Basic Research & Animal Models
Abstract Title: Recognition of human transthyretin aggregates by a novel monoclonal antibody

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Introduction: Biochemical characterization of TTR Y78F showed that this variant adopts a tetrameric conformation as normal TTR and retains the ability to bind T4, indicating a functional tetrameric structure. Under acidic pH the tyr---phe substitution at position 78 is more prone to form fibrils as compared with non mutated TTR. It was hypothesized that Y78F exhibits the characteristics of an intermediate structure in the fibrillogenesis pathway and might represent an early event in TTR amyloidogenesis. Interestingly, this mutation designed in silico was found associated with peripheral neuropathy and cardiopathy.

Objective: To obtain a monoclonal antibody that identifies specifically conformational TTR oligomers.

Methodology: HM30 TTR mice were immunized with recombinant mutant TTR Y78F and the best responder animal was used to perform fusion protocol. Obtained clones were screened for antibody production by testing in a sandwich and direct ELISA assay that selectively detects antibodies recognizing aggregated TTR protein. Oligomers/fibrils preparation was set by incubating recombinant WT TTR at room temperature under magnetic stirring for eight days. Aliquots were harvested at different time-points in order to evaluate the progression process through thioflavinT (ThT) and dot blot immunodetection.

Results: Selected clones recognized: (i) recombinant Y78F TTR; (ii) aggregates/fibrils obtained from oligomerization of recombinant TTR. One stable hybridoma named CE11A11, thereafter referred to as CE11, of the IgM isotype was positive for both above described criteria. Additionally this Mab is able to monitorize the fibril formation process by dot blot. The obtained results were corroborated by ThT analysis.

Conclusion: CE11A11 identifies specifically the presence of TTR aggregates and serves to detect amyloidogenic TTR but not native soluble WT-TTR.
Abstract Title: Patient-specific evaluation of ATTR in cellular models derived from induced pluripotent stem cells

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Mutations of the transthyretin (TTR) protein leads to systemic ATTR-Amyloidosis. Penetrance, onset, and organ specificity of individual TTR mutations show a large patient-specific variability that is hypothesized to be related to the individual genomic background of the patient. Primary target cells of the disease, mostly derived from liver, brain and heart, are limited for studies of pathogenesis. In this project, target cells were differentiated from ATTR patients by the methodology of induced pluripotent stem (iPS) cells. [1]

Urine from ATTR patients and healthy individuals was processed for isolation of renal epithelial cells, followed by reprogramming into iPSCs and differentiation toward hepatocyte-like cells (HLCs). qPCR was used to analyze gene expression. Protein expression was determined by western blot and ELISA. Cell culture supernatants were derived from HLCs and subjected to immunoprecipitation.

Growing evidence suggests the importance of hepatic chaperones for modulation of pathogenesis. Chaperone expression in HLCs derived from ATTR patients was compared to chaperone gene expression of healthy individuals. Chaperones that are predominantly located extracellularly were differently expressed. Expression of the chaperones showed a high correlation with TTR. After TTR knockdown, the correlation was mainly affected in ATTR-HLCs suggesting that variant TTR expression triggers aberrant chaperone expression. Serpin peptidase inhibitor clade A member 1 (SERPINA1) was the only extracellular chaperone that was markedly upregulated after TTR knockdown in ATTR-HLCs. Co-immunoprecipitation revealed that SERPINA1 physically interacts with TTR. In vitro assays indicated that SerpinA1 can interfere with TTR aggregation.

Taken together, our results suggest that extracellular chaperones play a crucial role in ATTR pathogenesis, in particular SerpinA1, which may affect amyloid formation.


Last co-author: Hartmut Schmidt, Universitätsklinikum Münster
Abstract Title: Role of TTR on neuronal progenitor cells derived from induced pluripotent stem cells

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Co-Authors: Hartmut Schmidt, Universitätsklinikum Münster

The role of TTR in neuronal cell tissues is far from being understood. TTR synthesis was demonstrated in motor neurons of the spinal cord, Schwann cells of peripheral nerves, sciatic nerve, dorsal root ganglion cells, and the brain cortex. In addition to the known functions of TTR as a transporter of thyroxine (T4) and retinol, the neurogenic potential of TTR was recently shown.

In this study we explored the role of TTR in induced pluripotent stem cell-derived (iPSC) neural progenitor cells (NPCs) obtained from ATTR patients.

Urine from ATTR patients (n=3) and healthy individuals (n=3) was processed for isolation of renal epithelial cells, followed by reprogramming into iPSCs. iPSCs were converted into single cells and seeded in STEMdiffTM Neural Induction Medium. Medium was replaced daily and cells were expanded for two passages up to day 8. On day 7, plasma-derived TTR (50 µg/ml) and 10% ATTRV30M plasma was added to the medium. Cells were analyzed by qRT-PCR analysis and viability assays.

Differentiation of iPSC-derived NPCs of ATTR patients and healthy individuals generated cells with a prominent neurite spine-like morphology that are highly positive for the neuronal marker Nestin. Comparison of both groups revealed similar efficacy of neuronal differentiation with no significant difference in gene expression of the neuronal markers MAP2, PAX6 or TUJ1. Of note, expression of TTR and LRP2 was similar in both NPCs. Treatment of healthy donor NPCs with TTR resulted in an 18.7±8-fold downregulation of motor neuron and pancreas homeobox 1 (MNX1). Treatment of cells with 10% ATTRV30M and healthy donor plasma over 3 days resulted in a moderate toxicity. No difference was observed with regard of the cell viability after treatment with ATTR and healthy donor plasma (49.5% ± 6.0% and 53.8% ± 8.4% respectively, relative to untreated control).

Our results demonstrate that iPSC-derived NPCs are an excellent tool to study the neurogenic potential of TTR. We show that motoneuron-specific marker gene MNX1 is downregulated after TTR treatment suggesting apoptosis. Our results contribute to the understanding of neuropathogenesis in ATTR patients.

This study was sponsored by Pfizer
Mutated transthyretin (TTR), mainly (>95%) synthesized by hepatocytes, causes hereditary TTR amyloidosis (ATTR). Deposition of destabilized TTR in various tissues is followed by disease, commonly manifested by polyneuropathy and/or cardiomyopathy. Using induced pluripotent stem cells (iPSCs) we recently could identify alpha-1-antitrypsin (SERPINA1, AAT1) as being related to ATTR [1]. In this study we further explored the role of SERPINA1.

The human hepatoma cell line HepG2 was used for analysis of knockdown and Oncostatin-M (OSM) treatment. TTR V30M mice were treated with OSM and antisense oligonucleotide (ASO) directed against mSERPINA1. TTR deposition in tissues was evaluated by immunohistochemical staining. SERPINA1 and TTR were examined by RT-qPCR and ELISA.

In HepG2 cells, knockdown of SERPINA1 mRNA was followed by induction of TTR mRNA and protein (factor 2.2 ± 0.4 and 3.0 ± 0.4, respectively). In contrast OSM, a known inducer of SERPINA1, resulted in downregulation of TTR in a dose-dependent manner. At 100 µM OSM, a 2.0 ± 0.9-fold and 6.0 ± 0.1–fold downregulation of TTR mRNA and protein, respectively, was observed. Inspection of sera derived from TTR knockout mice revealed significantly higher SERPINA1 levels (4.7 ± 0.3 mg/ml) in comparison to wildtype mice (3.2 ± 0.6 mg/ml). In addition, V30M mice also displayed lower SERPINA1 levels (2.9 ± 0.4 mg/ml). OSM treatment of V30M mice for 24 h could induce SERPINA1 by a factor of 1.5 ± 0.1 and 1.9 ± 0.1, while TTR was concomitantly downregulated by 1.6 ± 0.1 and 2.3 ± 0.2 (mRNA and protein, respectively). Downregulation of mSERPINA1 following ASO treatment resulted in an increased TTR deposition in V30M mice (factor 2.46 ± 0.38) in various tissues, including dorsal root ganglion (DRG).

SERPINA1 knockdown, OSM stimulation and ATTR V30M mice suggest a previously unrecognized inverse correlation of SERPINA1/TTR expression at the transcriptional and protein level. The molecular mechanism of the tight SERPINA1/TTR regulation in the liver is however not known. Ongoing experiments will reveal whether SERPINA1 represents a novel player to modulate disease in ATTR.


This study was sponsored by Pfizer
Abstract Title: Development of NTLA-2001, a CRISPR/Cas9 genome editing therapeutic for the treatment of ATTR

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Jessica Seitzer, Intellia Therapeutics
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Introduction: Transthyretin amyloidosis (ATTR) is a progressive disease caused by accumulation of amyloid deposits of misfolded transthyretin (TTR) protein in multiple tissues including the heart, nerves and gastrointestinal tract. Reduction of TTR monomer via stabilization of circulating tetramer and silencing of TTR gene expression in hepatocytes of ATTR patients have emerged as successful therapeutic strategies for chronically-administered medicines. As such, specific disruption (or knockout) of the TTR gene in hepatocytes using the CRISPR/Cas9 gene editing system is a potentially attractive next-generation treatment for ATTR, which may durably reduce the expression of TTR without the need for chronic therapy.

Objectives: To develop NTLA-2001, a lipid nanoparticle (LNP) formulated CRISPR/Cas9 genome editing therapeutic targeting the human TTR gene for the treatment for ATTR.

Methods: We examined the ability of CRISPR/Cas9-LNP to durably reduce the expression of serum TTR in multiple rodent and non-human primate (NHP) preclinical model systems using NTLA-2001 components and/or species-specific surrogates.

Results: A single dose of LNP containing CRISPR/Cas9 and TTR-specific guide (TTR LNP) in mice resulted in >97% reduction in circulating serum TTR protein that was sustained for at least 12 months. Additionally, in a humanized mouse model of hATTR expressing the V30M mutant form of the human TTR protein, rescue of TTR deposition in multiple tissues after a single dose of TTR LNP was demonstrated. NHPs receiving a single dose of TTR LNP achieved a therapeutically meaningful serum TTR reduction (>95%) that correlated with robust whole liver editing. Different effective doses were well tolerated. In NHP, components of TTR LNP were cleared from plasma and liver with half-lives of 23 and 17 hours, respectively.

Conclusions: One-time gene disruption of the TTR gene by CRISPR/Cas9-LNP LNP resulted in durable and robust decrease in serum TTR protein levels in vivo. Favorable tolerability of the delivery system was aided by transient exposure to the CRISPR/Cas9 and LNP components. These findings show pharmacologic activity of TTR LNP and support further development of NTLA-2001 for the treatment for patients with ATTR polyneuropathy and/or cardiomyopathy.

Additional authors:
Seth Alexander, Tracy DiMezzo, Adam Amaral, Samantha Soukamneuth, Arti Kanjolia, Rubina Parmar, Ellen Rodhe, Reynald Lescarbeau, Cindy Shaw, Tanner Dirstine, Carri Boiselle, Kathryn Walsh, Bo Han, Maria Saraiva*, Eva Essig, John Leonard, Michael McCaman, Yong Chang

*Affiliation: University of Porto; Affiliation of all other authors: Intellia Therapeutics
Objective

Transthyretin (TTR) is one of the major amyloidogenic proteins and causes two types, hereditary TTR (ATTRm) and non-hereditary TTR (ATTRwt) amyloidosis. One is hereditary TTR (ATTRm) amyloidosis previously known as familial amyloid polyneuropathy. The other type of ATTR amyloidosis is wild-type TTR (ATTRwt) amyloidosis previously known as senile systemic amyloidosis. In recent years, several therapeutic strategies to reduce or stabilize amyloidogenic TTR have been proposed. However, we still don't have therapies directly inhibiting amyloid fibrils formation or disrupting disease-causing amyloid fibrils in ATTR amyloidosis. In this study, we developed a novel high throughput screening (HTS) method using highly amyloidogenic C-terminal fragments of TTR and performed drug screening using the HTS methods.

Methods

We used recombinant C-terminal region of TTR expressed by Escherichia coli. For the amyloid formation in cultured cells, cells were seeded in half-area 96-well culture plates. Cells were treated with TTR in Opti-MEM containing 1% penicillin/streptomycin solution. We detected amyloid deposits in cultured cells by 1-Fluoro-2,5-bis [(E)-3-carboxy-4-hydroxystyryl] benzene (FSB) staining and enzyme-linked immunosorbent assay (ELISA). We performed drug screening using a library consisting of 1,280 off-patent drugs. [Results and Discussion] The C-terminal region of TTR easily formed amyloid fibrils in cultured cells. We also developed a novel cell-based HTS method using 96-well half-area microplates for evaluating TTR amyloid formation in cultured cells. The Z’-factor of our novel cell-based HTS method was sufficient for screening of drugs. Using this HTS method, we found several candidate drugs to inhibit amyloid formation.

Conclusion

Our novel HTS method directly targeting TTR amyloid formation may be useful for discovery of novel drugs for ATTR amyloidosis.
Cardiac Amyloidosis
Abstract Title: Hemodynamic profiles in patients with cardiac amyloidosis

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Introduction:

Pulmonary arterial pressures are strong predictors of outcome in various types of heart failure (HF). However, their role in patients with cardiac amyloidosis (CA) is less clear.

Objectives:

We aimed to characterize hemodynamic profiles of CA patients and assess their association with outcomes.

Methodology:

The present study was conducted within a prospective, national CA registry. Patients underwent invasive hemodynamic, clinical, laboratory, echocardiography, and cardiac magnetic resonance assessment. The primary endpoint was combination of hospitalization for HF or death from cardiovascular causes.

Results:

63 CA patients were consecutively enrolled. 36 had cardiac transthyretin amyloidosis (ATTR) and 25 cardiac light-chain amyloidosis (AL). In 2 patients subtyping was not possible. Median age was 74y and 62% were male. Almost half were in New York Heart Association (NYHA) class ≥III (48%) and showed an elevated NT-proBNP with a median of 3222pg/mL.

Compared to AL, cardiac TTR patients were older (75y vs. 69y, p=0.004), more often male (81% vs. 40%, p=0.001), less symptomatic (NYHA≥III: 39% vs. 64%, p=0.021), and had lower NT-proBNP values (2324pg/mL vs. 5151pg/mL, p=0.004). Hemodynamic profiling revealed significantly increased intracardiac and pulmonary arterial pressures (PAP). On an average, pulmonary artery wedge pressure was 20mmHg mean PAP was 30mmHg, and mean right atrial pressure was 11mmHg. No differences between ATTR and AL patients could be detected. During follow-up, 28 study participants (44%) reached the combined endpoint. Cardiac AL patients had significantly more events as their ATTR counterparts (72% vs. 28%, p=0.001). In cardiac ATTR patients, mPAP was significantly associated with outcome [HR: 1.083, p=0.034], which was not the case in the AL group (HR: 1.024, p=0.186).

