggesting that an endosome escape mechanism is required for efficient gene silencing. The development of nanoscale particles targeting the TTR gene requires drug delivery to the liver resulting in reduction of serum TTR levels, reduced immunogenicity and toxicity and progressive improvement of their efficacy. ALN-TTR is a systemically administered lipid nanoparticle-formulation of a siRNA targeting wild-type and all mutant forms of TTR.

Overall methodology
ALN-TTR01 and ALN-TTR02 are first- and second-generation formulations of lipid nanoparticles to deliver siRNA. A stable nucleic acid lipid particle formulation was applied in the Phase 1 trials of ALN-TTR01 and second-generation nanoparticles called MC3 in the Phase 1 trials of ALN-TTR02. To evaluate these two formulations, the studies showed in this paper were performed in nonhuman primates and two sequential phase 1 trials. Both phase 1 trials were multicenter, randomized, single-blind, placebo-controlled, dose-ranging studies to evaluate the safety and efficacy of a single dose of ALN-TTR01 or ALN-TTR02 in patients with transthyretin amyloidosis or in healthy adult volunteers, respectively.

Results
Nonhuman Primate Studies
In nonhuman primates that received ALN-TTR01 in a single dose of 1.0 mg per kilogram, the mean percent transthyretin knockdown at the nadir level (7 days after administration) was approximately 50%, with recovery to the baseline level by day 28. By contrast, ALN-TTR02 showed an improvement in potency by a factor of more than 10, with more than 70% suppression persisting at day 28 in the high-dose group. The transthyretin knockdown and recovery were similar after each dose ALN-TTR02.

Patients with hereditary transthyretin amyloidosis and healthy volunteers
ALN-TTR01
Significant lowering of TTR serum level from baseline was seen in the group receiving higher doses, 1.0 mg/ kilogram as early as day 7, with a mean reduction of 38% in transthyretin levels on day 7. There was considerable variability among participants in the observed degree of transthyretin knockdown. By day 28, there was still more than 50% knockdown, and recovery to the baseline level did not occur until day 70. There was evidence that in the group of V30M mutation there was a strong correlation between
mutant and non-mutant transthyretin results, showing that both forms of the protein were reduced to the same extent, with the same kinetics of lowering and recovery.

**ALN-TTR02**

Substantial transthyretin knockdown was observed in all participants receiving doses of 0.15 to 0.5 mg/kilogram. Transthyretin knockdown was rapid, potent, and durable through day 28. There was a robust response seen at 0.15 and 0.3 mg per kilogram and modest incremental improvement in response at 0.5 mg per kilogram. There was little variability among participants in the kinetics of response, with more than 50% lowering by day 3, a nadir level by approximately day 10, and continued suppression of more than 50% at day 28, with full recovery occurring by day 70.

To further demonstrate the specificity of the effect of ALN-TTR02, transthyretin was also measured in a group of healthy volunteers in a phase 1 trial of ALN-PCS that is formulated in the same type of lipid nanoparticle used in ALN-TTR02. A single dose of 0.4 mg of ALN-PCS/kilogram had no effect on TTR, which showed that the effect of ALN-TTR02 on TTR was due to specific targeting by the RNA and not a nonspecific effect of the formulation of lipid nanoparticles.

**Safety**

There was a reversible decline in levels of retinol-binding protein and vitamin A associated with transthyretin knockdown, consistent with TTR binding of retinol-binding protein. No significant change in thyroid function in participants with up to 94% suppression of transthyretin.

The use of ALN-TTR01 and ALN-TTR02 did not result in any significant changes in hematologic, liver, or renal measurements and there were no drug-related serious adverse events. Mild-to-moderate infusion-related reactions were observed in the two trials. Antibodies to the pegylated lipid component of ALN-TTR02 were not detected in any participants.

**Key messages**

- Hereditary transthyretin amyloidosis is a life threatening disorder that represents the most common form of familial amyloidosis. The amyloidogenic mutated protein is synthesized by the liver. Wild-type TTR has also intrinsic amyloidogenic properties.
- Eliminating mutant TTR with liver transplantation showed that circulating mutated protein causes the systemic disease.
- The inhibition of TTR synthesis by the liver is the basis for gene silencing with small interfering RNAs.
- ALN-TTR01 and ALN-TTR02 are first- and second-generation formulations of lipid nanoparticles to deliver siRNAs targeting the TTR gene.
- The ability of ALN-TTR01 and ALN-TTR02 to suppress hepatic TTR production is different. ALN-TTR02 has excellent potency.
- With this approach both mutant and non-mutant protein levels were reduced, representing a potential benefit in wild-type TTR cardiomyopathy.
These results encourage pursuing with clinical trials based on siRNAs and oligonucleotide platforms in ATTR diseases.

Main limitation
Human studies with ALN-TTR started in 2010. The safety of long-term suppression of transthyretin in patients with transthyretin amyloidosis remains to be determined.

Note
At the end of 2013, Alnylam Pharmaceutics, Inc. receives Fast Track designation for Patisiran (ALN-TTR02) for the treatment of TTR-mediated amyloidosis.

Please send your comments and questions to our experts, through the NDT-Educational blog