Conclusion:

Despite differences in the severity of symptoms, no differences with regards to hemodynamic profiles could be detected. Furthermore, intracardiac filling and pulmonary arterial pressures seem to be of greater clinical importance in cardiac ATTR as compared to cardiac AL.
<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients (n=63)</th>
<th>TTR amyloidosis (n=36)</th>
<th>AL amyloidosis (n=25)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical parameters</strong></td>
<td></td>
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</tr>
<tr>
<td>Age, years (IQR)</td>
<td>74.0 (63.0 – 78.0)</td>
<td>75.0 (70.0 – 81.5)</td>
<td>69.0 (55.0 – 75.5)</td>
<td>0.004</td>
</tr>
<tr>
<td>Sex, male gender, n (%)</td>
<td>39 (61.9)</td>
<td>29 (80.6)</td>
<td>10 (40.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>NYHA functional class ≥ III, n (%)</td>
<td>30 (47.6)</td>
<td>14 (38.9)</td>
<td>16 (64.0)</td>
<td>0.021</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg (IQR)</td>
<td>120 (112 – 140)</td>
<td>124 (114 – 139)</td>
<td>118 (103 – 143)</td>
<td>0.360</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg (IQR)</td>
<td>68.8 (61.8 – 78.0)</td>
<td>67.0 (61.0 – 76.0)</td>
<td>69.0 (63.5 – 78.5)</td>
<td>0.596</td>
</tr>
<tr>
<td>NT-proBNP, pg/mL (IQR)</td>
<td>3222 (1493 – 7213)</td>
<td>2324 (1351 – 5777)</td>
<td>5151 (2457 – 10799)</td>
<td>0.004</td>
</tr>
<tr>
<td>Troponin t, ng/mL (IQR)</td>
<td>0.07 (0.03 – 0.15)</td>
<td>0.07 (0.03 – 0.12)</td>
<td>0.07 (0.03 – 0.21)</td>
<td>0.777</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73m² (IQR)</td>
<td>65.5 (45.9 – 82.2)</td>
<td>60.8 (44.3 – 82.1)</td>
<td>69.7 (55.6 – 84.4)</td>
<td>0.278</td>
</tr>
<tr>
<td>Combined endpoint, n (%)</td>
<td>28 (44.4)</td>
<td>10 (27.8)</td>
<td>18 (72.0)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Co-morbidities</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Atrial fibrillation, n (%)</td>
<td>34 (54.0)</td>
<td>26 (72.2)</td>
<td>8 (32.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Arterial hypertension, n (%)</td>
<td>36 (57.1)</td>
<td>24 (66.7)</td>
<td>10 (40.0)</td>
<td>0.039</td>
</tr>
<tr>
<td>Coronary artery disease, n (%)</td>
<td>13 (20.6)</td>
<td>10 (27.8)</td>
<td>3 (12.0)</td>
<td>0.139</td>
</tr>
<tr>
<td>Polyneuropathy, n (%)</td>
<td>18 (28.6)</td>
<td>14 (38.9)</td>
<td>4 (16.0)</td>
<td>0.080</td>
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<tr>
<td>Cardiopulmonary resuscitation, n (%)</td>
<td>5 (7.9)</td>
<td>2 (6.6)</td>
<td>3 (12.0)</td>
<td>0.367</td>
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<tr>
<td>Diabetes mellitus type II, n (%)</td>
<td>10 (15.9)</td>
<td>5 (13.9)</td>
<td>4 (16.0)</td>
<td>0.819</td>
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<td><strong>Medication</strong></td>
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<td>Beta Blocker, n (%)</td>
<td>35 (55.6)</td>
<td>18 (51.4)</td>
<td>15 (60.0)</td>
<td>0.511</td>
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<td>ACE inhibitor, n (%)</td>
<td>14 (22.2)</td>
<td>8 (22.2)</td>
<td>6 (24.0)</td>
<td>0.918</td>
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<tr>
<td>Angiotensin receptor blocker, n (%)</td>
<td>19 (30.2)</td>
<td>12 (33.3)</td>
<td>6 (24.0)</td>
<td>0.391</td>
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<tr>
<td>Loop diuretic, n (%)</td>
<td>20 (76.9)</td>
<td>20 (55.6)</td>
<td>20 (80.0)</td>
<td>0.064</td>
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<td>Thiazide, n (%)</td>
<td>15 (23.8)</td>
<td>8 (22.2)</td>
<td>5 (20.0)</td>
<td>0.791</td>
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<td>Mineralocorticoid antagonist, n (%)</td>
<td>36 (57.1)</td>
<td>18 (60.0)</td>
<td>17 (68.0)</td>
<td>0.199</td>
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<td>Antiarrhythmic agent, n (%)</td>
<td>11 (17.5)</td>
<td>4 (11.1)</td>
<td>5 (20.0)</td>
<td>0.359</td>
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<td>Oral anticoagulant, n (%)</td>
<td>36 (57.1)</td>
<td>24 (66.7)</td>
<td>10 (40.0)</td>
<td>0.028</td>
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<td>Antiplatelet agent, n (%)</td>
<td>17 (27.0)</td>
<td>9 (25.0)</td>
<td>8 (32.0)</td>
<td>0.594</td>
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<td>EGCQ, n (%)</td>
<td>19 (30.2)</td>
<td>16 (44.4)</td>
<td>3 (12.0)</td>
<td>0.007</td>
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<td>Tafamidis, n (%)</td>
<td>5 (7.9)</td>
<td>5 (13.9)</td>
<td>0 (0.0)</td>
<td>0.052</td>
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<td>Immunotherapy, n (%)</td>
<td>6 (9.5)</td>
<td>0 (0.0)</td>
<td>6 (24.0)</td>
<td>0.002</td>
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### Invasive hemodynamic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (IQR)</th>
<th>Value (IQR)</th>
<th>Value (IQR)</th>
<th>Value (IQR)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic pulmonary arterial pressure, mmHg (IQR)</td>
<td>44.5 (37.8 – 53.3)</td>
<td>48.0 (39.0 – 53.0)</td>
<td>44.0 (37.0 – 67.0)</td>
<td>0.713</td>
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<td>Diastolic pulmonary arterial pressure, mmHg (IQR)</td>
<td>21.5 (16.8 – 26.0)</td>
<td>22.0 (16.0 – 24.0)</td>
<td>21.0 (16.0 – 30.0)</td>
<td>0.311</td>
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<tr>
<td>Mean pulmonary arterial pressure, mmHg (IQR)</td>
<td>30.0 (25.0 – 37.0)</td>
<td>30.0 (26.0 – 35.5)</td>
<td>31.0 (25.0 – 44.0)</td>
<td>0.398</td>
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<td>Right atrial pressure, mmHg (IQR)</td>
<td>11.0 (7.0 – 16.0)</td>
<td>11.0 (7.0 – 22.0)</td>
<td>11.0 (7.00 – 17.5)</td>
<td>0.620</td>
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<td>Pulmonary artery wedge pressure, mmHg (IQR)</td>
<td>20.0 (17.0 – 25.0)</td>
<td>19.0 (16.3 – 22.0)</td>
<td>21.0 (16.5 – 30.0)</td>
<td>0.148</td>
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<td>Cardiac output, L/min (IQR)</td>
<td>4.4 (3.8 – 5.1)</td>
<td>4.6 (3.9 – 5.2)</td>
<td>4.0 (3.5 – 5.2)</td>
<td>0.306</td>
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<td>Stroke volume, mL (IQR)</td>
<td>56.9 (46.2 – 76.3)</td>
<td>61.3 (48.5 – 77.6)</td>
<td>57.4 (45.9 – 71.5)</td>
<td>0.554</td>
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<td>Arterial oxygen saturation, % (IQR)</td>
<td>95.7 (93.5 – 96.4)</td>
<td>96.0 (93.0 – 96.4)</td>
<td>96.0 (93.9 – 96.7)</td>
<td>0.786</td>
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<td>Pulmonary pulse pressure, mmHg (IQR)</td>
<td>24.0 (19.0 – 30.3)</td>
<td>24.0 (20.0 – 30.0)</td>
<td>25.0 (19.9 – 36.0)</td>
<td>0.752</td>
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<tr>
<td>Pulmonary vascular resistance, dyn s cm⁻⁵ (IQR)</td>
<td>183 (127 – 264)</td>
<td>185 (130 – 244)</td>
<td>192 (129 – 273)</td>
<td>0.929</td>
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<td>Transpulmonary gradient, mmHg (IQR)</td>
<td>11.0 (8.0 – 13.0)</td>
<td>11.0 (9.0 – 13.0)</td>
<td>10.0 (7.0 – 13.5)</td>
<td>0.571</td>
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<td>Diastolic pressure gradient, mmHg (IQR)</td>
<td>1.0 (-1.0 – 3.3)</td>
<td>1.0 (-1.0 – 4.0)</td>
<td>0.0 (-2.0 – 3.0)</td>
<td>0.465</td>
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### Cardiac magnetic resonance imaging parameters

<table>
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<tr>
<th>Parameter</th>
<th>Value (IQR)</th>
<th>Value (IQR)</th>
<th>Value (IQR)</th>
<th>Value (IQR)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOLLI-ECV, % (IQR)</td>
<td>47.8 (41.7 – 60.0)</td>
<td>48.0 (41.7 – 57.5)</td>
<td>47.2 (41.4 – 66.0)</td>
<td>0.935</td>
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<td>Left atrial area, cm² (IQR)</td>
<td>31.0 (26.0 – 36.0)</td>
<td>32.0 (28.0 – 41.0)</td>
<td>27.0 (22.5 – 33.0)</td>
<td>0.025</td>
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<tr>
<td>Right atrial area, cm² (IQR)</td>
<td>28.0 (24.0 – 35.5)</td>
<td>29.0 (25.0 – 38.0)</td>
<td>24.0 (22.5 – 30.0)</td>
<td>0.014</td>
<td></td>
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<tr>
<td>Left ventricular ejection fraction, % (IQR)</td>
<td>57.0 (50.5 – 66.0)</td>
<td>55.0 (50.0 – 50.0)</td>
<td>63.0 (52.5 – 67.5)</td>
<td>0.067</td>
<td></td>
</tr>
<tr>
<td>Left ventricular end-diastolic volume, mL (IQR)</td>
<td>135 (101 – 169)</td>
<td>155 (127 – 181)</td>
<td>105 (78.5 – 139)</td>
<td>0.001</td>
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<tr>
<td>Interventricular septum, mm (IQR)</td>
<td>19.0 (16.0 – 22.0)</td>
<td>20.0 (17.0 – 23.0)</td>
<td>17.0 (13.5 – 20.0)</td>
<td>0.011</td>
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</tr>
<tr>
<td>Pulmonary artery diameter, mm (IQR)</td>
<td>28.0 (25.0 – 32.0)</td>
<td>29.0 (26.0 – 33.0)</td>
<td>26.0 (23.5 – 29.0)</td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>Right ventricular ejection fraction, % (IQR)</td>
<td>51.0 (41.0 – 62.0)</td>
<td>49.0 (41.0 – 61.0)</td>
<td>52.0 (42.0 – 62.0)</td>
<td>0.867</td>
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</tr>
<tr>
<td>Right ventricular end-diastolic volume, mL (IQR)</td>
<td>153 (119 – 189)</td>
<td>168 (141 – 191)</td>
<td>123 (108 – 165)</td>
<td>0.024</td>
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<tr>
<td>Pleural effusion, n (%)</td>
<td>22 (24.9)</td>
<td>9 (25.0)</td>
<td>13 (52.0)</td>
<td>0.019</td>
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<tr>
<td>Pericardial effusion, n (%)</td>
<td>26 (41.3)</td>
<td>12 (33.3)</td>
<td>14 (56.0)</td>
<td>0.048</td>
<td></td>
</tr>
</tbody>
</table>

TTR indicates transthyretin, AL, light chain; IQR, interquartile range; NYHA, New York Heart Association; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; eGFR, estimated glomerular filtration rate; ACE, angiotensin converting enzyme; ECGG, epigallocatechin gallate; MOLLI-ECV, modified Look-Locker inversion recovery sequence derived extracellular volume.

* including 2 patients with CA of unknown subtype
<table>
<thead>
<tr>
<th>Variable</th>
<th>Crude hazard ratio</th>
<th>95% Confidence interval</th>
<th>P value</th>
<th>Adjusted hazard ratio⁺</th>
<th>95% Confidence interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td><strong>Clinical parameters</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Age, years</td>
<td>1.003</td>
<td>0.963 – 1.045</td>
<td>0.889</td>
<td></td>
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<tr>
<td>Sex, male gender</td>
<td>0.850</td>
<td>0.391 – 1.850</td>
<td>0.683</td>
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<tr>
<td>NYHA functional class ≥ III</td>
<td>4.506</td>
<td>1.788 – 11.354</td>
<td>0.001</td>
<td>2.402</td>
<td>1.487 – 3.880</td>
<td>0.016</td>
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<tr>
<td>Systolic blood pressure, mmHg</td>
<td>0.978</td>
<td>0.959 – 0.996</td>
<td>0.020</td>
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<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>0.983</td>
<td>0.950 – 1.017</td>
<td>0.319</td>
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<tr>
<td>NT-proBNP, pg/mL⁺</td>
<td>2.510</td>
<td>1.641 – 3.839</td>
<td>&lt;0.001</td>
<td>3.184</td>
<td>1.239 – 8.176</td>
<td>&lt;0.001</td>
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<tr>
<td>Troponin t, ng/mL†</td>
<td>0.998</td>
<td>0.984 – 1.012</td>
<td>0.759</td>
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<tr>
<td>eGFR, mL/min/1.73m²</td>
<td>0.980</td>
<td>0.964 – 1.012</td>
<td>0.759</td>
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<tr>
<td><strong>Co-morbidities</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Atrial fibrillation</td>
<td>0.381</td>
<td>0.178 – 0.817</td>
<td>0.013</td>
<td>0.381</td>
<td>0.178 – 0.817</td>
<td>0.013</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>0.877</td>
<td>0.413 – 1.860</td>
<td>0.732</td>
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<tr>
<td>Coronary artery disease</td>
<td>1.716</td>
<td>0.754 – 3.904</td>
<td>0.198</td>
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<tr>
<td>Polyneuropathy</td>
<td>0.981</td>
<td>0.427 – 2.251</td>
<td>0.964</td>
<td></td>
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<tr>
<td>Cardiopulmonary resuscitation</td>
<td>2.312</td>
<td>0.689 – 7.764</td>
<td>0.175</td>
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<tr>
<td>Diabetes mellitus type II</td>
<td>1.230</td>
<td>0.494 – 3.063</td>
<td>0.657</td>
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<tr>
<td><strong>Concomitant medication</strong></td>
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<tr>
<td>Beta Blocker</td>
<td>0.819</td>
<td>0.384 – 1.747</td>
<td>0.605</td>
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<tr>
<td>ACE inhibitor</td>
<td>1.063</td>
<td>0.427 – 2.646</td>
<td>0.896</td>
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<tr>
<td>Angiotensin receptor blocker</td>
<td>0.670</td>
<td>0.283 – 1.588</td>
<td>0.363</td>
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<tr>
<td>Loop diuretic</td>
<td>2.718</td>
<td>1.027 – 7.192</td>
<td>0.044</td>
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<tr>
<td>Thiazide</td>
<td>0.426</td>
<td>0.146 – 1.238</td>
<td>0.117</td>
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<tr>
<td>Mineralocorticoid antagonist</td>
<td>3.447</td>
<td>1.388 – 8.561</td>
<td>0.008</td>
<td>3.447</td>
<td>1.388 – 8.561</td>
<td>0.008</td>
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<tr>
<td>Antiarrhythmic agent</td>
<td>1.382</td>
<td>0.515 – 3.705</td>
<td>0.521</td>
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<tr>
<td>Oral anticoagulant</td>
<td>0.658</td>
<td>0.308 – 1.403</td>
<td>0.279</td>
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<tr>
<td>Antiplatelet agent</td>
<td>1.338</td>
<td>0.609 – 2.942</td>
<td>0.469</td>
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<tr>
<td>EGCG</td>
<td>0.534</td>
<td>0.216 – 1.322</td>
<td>0.175</td>
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<tr>
<td>Tafamidis</td>
<td>0.041</td>
<td>0.000 – 8.492</td>
<td>0.241</td>
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<tr>
<td>Immunotherapy</td>
<td>1.424</td>
<td>0.425 – 4.767</td>
<td>0.567</td>
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</table>
### Invasive hemodynamic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (Mean ± SD)</th>
<th>Value (IQR)</th>
<th>p-value</th>
<th>Value (Mean ± SD)</th>
<th>Value (IQR)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pulmonary arterial pressure, mmHg</td>
<td>1.053 ± 0.002</td>
<td>1.019 – 1.087</td>
<td>0.002</td>
<td>1.052 ± 0.002</td>
<td>1.018 – 1.087</td>
<td>0.002</td>
</tr>
<tr>
<td>Right atrial pressure, mmHg</td>
<td>1.064 ± 0.025</td>
<td>1.008 – 1.124</td>
<td>0.025</td>
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<tr>
<td>Pulmonary artery wedge pressure, mmHg</td>
<td>1.084 ± 0.008</td>
<td>1.021 – 1.151</td>
<td>0.008</td>
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<tr>
<td>Cardiac output, L/min</td>
<td>0.804 ± 0.144</td>
<td>0.600 – 1.078</td>
<td>0.144</td>
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<tr>
<td>Stroke volume, mL</td>
<td>0.985 ± 0.063</td>
<td>0.969 – 1.001</td>
<td>0.063</td>
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<tr>
<td>Pulmonary pulse pressure, mmHg</td>
<td>1.031 ± 0.063</td>
<td>0.998 – 1.065</td>
<td>0.063</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Pulmonary vascular resistance, dyn-s·cm⁻¹</td>
<td>1.002 ± 0.089</td>
<td>1.000 – 1.003</td>
<td>0.089</td>
<td></td>
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<tr>
<td>Transpulmonary gradient, mmHg</td>
<td>1.043 ± 0.061</td>
<td>0.996 – 1.090</td>
<td>0.061</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic pressure gradient, mmHg</td>
<td>1.027 ± 0.555</td>
<td>0.940 – 1.122</td>
<td>0.555</td>
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</table>

### Cardiac magnetic resonance imaging

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (Mean ± SD)</th>
<th>Value (IQR)</th>
<th>p-value</th>
<th>Value (Mean ± SD)</th>
<th>Value (IQR)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOLLI ECV, %</td>
<td>1.039 ± 0.016</td>
<td>1.007 – 1.072</td>
<td>0.016</td>
<td>1.039 ± 0.012</td>
<td>1.008 – 1.070</td>
<td>0.012</td>
</tr>
<tr>
<td>Left atrial area, cm²</td>
<td>0.979 ± 0.461</td>
<td>0.927 – 1.035</td>
<td>0.461</td>
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</tr>
<tr>
<td>Right atrial area, cm²</td>
<td>0.953 ± 0.096</td>
<td>0.900 – 1.009</td>
<td>0.096</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>1.024 ± 0.201</td>
<td>0.988 – 1.061</td>
<td>0.201</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left ventricular end-diastolic volume, mL</td>
<td>0.987 ± 0.007</td>
<td>0.977 – 0.996</td>
<td>0.007</td>
<td>0.986 ± 0.004</td>
<td>0.976 – 0.995</td>
<td>0.004</td>
</tr>
<tr>
<td>Interventricular septum, mm</td>
<td>1.036 ± 0.486</td>
<td>0.938 – 1.145</td>
<td>0.486</td>
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<tr>
<td>Pulmonary artery diameter, mm</td>
<td>0.973 ± 0.590</td>
<td>0.883 – 1.074</td>
<td>0.590</td>
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<tr>
<td>Right ventricular ejection fraction, %</td>
<td>1.003 ± 0.862</td>
<td>0.969 – 1.038</td>
<td>0.862</td>
<td></td>
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<tr>
<td>Right ventricular end-diastolic volume, mL</td>
<td>0.992 ± 0.098</td>
<td>0.982 – 1.002</td>
<td>0.098</td>
<td></td>
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<tr>
<td>Pleural effusion</td>
<td>2.272 ± 0.060</td>
<td>0.967 – 5.336</td>
<td>0.060</td>
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<tr>
<td>Pericardial effusion</td>
<td>3.060 ± 0.015</td>
<td>1.242 – 7.538</td>
<td>0.015</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

TTR indicates transthyretin; AL, light chain; IQR, interquartile range; NYHA, New York Heart Association; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; eGFR, estimated glomerular filtration rate; ACE, angiotensin converting enzyme; EGCG, epigallocatechin gallate; MOLLI-ECV, modified Look-Locker inversion recovery sequence derived extracellular volume.

* including 2 patients with CA of unknown subtype
The first graph shows the relationship between time to combined endpoint (weeks) and cardiac death or heart failure hospitalization (%) for patients with or without pulmonary hypertension. The p-value for this comparison is 0.034.

The second graph also illustrates the relationship between time to combined endpoint (weeks) and cardiac death or heart failure hospitalization (%) for patients with or without pulmonary hypertension. The p-value for this comparison is 0.186.
Abstract Title: Study Design and Rationale of APOLLO-B: A Study to Evaluate Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy

Author: John Vest, Alnylam Pharmaceuticals

Co-Authors: Verena Karsten, Alnylam Pharmaceuticals, Cambridge MA 02142
Jihong Chen, Alnylam Pharmaceuticals, Cambridge MA 02142
Nancy Silliman, Alnylam Pharmaceuticals, Cambridge MA 02142
Mr, Dr, Matthew T White, Alnylam Pharmaceuticals, Cambridge MA 02142
Mr, Dr, Richard Riese, Alnylam Pharmaceuticals, Cambridge MA 02142

Introduction:

ATTR amyloidosis is a progressive, life-threatening disease due to accumulation of amyloid fibrils in multiple organs and tissues. Patisiran is an RNA interference (RNAi) therapeutic approved in certain countries for the treatment of hATTR amyloidosis with polyneuropathy. Patisiran is being further developed for the treatment of ATTR (hATTR and wtATTR) amyloidosis with cardiomyopathy (CM), based on preliminary evidence generated from exploratory analyses in a cardiac subpopulation of the Phase 3 APOLLO study.

Objectives:

Describe the rationale and design of APOLLO-B, a Phase 3, randomized, double-blind, placebo-controlled, multicenter study to evaluate the efficacy and safety of patisiran in patients with ATTR amyloidosis with CM.

Methodology:

APOLLO-B will enroll approximately 300 adult patients with ATTR amyloidosis with CM. Key inclusion criteria: medical history of heart failure, NT-proBNP >600 ng/L and <8500 ng/L at screening. Patients can either be tafamidis-naïve or on tafamidis for ≥6 months with evidence of disease progression at baseline. Key exclusion criteria: NYHA class III and at high risk based on published criteria (Gillmore et al., 2018), NYHA Class IV or PND score ≥IIIa. Patients will be randomized (1:1) to receive either patisiran 0.3 mg/kg IV or placebo IV, q3W during the 12-month double-blind period. During the subsequent 12-month open-label extension period, all patients will receive patisiran. The primary objective is to determine the efficacy of patisiran vs placebo based on the change from baseline in 6-MWT at 12 months.

Secondary objectives are to evaluate the efficacy of patisiran vs placebo on healthrelated quality of life (change from baseline in Kansas City Cardiomyopathy Questionnaire Overall Summary score) and mortality and hospitalizations over 12 months. Safety of patisiran will be assessed throughout the study.

Results: APOLLO-B is planned to begin enrollment in the second half of 2019.

Conclusions:

APOLLO-B will investigate the efficacy and safety of patisiran in patients with ATTR amyloidosis with CM, with the potential to address a significant unmet need in the ATTR amyloidosis community.

Abstract Title: Increased mortality associated with transthyretin cardiac amyloidosis in Afro-Caribbean patients with unexplained left ventricular hypertrophy

Author: Rishika Banydeen, University Hospital of Martinique

Co-Authors: Jules Lassus, University Hospital of Martinique
Astrid Monfort-Brafine, University Hospital of Martinique
Jocelyn Inamo, University Hospital of Martinique

Introduction & Objectives

Transthyretin cardiac amyloidosis (ATTR) is usually associated with an increased frequency of heart failure but not with increased mortality in individuals of African ancestry. Unexplained left ventricular hypertrophy (LVH) raises ATTR suspicion. A recent study in the French Caribbean island of Martinique, with a predominant Afro-Caribbean population, found a high ATTR frequency (32.9%) among 167 LVH patients. This current preliminary report addresses mortality risk in those patients.

Methods

The prospective TEAM-study, conducted in 2 hospitals of Martinique from January 2013 to June 2014, screened patients, with either left ventricular wall thickness ≥ 15 mm or wall sparkling, for ATTR. Vital status was determined for all patients up to July 2018, mainly by checking the national medical information system (PMSI), which records hospitalization and ambulatory visits in all French public hospitals. When necessary, patients were contacted by telephone to collect vital status data, and cause of death when applicable. Survival analysis consisted in Kaplan-Meier and Cox analyses.

Results

Vital status could be determined for 113 out of 167 patients screened for the TEAM-study: 41 with ATTR, 72 with unexplained LVH. No significant difference was found between the 113 patients considered for survival analysis and the others. Mean follow-up was 46.1 months. Death occurred in 24 (58.5%) of ATTR patients and 17 (23.6%) of those with unexplained LVH. Median survival after diagnosis was 2.8 years in ATTR patients, and exceeded mean follow-up in patients with unexplained LVH. Factors associated with survival in univariate analyses were ATTR diagnosis, NYHA status, troponine and ProBNP values, cardiac output, and Sokolow indice. In multivariate analysis, ATTR was associated with a higher mortality risk (HR=3.21, CI 95% [1.47; 7.04]), after adjusting on NYHA.

Conclusion

ATTR diagnosis in patients with severe LVH (wall thickness ≥15 mm) implies reduced survival. Precocious systematic screening of cardiac amyloidosis, namely ATTR, in at-risk populations, such as Afro-Caribbeans, presenting with moderate LVH and/or red flag symptoms could positively benefit patient survival, especially with the advent of new potent drugs on the global market.

The list of authors for the attached abstract are as follows:
Last names are in capital letters.

R.Banydeen, J.Lassus, N.Ozier-Lafontaine, A.Monfort-Brafine and J.Inamo are affiliated to the University Hospital of Martinique in Martinique, France.

A. Atallah is affiliated to Basse-Terre Hospital in Guadeloupe, France.
INTRODUCTION: Inotersen (INO) significantly slows the progression of TTR FAP. In the pivotal trial leading to its approval, 300mg subcutaneous (SC) INO was given weekly, after an initial loading dose of 300 mg x 3 in week 1. Mean TTR change from baseline was -72%, with almost all the reduction by 6 weeks. Most patients (PT) with clinically significant amyloid cardiomyopathy (AC) have ATTRw, and it is unknown whether their TTR response to inotersen will differ from ATTRm. METHODS INOCARD is an open label study of INO, given without a loading dose, to determine the drug’s effect on multiple cardiac parameters in PT with AC over 2 years. 300 mg SC INO is given weekly, without loading dose. TTR is measured at baseline, week 4, 6 and 8 and then monthly. RESULTS: 13 PT (1 woman) age 63-82 were studied. 10 had ATTRwt, 2 had ATTRm due to the V122I mutation and 1 Ala60. Mean baseline TTR level was 24.7 mg/dl, with 43.2% reduction at week 6 and nadir by 4 months. (TABLE). The 2 patients with V122Ile mutation had the most rapid decline in TTR, and the lowest nadir (GRAPH). CONCLUSIONS: INO 300 mg SC without a loading dose in ATTR AC produces similar suppression of TTR as in FAP, but over a longer time. Whether the longer time to nadir represents lack of a loading dose or the predominance of ATTRwt patients is unclear. The observation that the 2 Val122Ile patients had a more rapid fall may indicate a differential response between ATTRm and ATTRw TTR suppression.
Abstract Title: Prevalence of prior history of soft tissue structures affectation in patients with cardiac amyloidosis

Author: Raquel Vázquez-García, Complexo Hospitalario Universitario A Coruña

Co-Authors: Gonzalo Barge-Caballero, Complexo Hospitalario Universitario A Coruña

Eduardo Barge-Caballero, Complexo Hospitalario Universitario A Coruña

Patricia Pardo-Martínez, Complexo Hospitalario Universitario A Coruña

Couto-Mallón, David; Paniagua-Martín, María J; Larrañaga-Moreira, José M; Blanco-Canosa, P; Grille-Cancela, Zulaika; Vázquez-Rodríguez, José M, Crespo-Leiro, María G.

INTRODUCTION

Carpal tunnel syndrome secondary to deposition of amyloid in the flexor tenosynovium has been described in patients with transthyretin (ATTR-CA) and light chain cardiac amyloidosis (AL-CA). Its diagnosis usually precedes the clinical presentation of cardiomyopathy in 5-10 years. Affectation of other soft tissue structures has also been described. However, the prevalence of prior history of these conditions and the time from its diagnosis to the development of cardiac manifestations are not well known.

OBJECTIVES

To describe and compare the prevalence of prior history of several soft tissue structures affectation in patients with ATTR-CA and AL-CA, and to establish the temporal relationship between these manifestations and the clinical presentation of cardiomyopathy.

METHODOLOGY

Medical records of all patients with documented CA at our institution between 1998-2018 were systematically reviewed. Previous lumbar canal stenosis diagnosis (LCS) by computed tomography or magnetic resonance imaging (MRI), carpal tunnel syndrome diagnosis (CTS) by nerve conduction study, Dupuytren’s contracture surgery (DC), and rotator cuff, proximal biceps or quadriceps tendinopathy diagnosis (RC, PB and Qu) by x-ray, ultrasound or MRI were collected.

RESULT

We included 105 patients with CA, 65 ATTR-CA (61 wild-type, 4 hereditary) and 40 AL-CA. Prior history of LCS, DC and RC was significantly more frequent in ATTR-CA (15.4% vs 2.5%, p=0.036; 9.2% vs 0.0%, p=0.048; 18.5% vs 5%, p=0.049). No significant differences were observed regarding CTS, PB and Qu. The mean time from LCS, CTS, DC, RC, PB and Qu to the diagnosis of cardiac involvement was 9.1±4.9, 6.9±5.2, 8.5±4.8, 5.6±5.4, 10.3±7.0 and 3.8±3.2 years, respectively.
CONCLUSION

Prior history of soft tissue structures affection in CA is frequent and precedes the diagnosis of cardiomyopathy in several years. LCS, DC and RC are significantly more prevalent in ATTR-CA than in AL-CA, so they could serve to guide differential diagnosis.

<table>
<thead>
<tr>
<th></th>
<th>ATTR (n=65)</th>
<th>AL (n=40)</th>
<th>Total (n=105)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at diagnosis (years)</strong></td>
<td>79.1 ± 8.0</td>
<td>66.9 ± 10.4</td>
<td>74.4 ± 10.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Female sex</strong></td>
<td>7 (10.8)</td>
<td>19 (47.5)</td>
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<td>Dupuytren’s contracture surgery</td>
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<td>Proximal biceps tendinopathy</td>
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Data are shown as mean ± SD or n (percentage)
Abstract Title: Multimodality imaging and low intensity signals for the diagnosis of hTTR Cardiac Amyloidosis

Author: Michel SLAMA, CRMR NNERF Cardiology Department Hopital Bichat Paris France

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Introduction: In the setting of hTTR polyneuropathy, question arises of the extent of the cardiac assessment that should be proposed to patients with no cardiac symptoms. In most studies, cardiac involvement is defined as “myocardial thickness> 13mm”. Multimodality imaging, and "low intensity signals” like cardiac denervation allow for a more subtle assessment.

Clinical case: A 63 years old lady was referred for cardiac assessment after being diagnosed with hereditary TTR polyneuropathy.

The first symptoms of polyneuropathy appeared around the age of 55, EMG was positive at 61; she was operated for carpal tunnel syndrome at 62. Biology showed minor isolated MGUS IgG Kappa. There was no family history of hTTR amyloidosis. Genetic testing showed a double mutation: ILE107VAL and VAL122ILE. Accessory salivary glands biopsy was negative twice (2018-2019).

Clinical assessment showed markedly reduced physical capacity due to peripheral neuropathy, 6 min walk test limited to 390 m, NYHA class 1 without chest pain or syncope. Physical examination was normal, heart rate 95 bpm, blood pressure 108/63 mmHg with asymptomatic orthostatic hypotension.

The ECG was normal, but showed sinus tachycardia 99 bpm, with no response to atropine infusion, typical for parasympathetic denervation, and blunted heart rate variability (SDNN 43 msec) on 24h Holter recording.

NTproBNP was normal (99 ng/l) as well as Troponin Ic (<0.015µg/l).

Echo was normal with IV septum thickness of 9mm, ejection fraction 67%, GLS -21%, thin valves.

CMR was normal with IV septum thickness of 8.5mm, and no LGE

MIBG uptake was lower-normal with a heart to mediastinum ratio of 1.7 (normal value 2.02± 0.36)

DPD “bone” scintigraphy showed an intense cardiac uptake Perugini grade 3 (heart to lung ratio 7.4).
Conclusion: This case exemplifies low intensity signals of cardiac amyloidosis, such as elevated resting heart rate and parasympathetic cardiac denervation. The fact that cardiac DPD bone scan was positive when all the other modalities were normal is of major importance in this case as biopsies were negative. It illustrates the importance of complete multimodality imaging for detection of cardiac involvement in hereditary TTR amyloidosis.

Please also list as co authors Dr Cecile CAUQUIL, Dr Celine LABEYRIE and Pr David ADAMS both CRMR NNERF, Neurology Department Hopital Bicetre Paris France, and Pr Francois ROUZET, Nuclear Medecine Department, Hopital Bichat Paris France
Abstract Title: The Transthyretin Stabilizing Mutation (T119M) is Not Associated with Extended Lifespan or Protection Against Vascular Diseases: Analysis of the UK Biobank Cohort

Author: Margaret M. Parker, Alnylam Pharmaceuticals
Co-Authors: Simina Ticau, Alnylam Pharmaceuticals
James Butler, Alnylam Pharmaceuticals
David Erbe, Alnylam Pharmaceuticals

Introduction: Transthyretin (TTR) is a hepatically produced tetrameric protein mainly responsible for transporting vitamin A. Destabilized TTR can accumulate and form amyloid fibrils resulting in a progressive, fatal disease known as transthyretin-mediated amyloidosis, which includes wild-type (no TTR mutation) and hereditary (TTR mutation) amyloidosis. A non-pathogenic mutation (T119M) with stabilizing properties is the basis for one of the therapeutic strategies to reduce destabilized TTR tetramer. Recently, an association of T119M with extended lifespan and lower risk of cerebrovascular disease was reported in a Danish cohort.

Objectives: To evaluate whether improved clinical outcomes previously associated with the T119M mutation could be replicated in the UK Biobank.

Methodology: TTR T119M carriers were identified in UK Biobank, a prospective cohort study with genetic, physical and health data on ~500,000 individuals across the UK. Association between T119M genotype and diagnosis of vascular disease, cardiovascular disease, cerebrovascular disease and mortality were analyzed using logistic regression and Cox proportional hazard regression that controlled for potential confounders.

Results: Allele frequency of T119M within the unrelated, white population from UK Biobank (n=337,148) was 0.4% (2,499 heterozygotes, 3 homozygotes). Using logistic regression comparing T119M carriers and non-carriers, there was no association between T119M and vascular disease (36,024 events; OR=1.08; p=0.27), cardiovascular disease (28,738 events; OR=1.08; p=0.31), cerebrovascular disease (10,077 events; OR=1.1; p=0.42) or death (13,680 deaths; OR=1.2; p=0.06). Numerical trends towards increased risk for carriers were observed (OR>1). Cox proportional hazard regression analysis showed similar results. Age at death and vascular disease diagnosis was similar between T119M carriers and non-carriers (p=0.12 and p=0.38, respectively). There was no difference in mean survival time following vascular disease diagnosis (carrier=5.7 yrs; non-carrier=5.9 yrs; p=0.81).

Conclusion: In this analysis, there was no association between the TTR T119M genotype and risk of vascular disease or death in a large cohort indicating that TTR tetramer stabilization through T119M is not protective.

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Abstract Title: Neurofilament Light Chain (NfL) as a Potential Biomarker in Hereditary Transthyretin-Mediated (hATTR) Amyloidosis

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Akshay Vaishnaw (Alnylam Pharmaceuticals)
Paul Nioi (Alnylam Pharmaceuticals)

Introduction: Hereditary transthyretin-mediated (hATTR) amyloidosis is a rare, rapidly progressing, life-threatening disease caused by deposition of aggregated TTR amyloid in organs and tissues. The majority of patients develop a mixed phenotype that includes polyneuropathy and cardiomyopathy. Since TTR amyloid accumulates over time in nerves and other tissues, it is likely that tissue damage occurs prior to overt symptomology. Clinically validated and non-invasive plasma biomarkers proximal to the neuropathology may facilitate earlier diagnosis and treatment initiation, as well as aid in monitoring disease progression/regression.

Objective: Evaluate the impact on circulating protein biomarkers in response to patisiran treatment in patients with hATTR amyloidosis with polyneuropathy.

Methodology: Proteomic analysis (by proximity extension) was used to measure 1164 proteins in plasma samples collected from consenting patients in the Phase 3 APOLLO trial who received either placebo or patisiran 0.3 mg/kg IV q3w. A linear mixed model determined the impact of patisiran treatment on the time profile of each protein level at 0, 9, and 18 months.

Results: 66 proteins showed a significant change in levels following patisiran treatment (p<4.18x10^-5); change in neurofilament light chain (NfL), a marker of neuronal damage, was most significant (p<10^-20; Figure A). Plasma NfL was significantly reduced with patisiran at 9 and 18 months compared to placebo (Figure B). Improvement in mNIS+7 at 18 months compared to baseline significantly correlated with reduced NfL levels for the same time interval (R=0.40, p<10^-7; Figure C).

Conclusions: NfL may serve as a biomarker of nerve damage and polyneuropathy due to TTR amyloid deposition, as seen by the correlation with patisiran and change in mNIS+7. This may offer potential for earlier diagnosis of polyneuropathy in patients with hATTR amyloidosis and monitoring disease progression/regression over time, with or without treatment.
Long-Term Efficacy and Safety of Inotersen for Hereditary Transthyretin Amyloidosis: 2-Year Results From the NEURO-TTR Open-Label Extension Study

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Marcia Waddington Cruz, Federal University of Rio de Janeiro, University Hospital, Rio de Janeiro, Brazil

Annabel Wang, University of California, Irvine, Orange, CA, USA

Michael Polydefkis, Johns Hopkins University, Baltimore, MD, USA

Introduction: Hereditary transthyretin amyloidosis (hATTR) is a rare, progressive, and fatal disease that causes debilitating sensorimotor neuropathy and autonomic neuropathy, often with overlapping cardiomyopathy. Efficacy and safety of inotersen, an antisense oligonucleotide inhibitor of transthyretin protein production, were demonstrated in the randomised, controlled phase 3 study NEURO-TTR (NCT01737398) in patients with hATTR polyneuropathy.

Objective: To assess efficacy and safety of inotersen after 2 years in the ongoing NEURO-TTR open-label extension study (OLE).

Methodology: Patients who completed NEURO-TTR were eligible to enrol in the OLE (NCT02175004). Efficacy assessments include modified Neuropathy Impairment Score +7 neurophysiological tests composite score (mNIS+7), Norfolk Quality of Life–Diabetic Neuropathy questionnaire total score (Norfolk QOL-DN), and Short Form 36 Health Survey version 2 (SF-36) Physical Component Summary score (PCS). Safety monitoring includes regular platelet and renal testing.

Results: Of 139 patients who completed NEURO-TTR, 135 (97.1%) enrolled in the OLE. As of 5/31/2018, the longest cumulative inotersen exposure was 5.2 years. Patients who switched from placebo to inotersen in the OLE demonstrated slowing of neurological disease progression by mNIS+7 and Norfolk QOL-DN as early as 6 months after starting inotersen (mean change from OLE baseline to month 6/year 2: 6.22/5.08 in mNIS+7 and 0.54/2.26 in Norfolk QOL-DN). Patients who continued inotersen showed sustained benefit (mean change from OLE baseline to year 2: 11.18 in mNIS+7 and 5.22 in Norfolk QOL-DN). Patients who continued inotersen also showed stabilization of health-related quality of life as measured by SF-36 PCS (mean change from OLE baseline to year 2: 0.08). There was no evidence of increased risk for grade 4 thrombocytopenia or severe renal events with increased duration of inotersen exposure; no new safety concerns have been identified.

Conclusion: This study demonstrates continued efficacy and safety of inotersen, which improved, halted, or slowed progression of hATTR polyneuropathy. Better outcomes in neuropathy and quality of life were observed in patients treated earlier.

Additional Co-Authors in order:

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Mr. Morie Gertz, Dr, Mayo Clinic, Rochester, MN, USA
Ms. Teresa Coelho, Dr., Centro Hospitalar do Porto, Porto, Portugal
Introduction: hATTR amyloidosis is a rapidly progressive disease with heterogeneous clinical manifestations leading to severe morbidity. Patisiran, an RNAi therapeutic approved in certain countries, demonstrated significant improvement in polyneuropathy (PN) and quality of life (QOL) vs placebo in patients with hATTR amyloidosis with PN in APOLLO. Vutrisiran, an investigational RNAi therapeutic, is in development for the treatment of ATTR (hATTR and wtATTR) amyloidosis. Objectives: Describe the rationale and design of HELIOS-A, a global Phase 3, randomized, open-label study to evaluate the efficacy, safety, and PK/PD of vutrisiran in patients with hATTR amyloidosis (NCT03759379).

Methodology: HELIOS-A will enroll approximately 160 patients with hATTR amyloidosis. Key inclusion criteria: symptomatic PN (NIS 5-130), PND score ≤IIb and KPS ≥60%. Key exclusion criteria: previous liver transplant or NYHA class >II. Patients will be randomized (3:1) to receive either vutrisiran 25 mg SC q3M or patisiran 0.3mg/kg IV q3W during the 18-month treatment period. All patients will receive vutrisiran during the 18-month extension period. The APOLLO placebo arm will be an external control for all primary and most secondary efficacy analyses. Within-study patisiran arm will be used to establish non-inferiority of vutrisiran vs patisiran for TTR reduction. The primary objective is to determine efficacy of vutrisiran on mNIS+7 and Norfolk QOL-DN compared to APOLLO placebo arm. Primary analysis will be at 9 months, with additional analysis at 18 months. Secondary objectives include: efficacy of vutrisiran on gait speed (10-MWT), nutritional status (mBMI), and disability (R-ODS) at 9 and 18 months, effect on serum TTR levels at 9 and 18 months, and mortality and hospitalization over 18 months for overall population and in patients with cardiac involvement. Safety will be assessed throughout the study and up to 1 year after last dose. Results: HELIOS-A is currently recruiting, with an estimated primary completion date of early 2021.

Conclusions: HELIOS-A will investigate the efficacy, safety, and PK/PD of vutrisiran in patients with hATTR amyloidosis, with the potential to address a significant unmet need and offer additional treatment choices for patients with hATTR amyloidosis.
Abstract Title: THE BURDEN OF TRANSTHYRETIN AMYLOIDOSIS AND AMBULATORY DISABILITY ON HEALTH-RELATED QUALITY OF LIFE: RESULTS FROM AN OBSERVATIONAL STUDY

Author: Theodora Weisz, Akcea Therapeutics
Co-Authors: Michael Pollock, Akcea Therapeutics
Lovely Andrew, Optum

Authors: Andrew Lovley, Asia Sikora Kessler, Spencer Guthrie, Michael Pollock, Kristen L. McCausland

Introduction: Disease progression of transthyretin (ATTR) amyloidosis can result in peripheral neuropathy, beginning with sensorimotor impairment in the lower limbs and leading to increasing ambulatory disability.

Objectives: To assess the burden of ATTR amyloidosis and ambulatory disability on patients’ health-related quality of life (HRQOL) and to compare the HRQOL profile of patients from different study samples.

Methodology: Baseline SF-36v2® Health Survey (SF-36v2) data were collected from 93 adults with ATTR amyloidosis enrolled in a longitudinal online observational study and 172 adults enrolled in a randomized clinical trial (ClinicalTrials.gov ID: NCT01737398). Mean SF-36v2 scores from both patient samples were compared with scores from a United States general population (USGP) sample (N=4036). Regression models were used to adjust the USGP sample to match the age and gender distribution of the patient samples, and univariate analysis of variance models were used to compare mean SF-36v2 scores. Similar approaches were run among subgroups with varying ambulatory disability. Results were interpreted using minimally important difference (MID) thresholds established for the SF-36v2 domain and summary scores.

Result: Patients with ATTR amyloidosis from the observational study scored worse on most domains of the SF-36v2, compared with the USGP, by more than an MID: physical functioning, role-physical, general health, vitality, social functioning, role-emotional, and the physical and mental component summary scores (ps<0.003), with the greatest burden in general health. Patients from the clinical trial showed a similar HRQOL profile to those in the observational study, with larger deficits in physical functioning and role-physical. Patients from the observational study who reported ambulatory disability showed profound burden on all scores of the SF-36v2, except mental health, when compared to the USGP (ps<0.04); differences between the two patient samples with ambulatory disability were not statistically significant.

Conclusion: Patients with ATTR amyloidosis, regardless of data source, experience clinically meaningful deficits in multiple areas.

Please note that Theodora Weisz is not an author but presenter and responsible for the submission of the poster. Other authors are noted on the text and below: Authors: Andrew Lovley, Asia Sikora Kessler, Spencer Guthrie, Michael Pollock, Kristen L. McCausland
Transthyretin amyloidosis (ATTR) represents a life-threatening progressive disease. Patients mostly die within 5 to 15 years after onset of symptoms. Until recently, the only treatment option for ATTR was liver transplantation. By the end of 2018, Patisiran (Onpattro®), a small interfering RNA (siRNA) for knockdown of serum TTR, has been approved by FDA and EMA. Patisiran is a lipid nanoparticle (LNP) encapsulated siRNA that was postulated to be opsonized by hepatocytes via apolipoprotein E (APOE) -mediated uptake.

In this study, we raised the question whether APOE polymorphism affects the efficacy of Patisiran in ATTR patients.

Informed written consent was obtained from ATTR patients treated with Patisiran. Human hepatoma cell line HepG2 was transfected with APOE siRNA for 24 hours. The next day, Patisiran was added to the cell culture medium. APOE genotype was determined by sequence analysis. Knockdown was analyzed by qRT-PCR and ELISA.

APOE siRNA treatment of HepG2 caused a sustained APOE protein downregulation (92.7% ± 0.7% and 94.3% ± 0.4% after 24 and 48 hours, respectively). TTR mRNA and protein was significantly downregulated after APOE knockdown in HepG2 cells as compared to control. Knockdown of serum TTR in ATTR patients treated with Patisiran was robust at week 3 after treatment (84.7% ± 5.6%). Analysis of APOE in ATTR patients revealed the three most frequent genotypes E3/3 (n=6), E3/4 (n=5) and E3/2 (n=3). APOE stratification of ATTR patients did not show significant differences in TTR plasma concentrations following Patisiran treatment (week 3, week 6 and week 12). In addition, APOE levels did not differ between genotypes.

Our in vitro results show that Patisiran-mediated TTR knockdown is highly dependent on APOE expression. APOE polymorphism does not seem to affect efficacy of Patisiran treatment.
Abstract Title: The first six months of Patisiran in hereditary Transthyretin-amyloidosis: real-life experiences from a non-endemic center

Author: Maike F. Dohrn, RWTH Aachen University Hospital, Department of Neurology

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Burkhard Gess, RWTH Aachen University Hospital, Department of Neurology
Jörg B. Schulz, RWTH Aachen University Hospital, Department of Neurology

Introduction: Hereditary Transthyretin-amyloidosis (hATTR) is an autosomal dominant systemic disease with a progressively disabling and lethal course, if untreated. Currently approved treatment options comprise liver transplantation, tetramer stabilization, and protein knockdown. At our non-endemic center, three patients have so far received Patisiran, a new siRNA substance.

Objectives: We will herein depict our first six months of real-life experience with Patisiran in a clinical setting.

Methodology: All three patients were diagnosed and treated at the neuromuscular outpatient clinic of the RWTH Aachen University Hospital. Follow-up examinations included a detailed symptom history, a clinical examination, NCV-studies, quantitative sensory testing, Schellong’s test, Sudoscan, electrocardiogram, and laboratory studies of blood and urine.

Result: One early- and two late-onset patients have so far been treated with Patisiran at our clinical center. Two carry the most preponderant TTR-mutation Val50Met and patient 3 carries Glu109Gln, each heterozygously. All three patients were previously treated with Tafamidis meglumine. Whereas patient 3 did not respond to Tafamidis, the first two patients only deteriorated after a long period of disease stabilization (65 and 34 months). According to our follow-up examinations, no further disease progression was noted within the first three to six months of Patisiran treatment. Side effects were only observed in patient 1, who had an asthma attack during the second and a delayed flush after the first four applications. Patient 2 has successfully been transferred into a home-nursing program.

Conclusion: Patisiran has been well tolerated and appears to be an effective treatment option even in patients with disease progression under Tafamidis meglumine. To overcome the elaborate process of drug application, the home-nursing program is a promising option to disburden both patients and clinical centers.
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</tr>
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<td><strong>Secondary treatment/duration (so far)</strong></td>
<td>Patisiran (6 months)</td>
<td>Patisiran (5 months)</td>
<td>Patisiran (2 months)</td>
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<tr>
<td><strong>Patisiran dosage</strong></td>
<td>26mg</td>
<td>19mg</td>
<td>20mg</td>
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<tr>
<td><strong>Reasons to switch treatment modalities</strong></td>
<td>disease progression following a long period of stabilization</td>
<td>no response to Tafamidis meglumine</td>
<td>-</td>
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<tr>
<td><strong>Reasons of delay after deciding to switch</strong></td>
<td>reimbursement declined (inspite of approval)</td>
<td>patient’s hesitation</td>
<td>-</td>
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<tr>
<td><strong>Immediate side effects</strong></td>
<td>asthma attack at the second application</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Late side effects</strong></td>
<td>flush one day after application</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Technical difficulties</strong></td>
<td>supply shortage due to holiday air entrapment in the infusion system</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Disease course (so far)</strong></td>
<td>stable</td>
<td></td>
<td></td>
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<tr>
<td><strong>Diagnostic approach to monitor disease progression</strong></td>
<td>symptom history</td>
<td>clinical examination</td>
<td>NCV-studies</td>
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<td>quantitative sensory testing</td>
<td>Schellong’s test</td>
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<td>Sudoscan</td>
<td>Electrocardiogram</td>
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<tr>
<td><strong>Intervals of monitoring</strong></td>
<td>three months</td>
<td>three months</td>
<td>three months</td>
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<tr>
<td><strong>Future perspectives</strong></td>
<td>continues to receive Patisiran in our outpatient clinic</td>
<td>included into a home-nursing program</td>
<td>announced to be included into a home-nursing program</td>
</tr>
</tbody>
</table>

Table 1: Patient overview
By the end of 2018, two novel compounds, Patisiran and Inotersen, have been approved by FDA and EMA for treatment of hereditary transthyretin amyloidosis (ATTR) stage 1 and 2. Patisiran (Onpattro®) represents a lipid nanoparticle (LNP)-encapsulated siRNA, whereas Inotersen (Tegsedi®) is an antisense DNA. Although the compounds exert quite different molecular mechanisms for TTR knockdown, high reductions of serum TTR have been reported for both oligonucleotides in recent phase 3 clinical trials.

In this study, we examined the effect of Patisiran and Inotersen on serum TTR levels in an ATTR patient cohort at our clinical center. Informed written consent was obtained from all patients. Patisiran was administered by intravenous infusion every three weeks, and Inotersen by subcutaneous injections once weekly. Blood samples were collected prior to and during treatment and stored for subsequent analysis of serum TTR using an in-house ELISA.

Treatment with Patisiran and Inotersen resulted in a significant reduction of serum TTR levels compared to pre-treatment (73 ± 4% and 73 ± 6%, respectively). TTR downregulation was maintained on-treatment in follow-up sera during a period of several months. Adverse events were observed, but did not differ significantly between the compounds. Interestingly, in five ATTR patients who switched from Inotersen to Patisiran after a washout period of several weeks, we observed similar rates of TTR downregulation.

In conclusion, our first real-life experiences demonstrate that both compounds, Patisiran and Inotersen, induce a robust downregulation of serum TTR similar to the values reported in clinical trials. Moreover, our results suggest that both compounds are equally effective in the same patient and do not affect their particular mechanism of action after a washout period of several weeks. Long-term follow-up is needed to evaluate the effect of both compounds on clinical outcomes.
Abstract Title: Treatment of Hereditary amyloidosis mediated by transthyretin (hATTR) in Huelva, Spain

Author: Álvaro Gragera-Martínez, Juan Ramon Jimenez University Hospital

Co-Authors: Francisco Muñoz-Beamud, Juan Ramon Jimenez University Hospital
            Cristina Borrachero-Garro, Juan Ramon Jimenez University Hospital
            Andrés Gonzalez-Macia, Juan Ramon Jimenez University Hospital

Introduction

Treatment of hereditary amyloidosis mediated by transthyretin (hATTR) for many years has been symptomatic.

At the beginning of the 90s, orthotopic liver transplant (OLT) was the standard of care for patients with hATTR. Replacing the liver, where more than 95% of the protein is synthesized, the disease seemed to stop. Due to the problems and comorbidities associated with liver transplantation, now it is not the therapy of choice.

In recent years, new drugs, called stabilizers of the transthyretin molecule have been developed. Tafamidis is currently the only approved stabilizer in Europe.

2 new distinct oligonucleotide-based drugs therapies for hATTR have being developed. Inotersen and Patisiran.

These drugs are more effective in early disease, since the treatments available so far, aim to prevent additional amyloid deposition.

Objective

To describe the pharmacological treatment received by patients with Andrade’s disease who are treated at the Juan Ramón Jiménez University Hospital in Huelva.

Methodology

A retrospective study was performed from January to June. Medical records (treatment and time that has elapsed since the beginning of the treatment until the current time) have been collected.

Results

Throughout these years 39 patients have received treatment.

A total of 25 patients have underwent under OLT. Regarding drug treatment, 10 patients have received Tafamidis. Due to progression of the disease, one underwent liver transplantation and one patient switched to Patisiran; so currently 8 patients are receiving Tafamidis. 9 patients had received Patisiran, 5 patients with rapid progression of the disease, another patient previously treated with Tafamidis and who presented disease progression, and finally 3 patients, with previous OLT, in a current clinical trial, in which the disease has progressed.

Conclusions
Currently in Spain there is only one treatment approved and commercialized: Tafamidis, a drug indicated for FAP 1 stage

We have developed a great experience with Patisiran. It is presented as promising, allowing an improvement in the quality of life and life expectancy of patients.

New alternatives are being explored for transplanted patients
Abstract Title: Clinical efficacy and management of thrombocytopenia and glomerulonephritis in patients with hereditary ATTR amyloidosis treated with inotersen: Results from >5 years of clinical trial and post-marketing surveillance

Author: Morie Gertz, Mayo Clinic, Rochester, MN, USA

Co-Authors: Sami Khella, University of Pennsylvania, Philadelphia, PA, USA
Annabel Wang, University of California, Irvine, Orange, CA, USA
Teresa Coelho, Centro Hospitalar do Porto, Porto, Portugal

Introduction: Hereditary transthyretin amyloidosis (hATTR) is a progressive and fatal disease that causes debilitating autonomic and sensorimotor neuropathy. The efficacy and safety of inotersen, an antisense oligonucleotide inhibitor of transthyretin protein production, was demonstrated in the global phase 3 study NEURO-TTR (NCT01737398) in patients with hATTR polyneuropathy.

Objective: To review clinical efficacy and assess thrombocytopenia and glomerulonephritis (GN) in patients treated with inotersen across datasets of clinical and real-world experience.

Methodology: Patients with hATTR polyneuropathy who completed NEURO-TTR were eligible to enter an ongoing open-label extension (OLE; NCT02175004) study; in the OLE, some patients have received inotersen for >5 cumulative years. Patients with hATTR have also received inotersen through an expanded access program (EAP) in the United States and a Compassionate Use Program (ATU) in France. An ongoing, investigator-sponsored trial (IST) includes patients with hATTR or wild-type ATTR. Data from these 5 studies plus ~3 patient-years of postmarketing exposure were evaluated from 7/6/2018 to 1/5/2019.

Results: In total, 267 unique patients have been treated with inotersen in NEURO-TTR (N=112), OLE (N=50/135), EAP (N=67), ATU (N=2), and IST (N=36) as of 1/5/2019. Interim analyses of the OLE show that inotersen-treated patients have sustained slowing of neurologic disease progression and stabilization of health-related quality of life, with greater benefit observed in patients who were treated earlier. No cases of grade 4 thrombocytopenia and no severe acute GN have been reported since implementation of enhanced monitoring of platelet levels and kidney function in ongoing clinical trials and noninterventional studies.

Conclusion: Patients show sustained slowing of disease progression from extended dosing of inotersen, with greater disease stabilization in patients treated earlier. With enhanced safety monitoring, events of Grade 4 thrombocytopenia and acute GN have been successfully mitigated across a number of studies and treatment programs.

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Giampaolo Merlini, Amyloidosis Center, IRCCS Policlinico San Matteo, University of Pavia, Italy;
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Arvind Narayana, Akcea Therapeutics, Boston, MA, USA;
Noel Dasgupta, Indiana University School of Medicine, Indianapolis, IN, USA;
Merrill D. Benson, Indiana University School of Medicine, Indianapolis, IN, USA
Abstract Title: A feasibility assessment for an indirect treatment comparison of inotersen versus patisiran for the treatment of hATTR-PN

Author: Megan Lewis, Fiecon
Co-Authors: Evelina Bertranou, Fiecon
Mark Fisher, Fiecon
Sandra Nestler-Parr, Akcea Therapeutics

Introduction: Before conducting an indirect treatment comparison (ITC), it is important to assess the quality of available evidence and the validity of assumptions made to facilitate the comparison.

Objectives: To perform a feasibility assessment of an ITC of inotersen versus patisiran for the treatment of hereditary transthyretin-related amyloidosis with polyneuropathy (hATTR-PN).

Methodology: We identified 2 randomized clinical trials (RCTs), NEURO-TTR (inotersen vs placebo) and APOLLO (patisiran vs placebo) for inclusion in our assessment. Australian Pharmaceutical Benefits Advisory Committee guidelines were used to assess heterogeneity and treatment effect modifiers across RCTs.

Result: Both RCTs had similar study design with some differences between treatment duration (NEURO-TTR: 15 months vs APOLLO: 18 months) and route of administration (NEURO-TTR: subcutaneous vs APOLLO: intravenous). There was some variation between patient baseline characteristics including the time since diagnosis (NEURO-TTR: 3.3 and 3.5 years vs APOLLO: 1.4 and 1.3 years; placebo and treatments arms respectively), presence of cardiomyopathy (NEURO-TTR: 55% and 67% vs APOLLO: 47% and 61%; placebo and treatments arms respectively), pre-medication (NEURO-TTR: 0 vs APOLLO: premedication), comedication (NEURO-TTR: 0 vs APOLLO: comedication in the placebo arm) and a higher proportion of patients in stage 2 and 3 and higher baseline Norfolk-QoL-DN scores in the APOLLO trial. In addition, key outcomes were measured at different timepoints (NEURO-TTR: weeks 35 and 66 vs APOLLO: weeks 36 and 72).

Conclusion: Our assessment shows that the 2 RCTs are somewhat comparable, however, appropriate methodological considerations should account for differences in population, interventions and study design when conducting an ITC. The only published study of an ITC of inotersen and patisiran excluded key confounding variables such as time since diagnosis and presence of cardiomyopathy, did not describe the method of outcome interpolation across different timepoints, and did not explore the impact of comedication in the APOLLO trial. Overall, the minimum necessary requirements to undertake an ITC that will generate robust results and draw meaningful conclusions have not been evaluated to date.
Abstract Title: First treatment experiences with patisiran in two patients with hereditary transthyretin amyloidosis

Author: Helmar Lehmann, Department of Neurology, University Hospital Cologne, Germany

Co-Authors: Nicolai Grether, Department of Neurology, University Hospital Cologne, Germany
Gilbert Wunderlich, Department of Neurology, University Hospital Cologne, Germany

Objective: Patisiran is a RNA-silencing drug that inhibits the production of transthyretin via gene-knockdown approved for the treatment of hereditary transthyretin amyloidosis in the E.U. since 2018. We report here the use of patisiran in two patients with hereditary transthyretin amyloidosis.

Methods: Retrospective case study, single center

Results: The two patients are 65 and 81 years old (1 male, 1 female), and have been diagnosed with hTTR four and two years ago, respectively. Clinical symptoms include impairment of motion, hyporeflexia, sensory ataxia, loss of weight and carpal tunnel syndrome. In the first patient a Leu58/78His mutation, and in the second patient a Val30/50Met mutation could be detected. Both patients fulfill stage II of polyneuropathy severity. Patients were treated with 24mg and 21mg of patisiran, respectively. Up to now, there have been no adverse events or infusion-related events reported in the patisiran cohort. In contrast, patients report to tolerate patisiran-infusions well. Even though we started our first treatment with patisiran only in autumn 2018, one of the patients describes increased sensorimotor abilities, while the other patient has the impression of stabilization without further clinical progress.

Conclusion: Patisiran is well tolerated and can prevent disease progression in hereditary transthyretin amyloidosis.
Abstract Title: A snapshot of health-related quality of life in ATTR amyloidosis in the UK

Author: Thirusha Lane, University College London, UK

Co-Authors: Marianna Fontana, University College London, UK
Joan Caringal-Galima, University College London, UK
Carol J Whelan, University College London, UK
Dr Ana Martinez, University College London, UK
Prof Philip N. Hawkins, University College London, UK
Julian D. Gillmore, University College London, UK

Background: Progressively diminishing quality of life (QoL) is a known feature of transthyretin (ATTR) amyloidosis, however there are limited prospective data on this aspect of the disease.

Methods: As part of a protocolised model of care involving comprehensive annual evaluation, patients were asked to voluntarily complete the SF-36 health-related QoL questionnaire. The SF-36 is composed of eight health domains, each one scored out of a maximum of 100, with scores closer to 100 representing better QoL. Reduced scores on a repeat survey indicate deterioration in that health domain.

Results: Over a 5 year period, 1254 questionnaires were completed by 770 patients: 551 wild-type (WT), 106 with V122I variant-associated hereditary ATTR amyloidosis (hATTR), and 113 with non-V122I-hATTR (T60A=60, V30M=12, S77Y=8, and 33 with a variety of other mutations). A snapshot of QoL showed predictably reduced scores compared to the general population across all domains (Figure 1). Particularly diminished scores were seen in physical function (PF), role physical (RP), vitality (VT) and general health (GH) across all three groups. Interestingly, WT patients scored better than those with hereditary disease (hATTR) across six of the eight domains, those with hATTR scoring particularly poorly for PF, RP, bodily pain (BP) and social function (SF). Three hundred and fifty one patients completed at least one follow-up questionnaire over the study period, allowing examination of annual change in QoL. The greatest deteriorations over time were seen in the non-V122I group, in BP (-9 and -19 points at 1y and 2y respectively), and in GH (-20 points at 2y), whilst the V122I group scored the worse for mental health (MH, -10 and -13 points at 1y and 2y). All groups showed deterioration of 13 points in SF at 2y.

Conclusion: Patients with ATTR amyloidosis have significant impairment over several aspects of health status resulting in generally poor QoL. Those with hereditary forms of the disease appeared to show greater disease burden compared to WT patients. The effect of the various new treatments is eagerly awaited.
Abstract Title: Presence of Transthyretin Amyloid In Carpal Tunnel Biopsies

Author: Janet Gilbertson, National Amyloidosis Centre, UCL, Division of Medicine, London, UK

Co-Authors: Julian D Gillmore, National Amyloidosis Centre, UCL, Division of Medicine, London

Gilbertson Janet A (1), Botcher Nicola (1), Lane Thirusha (1), Youngstein Taryn (2), Fontana Marianna (1), Bland Jeremy (3), Hawkins Philip N (1), and Gillmore Julian D (1)

(1) National Amyloidosis Centre, UCL, Division of Medicine, London, UK.

(2) Imperial College, London UK

(3) East Kent Hospitals University NHS Foundation Trust, UK

Introduction:

Transthyretin (TTR) is a normal serum protein and acts a carrier protein for thyroxine and retinol. Genetic variants of TTR can be associated with widespread tissue aggregation and deposition of TTR amyloid, causing neuropathy and cardiomyopathy. In advancing age, wild-type (non-variant) TTR amyloid (ATTRwt) is deposited in synovial tissues, tendons and heart. ATTRwt amyloid in certain tissues may be asymptomatic, although extensive cardiac infiltration can lead to a cardiomyopathy. Wild-type ATTR amyloidosis is diagnosed in ~200 individuals in the UK per year, despite post-mortem studies showing presence of ATTRwt amyloid deposits in hearts of up to 30% of male individuals over the age of 80.

This discrepancy in conjunction with the knowledge that the majority of ATTRwt patients treated in the UK have a history of carpal tunnel (CT) decompression surgery, often 12 years prior to presentation with cardiac amyloidosis, prompted this preliminary study in which we sought to identify amyloid in excised tissue from the flexor retinaculum from patients over 50 years of age undergoing CT decompression surgery. If amyloid was identified, the amyloid fibril protein was determined by immunohistochemistry (IHC) using a panel of antibodies. The study is ongoing.

Methods:

Samples from the flexor retinaculum were taken from 31 males and 34 females, median age of 61 and 67 years respectively, who underwent routine CT decompression surgery. Samples were fixed and processed into a paraffin blocks for routine histology, Congo red staining and IHC. If amyloid was identified, IHC, using a panel of monospecific antibodies against known amyloid fibril proteins, was performed in order to amyloid type.

Results:

Of the 65 biopsies tested 39% (25 cases) contained amyloid. Of those, 72% (18 cases) were from males, with a median age of 61 (range 55-88) years and 28% were from (7 cases) females, with a median age 67, (range 52-92) years. The amyloid type in 24 cases was ATTR; amyloid was too scanty to type by IHC in 1 case.
Conclusions:

This preliminary study has shown ~40% of samples taken for routine CT decompression contain TTR amyloid, this may be a simple procedure to identify individuals at risk of developing cardiac ATTR amyloidosis, at an early age.

Please add all authors in order as follows, (first and last author have been added in set format above) –
Introduction: Patisiran® has been shown to be effective in double blind studies. So far follow-up has been published up to 30 months.

Objectives: To evaluate the longterm treatment in a patient carrying the Arg34 Thr variant.

Methodology: We report on a case who received since June 2013 Patisiran®.

Result: The reported case was listed for liver transplantation in August 2012. In June 2013 therapy with Patisiran® was initiated. Since then disease symptoms were stable, NT-proBNP significantly dropped and mBMI improved. Liver transplantation was not required up to today.

Conclusion: Patisiran® is safe and effective for now 74 months in this one subject reported here.
Gastrointestinal (GI) complications are common in neuropathic hereditary transthyretin (ATTRv) amyloidosis. The frequency of GI complications varies between different populations and mutations, but for ATTRV30M amyloidosis virtually all untreated patients develop GI disturbances. The pathogenesis is not fully understood, but it is generally suggested that GI symptoms arise due to motility disturbances of the GI tract caused by an autonomic neuropathy. In addition to the destruction of autonomic nerves, a depletion of enteric nerves and endocrine cells has been found. A decreased amount of interstitial cells of Cajal has also been noted in the upper GI tract and, since the interstitial cells of Cajal operate as intestinal ‘pacemaker cells’, a depletion of these cells may lead to the decreased GI tract motility and gastric paresis that is commonly seen in ATTRv amyloidosis.

These motility disturbances give rise to an impaired function of both the upper and lower GI tract, and have a profound impact on the patient’s quality of life, especially if faecal incontinence is present. Commonly reported upper GI symptoms include early satiety, nausea and vomiting, while constipation, alternating constipation and diarrhoea, and constant diarrhoea (often in combination with faecal incontinence) account for the lower GI tract disturbances. Difficulties in swallowing can also occur and have been attributed to autonomic, predominantly vagal, denervation of the oesophagus. Investigations have disclosed that gastric retention, small bowel bacterial overgrowth and malabsorption of fat and bile acids are commonly present, and therapy directed towards those disturbances can relieve and improve the symptoms. However, even with comprehensive investigations and appropriate treatment (Table 1), the long-term outcome can be disappointing.

GI disturbances leading to malabsorption and malnutrition have an adverse impact on survival, and malnourished patients are also poor candidates for liver transplantation. Thus, for ATTRv amyloidosis patients, the presence of GI disturbances is related to poor quality of life and poor prognosis. However, early diagnosis together with adequate disease-modifying treatment can help to prevent the development of these disabling complications.
<table>
<thead>
<tr>
<th>Symptom</th>
<th>Diagnostic tests</th>
<th>Treatment</th>
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<td>Nausea and vomiting</td>
<td>Scintigraphic measurement of gastric emptying</td>
<td>Dopamine 2 receptor antagonists</td>
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<td>ACTH test</td>
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ACTH: adrenocorticotropic hormone; mBMI: modified body mass index; SeHCAT: selenium homocysteine taurine.
Diagnosis, Epidemiology & Other
Abstract Title: Enlightmen with a Care Manual of ATTR-FAP Patients and Families

Author: Chieko Kukinaka, Kumamoto University

Co-Authors: Hiromi Kawasaki, Hiroshima University
Satoko Nakagomi, Hiroko Kokufu,

Background

ATTR-FAP is an intractable autosomal dominant inherited disease. Treatment and care are important because the disease has severe systemic disorders. For the patients and families, specialized medical staff are indispensable to improve their Quality of life.

Objectives

The purpose of this study is for medical staff to understand the symptoms more precisely. And, to educate medical staff so that medical staff can provide high quality care to patients and their families. We developed a care manual.

Methodology

To obtain information about clinical manifestations, we conducted a focused group interview for 4 doctors and 12 nurses who worked in neurology, transplantation surgery, ophthalmology, and cardiology. We also conducted interviews for 16 patients and 9 spouses what their hereditary disease was.

Interviews were conducted for them in May, 2014 and with patients and families from July, 2009 to February, 2012.

The way of analysis in the interview content was used a qualitative content analysis.

Based on above described methods, our previous research on care, and ATTR-FAP treatment guidelines, we newly created a care manual.

Result

For symptom management, we described medical staff needed to focus carefully on the patient’s symptoms of autonomic nervous system, heart, kidney, digestive tract, urinary tract and eyes. As for the care of physical symptoms, the important point of each symptom was specifically described in the care manual. Patient, children and spouses had a specific set of challenges. Medical staffs needed to carefully support them from the point of hereditary disease. If necessary, collaboration with genetic counselors are needed. We also wrote information on social resources and patient association.

Conclusions

We newly published a care manual for medical staff, patients and their family members. On the basis of the book, medical staff could help to improve the quality of care.

Co-authors include Dr. Ando and Dr. Yamashita.

I would like to present a poster.
The two themes are poster presentations.

Enlightmen with a Care Manual of ATTR-FAP Patients and Families

Report on ATTR-FAP International Meeting in Japan
Abstract Title: Link betwin ATTR wt versus Al-Amyloidosis.

Author: Maria del Carmen Nadal Massanet, AMILO (Asociación Española de Amiloidosis)

Co-Authors: Javier Melero Otero, AMILO (Asociación Española de Amiloidosis)

At First Meeting of Amyloidosis Alliance in Paris, we point the importance to add A-AL in our task-force to obtain the truly diagnosis of different kind of Amyloidosis.

In Spain, like others countries we are connected with AL patients and at Brussels Meeting of MPE it was spoken of the incidence worldwide on AL is more incidence population than hTTR and TTR. Eventhough, we tougth that is very important to look at this and include this type in our Association.

Last MPE in Munich, we share and talk about this task force with Ben Voltering-Tus and Daniel Drimer to look forward include this kind of Amyloidosis in the Snd Amyloidosis Alliance Meeting in Berlin.
Abstract Title: The disease of the 21st Century, TTR related Amyloidosis -
Updates on diagnosis and treatment of cardiac amyloidosis-

Author: Ando Yukio, Nagasaki International University

Co-Authors:

Amyloidosis is a disorder of protein metabolism in which soluble proteins are deposited in tissues as abnormal insoluble fibrils. Amyloid deposits are like nylon fibrils. Recently, 4 amyloidogenic precursor proteins of familial amyloidotic polyneuropathy (FAP, ATTRv amyloidosis) have been identified: mutated form of transthyretin (TTR), gelsolin, apolipoprotein AI, and beta-2 microglobulin. Of these, ATTRv amyloidosis induced by mutated form of TTR is most common in the worldwide. In addition, ATTRwt amyloidosis induced by wild type TTR has been focused in the recent attention.

Recently, in addition to big foci of ATTRv Val30Met in Kumamoto and Nagano districts, many sporadic ATTRv Val30Met patients with late onset have been reported. In this type of ATTRv amyloidosis, small fiber neuropathy including autonomic dysfunction is one of main manifestations in addition to cardiac and renal disorders, intestinal dysfunction, and ocular symptoms. To evaluate those manifestations, we established various novel examination systems by means of biochemical, radiological and histopathological methods.

We newly established Diagnostic Unit for Amyloidosis, Department of Laboratory Medicine, Kumamoto University Hospital. In addition to conventional diagnostic methods, we newly applied laser micro- dissection and liquid chromatography/ mass spectrometry mass spectrometry (LC/MS MS) system to screen TTR related amyloidosis and other types of amyloidosis. Using this system, we could screen a mutation of TTR gene and components of TTR related amyloid fibrils in tissues.

In Japan, 50 different points of mutation in TTR which lead ATTRv amyloidosis have been identified and most of them show small fiber neuropathy. As a therapy for ATTRv amyloidosis in addition to liver transplantation, stabilizers for tetrameric form of TTR, gene silencing methods have been proven to be effective to retard the progression of clinical manifestations of the disease. Therapeutic possibility of amyloid disruptors has been investigating by our group.
Abstract Title: AmyloScan® – Development of a new screening tool for ATTRv Amyloidosis

Author: Juliane Sachau, Division of Pain Research and Therapy, Department of Neurology, University Hospital Kiel

Co-Authors: Ralf Baron, Division of Pain Research and Therapy, Department of Neurology, University Hospital Kiel

Introduction

Hereditary variant transthyretin (ATTRv) amyloidosis, also known as hATTR amyloidosis, is characterized by a heterogeneous clinical presentation, including polyneuropathic and cardiac symptoms, as well as ocular, renal, and gastrointestinal involvement. A range of life-impacting neurological symptoms including burning neuropathic pain, loss of sensation in hands and feet, as well as autonomic dysfunction (diarrhea/constipation, sexual impotence, dizziness) can occur. Due to this highly heterogeneous presentation, only a minority of symptomatic ATTRv amyloidosis patients are correctly diagnosed. This is in particular fatal because the median survival for untreated patients is reported to be about 4.7 years from diagnosis. Early diagnosis would facilitate early treatment and improve prognosis. Thus, an easy-to-use screening tool to identify ATTRv amyloidosis early in the disease course is desperately needed.

Objective

The aim of this project is the development and validation of a simple screening tool to distinguish ATTRv amyloidosis with polyneuropathy from other neuropathies or diseases with pain in the extremities (AmyloScan®).

Methodology

A list of clinical symptoms, which occur characteristically for ATTRv amyloidosis, especially in the beginning of the disease, will be collected after performing an extensive literature analysis, connecting with attending physicians and interviewing patients. Several questionnaires and testing of neurological functions, e.g. bedside testing and autonomic testing, will be performed to characterize the pain and sensory symptoms as precisely as possible. In order to identify the most specific items, ATTRv amyloidosis patients will be compared to patients with sensory neuropathy and with chronic extremity pain of other origin.

Results

Items that differ significantly between polyneuropathy caused by ATTRv amyloidosis and sensory neuropathy or chronic extremity pain of other origin will be identified. These items will be part of the final AmyloScan®. A total cut-off score will be calculated by addition of the single scores of each item.

Conclusion

The results of this study might lead to the development of an easy-to-use screening tool to identify ATTRv amyloidosis early in the disease course.

This study is funded by Alnylam Pharmaceutic
Abstract Title: Most frequent symptoms of onset in the endemic focus of the province of Huelva

Author: Álvaro Gragera-Martínez, Juan Ramon Jimenez University Hospital

Co-Authors: Francisco Muñoz-Beamud, Juan Ramon Jimenez University Hospital
Borrachero-Garro Dra., Cristina
Andres Gonzalez-Macia, Juan Ramon Jimenez University Hospital

Introduction

The clinical expressiveness of hereditary amyloidosis mediated by transthyretin (hATTR), is very variable, associated with a wide range of clinical manifestations that may present in varying degrees and combinations; hence there are patients who express a galloping disease in which the clinical symptoms evolve in a short period of time, while others have a slower evolution over time, even not developing a complete symptomatology of the disease. Clinical manifestations of hATTR amyloidosis are heterogeneous and influenced by TTR genotype, but also by geographic location, and other genetic and environmental factors.

Objective

To know the disease symptoms onset in patients from the endemic focus of Valverde del Camino.

Methodology

A retrospective, observational study was performed. The variables collected were: age at the onset of symptoms, onset symptom

Results

In the database with 137 patients we only could collect clinical data of the patients who were still alive, in order to know the expressiveness of the disease. The clinical symptoms, on patients’ debut, are described overall (Figure 1) and subsequently by their age of onset (Figure 2).

Conclusions

Expressiveness of hATTR amyloidosis is clearly linked to the genetic variant that patients carry. Two phenotypes can be clearly distinguished, the cardiac phenotype, which gives rise to familial amyloid cardiomyopathy, with a predominance of the founding variant p.Val142Ile, the most frequent variant in America. However, in Europe, Spain and in the focus of Valverde del Camino, we find the variant p.Val50Met, which causes a more neuropathic phenotype. Although neurological and cardiological phenotypes have been classically defined, mixed phenotype is common across most of mutations.

The great variability in the expression of symptoms within this variant of the pathology makes difficult an early the diagnosis, follow-up of carrier asymptomatic patients and screening with other diseases that produce an axonal polyneuropathy clinic.

Through this study it has been possible to see how the predominant clinic of the disease in our geographic area is polyneuropathy, as expected in the variant p.Val50Met.
Abstract Title: Targeted genetic screening identifies pathogenic TTR alleles in almost 1% of patients with idiopathic cardiomyopathy and/or polyneuropathy

Author: Volha Skrahina, Centogene AG
Co-Authors: Christian Beetz, Centogene AG
Arndt Rolfs, Centogene AG

Introduction

Hereditary transthyretin-related amyloidosis (ATTRm) is a rare disorder, for which age at onset, penetrance and clinical manifestation vary widely. Presenting symptoms may include cardiomyopathy (CMP) and polyneuropathy (PNP). ATTRm is caused by heterozygous missense variants in TTR, and its prevalence has been suggested to be ~1 in 1,000,000. Treatment has become available recently.

Objectives

By genetically screening patients at risk for suffering from ATTRm, we aimed at (i) an improved definition of the epidemiology, and (ii) getting more insight into the clinical heterogeneity.

Methodology

Patients were enrolled by 54 centers from Austria, Germany and Switzerland. Inclusion criteria were: >18 year of age, and a diagnosis of PNP and/or CMP of unclear etiology. A structured case report form was used to document demographic data and detailed clinical information. All exons and exon/intron boundaries of the TTR gene were screened using next generation sequencing.

Results

Pathogenic TTR alterations were identified in 20 of 2,300 patients (0.87%, ‘TTR+’). Positivity rate was 0.5% (6 of 1,197) amongst patients with PNP only, 1.1% (8 of 712) amongst patients with CMP only, and 1.5% (6 of 391) amongst patients with both manifestations. The variants c.148G>A (p.V50M; n=6) and c.424G>A (p.V142I; n=5) were observed recurrently. The others were unique variants, which have previously been reported in ATTRm, and/or are very rare in the European reference population. Males accounted for 75% and 60% of ‘TTR+’ and ‘TTR-’ cases, respectively (p=0.25, Fisher’s exact test). Age at diagnosis was 62.8 (+/-11.9) years in ‘TTR+’ patients, compared to 55.4 (+/-13.0) years in ‘TTR-’ patients (p=0.014, Student’s T-test).

Conclusions

ATTRm appears to be much more frequent than previously anticipated. Patients with unspecific initial clinical symptoms can efficiently be identified by genetic analysis, whereby elderly males with both CMP and PNP benefit most.
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4 Neurogeriatrie, Jung-Stilling Klinikum Siegen, Siegen, Germany
5 Klinik für Kardiologie, Kerckhoff-Klinik GmbH, Bad Nauheim, Germany
6 Klinik für Neurologie, Klinikum Altenburger Land GmbH, Altenburg
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presentation will be by either Volha Skrahina or Christian Beetz
Abstract Title: ATTR Epidemiology in Huelva, Spain

Author: Álvaro Gragera-Martínez, Juan Ramon Jimenez University Hospital

Co-Authors: Francisco Muñoz-Beamud, Juan Ramon Jimenez University Hospital
Cristina Borrachero-Garro, Juan Ramon Jimenez University Hospital
Andrés Gonzalez-Macia, Juan Ramon Jimenez University Hospital

Introduction

Hereditary amyloidosis mediated by transthyretin (hATTR), also known as Andrade's disease is a hereditary, neurodegenerative, progressive, highly disabling and life-threatening disease in short term if an early treatment is not established.

It is a rare disease, with prevalence less than 1 / 100,000 habitants in worldwide. Huelva is one of the most important endemic foci of the disease worldwide and the second with more patients in Spain.

This disease is inherited with an autosomal dominant pattern and is characterized by a reduced penetrance, which varies according to age and geographic area. This poses big problems and big challenges when it comes to monitoring patients and their families.

Objectives

To know the penetrance of the disease in the endemic focus of Valverde del Camino.

To know the sociodemographic variables in these patients

Methodology

A retrospective, observational study was performed. For this work, medical records has been collected and statistically analyzed. Demographic details (sex, age at diagnosis of the disease, age at the onset of symptoms) were obtained.

Results

Table 1 shows the results of the sociodemographic variables, as well as the age of onset of symptoms in patients

In order to calculate the penetrance, the total number of patients have been collected with respect to the total number of carriers who do not have clinical symptoms. The penetrance of the disease at 35, 55 and 75 years of age has been calculated, because the penetrance is time dependent.

Conclusions

The overall penetrance of the disease in the endemic focus of Valverde del Camino is 42%. There is a great difference between men and women: penetrance in men is much higher at earlier ages, while, in women, most of them express the symptomatology of the disease at later ages. Characteristics of the endemic focus of Huelva are similar to those found in France, Sweden or Portugal. This difference between men and women with clinical symptoms agrees a large series of published cases, where 69% of the affections worldwide were men.
<table>
<thead>
<tr>
<th>Patients</th>
<th>No clinical symptoms</th>
<th>Symptomatic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>79</td>
<td>58</td>
<td>137</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>50</td>
<td>40</td>
<td>90 (66%)</td>
</tr>
<tr>
<td>Women</td>
<td>29</td>
<td>18</td>
<td>47 (34%)</td>
</tr>
</tbody>
</table>

| Mean age                 | 46                   | 75          |       |

<table>
<thead>
<tr>
<th>Mean age at symptoms onset</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Early onset</td>
<td></td>
<td></td>
<td>52</td>
</tr>
<tr>
<td>Middle onset</td>
<td></td>
<td></td>
<td>52</td>
</tr>
<tr>
<td>Late onset</td>
<td></td>
<td></td>
<td>70</td>
</tr>
</tbody>
</table>

| Variant p.Val50Met        |                      |             | 137   |

Table 1. Demographic data of subjects included in the study

<table>
<thead>
<tr>
<th>Penetrance</th>
<th>Men</th>
<th>Women</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>44%</td>
<td>38%</td>
<td>42%</td>
</tr>
<tr>
<td>35 years</td>
<td>27%</td>
<td>5%</td>
<td>17%</td>
</tr>
<tr>
<td>55 years</td>
<td>38%</td>
<td>18%</td>
<td>31%</td>
</tr>
<tr>
<td>75 years</td>
<td>39%</td>
<td>20%</td>
<td>37%</td>
</tr>
</tbody>
</table>

Table 2. Penetrance in Valverde del Camino cohort
Abstract Title: Epidemiological and clinical characteristics of Transthyretin familial amyloid polyneuropathy in the Republic of North Macedonia

Author: Ivan Barbov, University Clinic for neurology Skopje

Co-Authors: Goce Kalcev, University Clinic for neurology Skopje
            Frosina Stojkoska, University Clinic for neurology Skopje
            Gabriela Novotni, University Clinic for neurology Skopje

Introduction:
Familial amyloid polyneuropathy (FAP) associated with genetic variants of the transthyretin (TTR) is the most prevalent type of hereditary systemic amyloidosis, and more than 100 amyloidogenic point mutations in the TTR gene have been described.

Materials and Methods:
We present the epidemiological and clinical features of patients already diagnosed with TTR-FAP, with confirmed pathogen mutation in the TTR gene in the Republic of North Macedonia.

Results:
So far in the Republic of North Macedonia, 14 patients with TTR-FAP have been diagnosed. Additionally, 9 of the patients are in the first stage, 3 in the second stage and 2 in the third stage. 4 of the 14 patients are treated with Tafamidis, 20 mg. Moreover, pathogenic mutations in the TTR gene were confirmed in all 14 patients. 13 patients have confirmed Glu89Gln genetic pathogenic mutation (all are from Macedonian origin). These patients are from the eastern part of North Macedonia. 1 Muslim patient has a confirmed genetic pathogenic mutation Phe53Val. Only this patient comes from the western part of North Macedonia. On the other hand, it is interesting fact that the largest number of TTR-FAP patients from the neighboring Republic of Bulgaria, is from the southwestern part of the country, that is, around the border with the Republic of North Macedonia. This is an indication of an endemic area for the TTR-FAP on the Balkan. Also, Congo red staining of samples from subcutaneous fat tissue aspiration biopsy was positive in all 14 patients, which is in favor of amyloid deposits. In 10 of TTR-FAP patients, the symptoms of the nervous system are predominant and in 4 patients of the cardiovascular system. In the patients who have predominant nervous system symptoms, electromyography with the
Abstract Title: Founder effect and estimation of the most recent common ancestor of the Glu89Gln TTR mutation in the Bulgarian population

Author: Andrey Kirov, IMDL Genome Center “Bulgaria”, Sofia, Bulgaria

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Zornitza Pavlova, Genetic Medico-Diagnostic Laboratory Genica, Sofia, Bulgaria

Tihomir Todorov, Genetic Medico-Diagnostic Laboratory Genica, Sofia, Bulgaria

Familial transthyretin amyloidosis is an autosomal dominant genetic disorder caused by pathogenic genetic variants in the TTR gene resulting in amyloid plaques formation of the transthyretin protein. Depending on the system affection the manifestations may be different and high heterogeneity in the penetrance is observed. An endemic region in Bulgaria exists where the TTR mutation Glu89Gln is found with high frequency. This is a rare pathogenic variant that was probably introduced in the population by a common ancestor. This phenomenon, called “founder effect” was proved in carrier families by haplotype analysis of microsatellite markers showing linkage disequilibrium. The common ancestry of the carriers was demonstrated using additional data for their genealogies and microsatellite data from a control group of non-affected individuals. The results show that the pathogenic genetic variant Glu89Gln is linked to one haplotype, called “hypothetical founder haplotype” which was compared to published haplotype data from other European patients and no similarity was found. The fact that the founder haplotype has been subjected to decay was used to determine the theoretical age of the most recent common ancestor using DMLE+ v.2.0 software. The results demonstrated that the theoretical most recent common ancestor lived few hundreds generations ago. Some studies focused on mapping the human migrations in the Eastern Mediterranean in the period c.1250-c1150 BC showed the well-known hotspots and endemic regions connected by migration movements during this period, which support the hypothesis that the age of this genetic variant is ancient. Of course, further population genetics studies of carriers of the Glu89Gln mutation from other endemic regions are required in order to clarify the geographical distribution of the pathogenic genetic variant.

Additional Co-Authors: Teodora Chamova1, Mariana Gospodinova2, Ivailo Tournev1,3 , Vanyo Mitev4 and Albena Todorova4,5,6

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Abstract Title: Incidental finding of TTR gene variants in patients diagnosed with AL amyloidosis

Author: Dorota Rowczenio, National Amyloidosis Centre

Co-Authors: Helen Lachmann, National Amyloidosis Centre

Introduction

Clinical management and prognosis for patients with systemic amyloidosis depend entirely on correct identification of the fibril protein. AL amyloidosis, is treated with chemotherapy which has no role in hereditary ATTR amyloidosis.

Methods

Between 1992 and 2018, 4973 patients who were referred to the single UK specialist centre underwent TTR gene sequencing as part of their routine work up.

Results

Of the 4973 patients in whom the TTR gene was sequenced 37% had the final diagnoses of ATTR amyloidosis; 27% light chain (AL) amyloidosis; 0.7% other types of amyloidosis; 21.3% had no evidence of amyloid deposition and in 14% we have no data. Pathogenic TTR variants were an incidental finding in 6 patients who had been diagnosed with AL amyloidosis prior to genetic testing. TTR p.V50M (V30M) was found in a British 67 year old, referred with biopsy confirmed AL lambda amyloid causing macroglossia, proteinuria and renal impairment. She had a large amyloid load in the liver, spleen and kidneys on SAP scintigraphy. There was no evidence of cardiac involvement nor neuropathy. Five further patients (all of Afro-Caribbean ancestry) with biopsy proven AL amyloidosis (four of lambda and one of kappa sub type) were found to carry the p.V142I (V122I) variant. Four of these patients died of cardiac amyloidosis between the ages of 46 and 55 years.

The protective TTR p.T139M (T119) substitution was found in 11 subjects. None had ATTR amyloidosis; eight had AL lambda, one AL kappa and two had no evidence of amyloid deposition.

Conclusion

Finding of pathogenic TTR variants in 6 cases was incidental; none had a family history to suggest hereditary ATTR type and the diagnosis of AL amyloidosis and treatment with chemotherapy was entirely appropriate, although genetic testing is recommended for the family members, in particular of the p.V50M case. This variant causes peripheral and autonomic neuropathy and cardiac involvement and has a frequency of 0.03% in Europeans. The cardinal feature of p.V142I is the late-onset restrictive cardiomyopathy. The estimated allele frequency of this substitution among African Americans is 3.9% and cohort studies suggest allele carriers are at greater risk of heart failure even in the absence of overt amyloid deposits.
Abstract Title: External quality assessment (EQA) scheme for genetic testing in ATTR amyloidosis

Author: Dorota Rowczenio, National Amyloidosis Centre

Co-Authors: Julian Gillmore, National Amyloidosis Centre

Introduction

The TTR gene is highly polymorphic with more than 140 variants identified, of which 130 are amyloidogenic. With the exception of p.V142del and the 6 nucleotides duplication in exon 3 of the TTR gene all variants result from nucleotide substitutions. Recent advances in the treatment for ATTR amyloidosis, which has revolutionised patient care and prognosis, will result to a higher demand for genetic testing.

Objective and Methodology

Diagnostic genetic testing requires quality control procedures to ensure consistency and accuracy of results. To date there is no standardised criteria for genetic analysis and reporting in hereditary ATTR amyloidosis, which can influence genetic outcome and compromise patient care. We are proposing a new external quality assessment (EQA) scheme for genetic testing in ATTR amyloidosis across European laboratories. This will be done in collaboration with the European Molecular Genetics Quality Network (EMQN) and will help in establishing best practice guidelines for molecular diagnosis ensuring appropriate patient care.

Participating in EQA scheme will help in obtaining information on molecular techniques used, quality and performance of the laboratories undertaking genetic testing and help implementing the use of nomenclature in accordance with current recommendations (HGNC and HGVS nomenclature guidelines). This is particularly useful since laboratory practices are changing rapidly due to the implementation of next-generation sequencing (NGS) technologies and an increased demand for DNA screening in the era of treatment-oriented genetic testing.

Discussion

With advances in treatment for ATTR amyloidosis, in particular targeted gene silencing therapies, we are on the threshold of a new era in the treatment of ATTR amyloidosis. TTR gene screening should be used in the diagnostic work up of patients with systemic amyloidosis in order to provide information for family members, to allow selection of patients for appropriate disease-modifying therapies and liver transplantation, and to avoid misdiagnosis of AL amyloidosis which is typically treated with chemotherapy. Genetic testing provides early and accurate diagnosis and is a logical and feasible way to corroborate clinical diagnosis.
Abstract Title: INITIAL PATIENT MONTHLY COST OF TAFAMIDIS IN MASSACHUSETTS USA. A BARRIER TO ACCEPTANCE?

Author: Melissa Coyle, Brigham and Women’s Hospital Amyloidosis Program, Boston, MA, USA

Co-Authors: Sarah Cuddy, Brigham and Women's Hospital Amyloidosis Program, Boston, MA, USA
Rodney Falk, Brigham and Women's Hospital Amyloidosis Program, Boston, MA, USA

BACKGROUND AND METHODS: Based on the positive outcome of the ATTR-ACT trial, tafamidis was approved for use in the USA in May 2019 to treat wild-type or hereditary TTR cardiomyopathy (ATTR-CM). There are no labeling restrictions in prescribing tafamidis, provided there is evidence of TTR cardiac amyloidosis, and the efficacy and excellent safety and side-effect profile makes it a sought-after drug by patients, who see it as the only FDA-approved option for their heart disease. The USA list price of tafamidis is approximately $225,000 per year and most patients needing it are insured by Medicare, the Federal program for citizens over age 65. Medicare has a multilayered structure for drug payments, including an annual portion requiring a high patient co-payment. While these co-pays vary, depending on the type of coverage, patients can be subject to high co-pays for expensive agents. We determined the cost of the first month of drug in a consecutive group of patients with ATTR-CM.

RESULTS: Among 47 patients, all with either Medicare or commercial insurance, only 14 (29.8%) were required to pay less than $100 per month, with costs ranging from $2 monthly to $25 monthly. The remaining 33 patients had monthly costs ranging from $984 monthly to $3915 monthly, with a mean cost of $2112+/-$1610, and all >$1000 except one. One other patient, without prescription insurance, was asked to pay $22,500 monthly. Patients with low-costs tended to be very low-income patients and 5 patients opted not to take tafamidis because of the cost.

CONCLUSIONS: Although there are some potential assistance options, including from the manufacturer, the availability of some options varies throughout the year. The use of these programs also has restrictions that are related to the patient’s income level, the type of insurance coverage that they have (Medicare vs. commercial) and even the State in which the patient lives. Our initial experience prescribing tafamidis indicates that it requires a very high initial monthly copay in most patients. This potentially results in financial difficulty in this older patient population who already have high medical expenses and are often on a fixed income and may prevent some patients from receiving a life-prolonging agent.
Abstract Title: Report on ATTR-FAP International Meeting in Japan

Author: Chieko Kukinaka, Department of Nursing Faculty of Life Science Kumamoto University

Co-Authors: Taro Yamashita, Kumamoto University

Yukio Ando, Department of Amyloidosis Research Nagasaki International University, Kumamoto University, Michishirube(milestone) no kai

Background

There are two ATTR-FAP patient meetings in Japan. ‘Michishirube(milestone) no kai’(Kumamoto) and ‘Tanpopo(dandelion) no Kai’ (Nagano). In Japan, it is known that there are patients spread all over the country, but only a small number of people have joined the patient associations. We also know that there are patients in various countries all over the world. Therefore, with the opportunity of the International Amyloidosis Society being held in Japan, we planned an exchange meeting of ATTR-FAP patients and their families inside and outside Japan.

Objectives

The purpose was to ensure that patients and their families had up-to-date information on the pathogenesis from specialized medical doctors.

In addition, we aimed to encourage each patients and families to interact more.

Activity report

1. Program content

Two doctors gave a talk on "Overview of ATTR-FAP" and "Diagnosis and treatment of ATTR-FAP".

We had a lecture on "Genetic counseling of ATTR-FAP" from a certified genetic counselor.

After that, we had an introduction to the patient meetings of each country: Japan ‘Michishirube(milestone) no kai (Kumamoto)’, ‘Tanpopo(dandelion) no Kai (Nagano)’,”TFRD (Taiwan)”,”FAMY（Sweden）” and the Netherlands. Members of each area told us about the efforts of the patient meetings in their country.

Posters introducing each patient meeting were posted in Japanese and English in the hall.

Simultaneous interpretation was provided.

2. Participant

Total number of participants : 93

Number of patients : 27 （Japan: 24, Korea:1, Taiwan:1, Netherlands:1）
Number of families (spouses and children): 35 (Japan: 29, Korea: 1, Taiwan: 2, China: 2, Sweden: 1)

Medical experts: 31 (Japan: 24, Korea: 3, America: 3, United Kingdom: 1)

Type of medical experts: Medical doctor: 6, nurse: 6, certified genetic counselor: 2, researcher: 2, incurable disease counselor: 1, corporation staff: 8, journalist: 1, nursing student: 5

This meeting was supported by Sanofi Genzyme.
Abstract Title: Sensory and motor self-rehabilitation in hereditary transthyretin amyloidosis

Author: Agnes Morier, CHU Bicêtre

Co-Authors: Margaux Cornec, CHU Bicêtre
Cecile Doctor Cauquil, CHU Bicêtre
Celine Labeyrie, CHU Bicêtre
Professor Adams David CHU Bicêtre

Introduction: Rehabilitation is essential for management of hereditary transthyretin amyloidosis (hATTR), however a regular practice is sometimes difficult. To favour rehabilitation in patients with neuropathy we developed a self-rehabilitation booklet with specific exercises for motor and sensory loss that can easily be performed at home. In 2017, a new edition was published with a French and English version, easily available online.

Aims: determine the use and the impact of the self-rehabilitation booklet on hATTR patients’ daily life. Methods: an online survey was performed. The link was sent to patients to whom the booklet was given and published on social networks and patient association website. The collected data were: disease type, how the booklet was obtained, the impact of the booklet on their quality of life, how often they use it and if they would recommend its use.

Results: Twenty-three answers were obtained, including 8 patients with hATTR. The use of the booklet was beneficial in 84% of cases. Patients who uploaded the booklet via social networks or on the internet had lower motivation for self-rehabilitation. On the other hand patients who were given the booklet by their therapist in our unit found it useful and had regular practice.

Conclusion: Rehabilitation is important for patients with neuropathy including hATTR and can improve quality of life. The self-rehabilitation booklet helps patients continue exercises at home, explanations by a trained therapist increase the benefit of our booklet. The widespread of a self-rehabilitation booklet and a more detailed questionnaire on its’ use could evaluate the impact of self-rehabilitation in hATTR patients.
Abstract Title: Burden of illness of hATTR amyloidosis on patients and caregivers

Author: Thirusha Lane, University College London, UK

Co-Authors: Philip N Hawkins, University College London, UK

Julian Gillmore, University College London, UK

Introduction: Hereditary transthyretin (hATTR) amyloidosis is a rare disease, the progressive nature of which leads to significant morbidity, and is associated with a significant burden of illness on patients and caregivers.

Methods: Semi-structured interviews were conducted with 21 patients with hATTR amyloidosis and 11 caregivers, with the aim of understanding of how hATTR amyloidosis impacts the daily lives of patients and their carers.

Results: Patients reported reduction in ability to perform daily activities due to fatigue and shortness of breath: "I used to walk the dog ... every day, morning and at night. Now, when I ... start to walk I get really tired, my legs ache, get out of breath ..." For carers, fatigue often resulted from poor or disturbed sleep at night due to patients having pain or restless sleep, or needing assistance during the night. "If he's awake, I'm awake because I worry if ... he's dizzy when he gets up ... he's going to pass out ... in the bathroom ... And it's just a constant worry ... so, it's broken sleep most nights." The combination of gastrointestinal dysfunction and sensorimotor neuropathy affected social life, as described by one carer: '[We] ... were very sociable before he became ill ... we'd have people around for meals, or we'd go to them ... we used to love going out ... But we don't do any of that anymore. ... because [he has a] problem with ... what he can eat, and now ... he's ... less able to feed himself, he feels embarrassed in front of other people [...] He won’t go to other people’s houses because, if he needs to go to the toilet ... well, it’s his dignity ..." Some patients and carers did express feeling hopeful for the future: "At the moment I'm feeling very optimistic, I feel it's a good time for patients with TTR amyloidosis because we've been waiting for a drug that works well for many, many years and now suddenly it is becoming a reality."

Conclusion: The burden of hATTR amyloidosis is significant; as the disease progresses it impacts every aspect of the daily lives of patients and their families. Many patients and carers described a sense of hope that an effective treatment would become available, enabling them to retain a good quality life, and providing hope for future generations.
Figure 1. Snapshot of QoL showing scores in each domain for each of the three patient groups.