RNA interference therapy with patisiran for hereditary transthyretin amyloidosis

Léo Sumi

May 2019

Abstract

Onpattro^{TM} is a new drug approved in August 2018 by the FDA. It is made by Alnylam Pharmaceuticals for patients having polyneuropathy caused by hereditary transthyretin-mediated amyloidosis. Its active ingredient, patisiran, is an siRNA targeting transthyretin mRNA. This new drug is of great interest because it is the first siRNA drug on the market.

Transthyretin (TTR, **trans**ports **thy**roxine and **retin**ol) is a transport protein that carries the thyroid hormone, thyroxine (T4), and the retinol-binding protein (RBP) when it is bound to retinol (vitamin A). TTR is formed by four monomers of 127 amino acids. It is mostly produced by the liver and secreted into the bloodstream (Al Shaer et al. 2019). There is more than 100 genetic variants of the gene encoding TTR that are associated with autosomal dominant form of diseases, known as familial amyloidotic polyneuropathy (FAP) and familial amyloidotic cardiomyopathy (FAC) (Coelho et al. 2013).

Mutations in the *ttr* gene destabilize the tetrameric TTR protein. This lead to misfold of the monomers. The misfolding result to aggregation into TTR amyloid fibrils (ATTR). Tissue deposition of ATTR results in systemic ATTR amyloidosis. (Suhr et al. 2015)

The disease is mostly hereditary and is now known as hereditary ATTR (hATTR) amyloidosis (instead of FAP). The disease is progressive and life-threatening. Because of the accumulation of amyloid fibrils in multiple organs, the symptoms are multiple. ATTR fibrils can be found in the nerves, heart, and gastrointestinal tract. The diagnosis is difficult because of the heterogeneous clinical presentation, including sensory, motor, autonomic, and cardiac symptoms. (Adams et al. 2017)

Current treatment of hATTR amyloidosis try to reduce the amount of amyloidogenic protein circulating into the bloodstream. Orthotopic liver transplantation (OLT) has the reduction of mutant TTR as purpose. The liver, which is the main source of ATTR, from the OLT will produce wild-type TTR. The survival is improved for patients in the early stage of the disease with OLT. (Suhr et al. 2015)

OLT has many problems and is not the solution for hATTR amyloidosis treatment. It is only recommended for patients with early-stage hATTR amyloidosis and survival rate depend on many factors. There is the different problems associated to transplantation such as cost, donor availability, cardiac involvement, and toxicities associated with immunosuppression. Even if the OLT is a success, disease progression can continue: amyloid fibril deposition from wild-type TTR is possible. (Suhr et al. 2015)

Currently, two drugs: tafamidis and diflunisal are used against hATTR amyloidosis. These drugs are TTR tetramer stabilizers. The goal is to stabilize the TTR complex and prevent protein misfolding. In clinical studies both compounds have slowed progression of neurologic impairment and were generally well tolerated. However, progression of neuropathy symptoms or disability is still observed in some patients. The two compounds are not sufficient and a need for novel treatment options remains. (Adams et al. 2017)

Patisiran (Onpattro^{TM}) is a drug approaved in 2018 by the Food and Drug Administration (FDA) in the USA as a response for the need of a new treatment for hATTR amyloidosis. Patisiran make an impressive breakthrough in the drug discovery field as it is the first small interfering RNA (siRNA) drug to use RNA interference (RNAi) to downregulate protein expression. (Al Shaer et al. 2019)

RNAi is an endogenous cellular mechanism for gene expression post-transcriptional regulation using non-coding RNAs. During RNAi, short single-stranded RNAs of 20 to 30 nucleotides serve as RNA guide to selectively bind, through base-pairing, other RNAs in the cell. Target RNAs are generally mRNAs that will be inhibited in its translation, or even catalyzed for its destruction.

siRNAs is one of the class (with miRNAs and piRNAs) of small non-coding RNAs causing RNAi. In the cell siRNAs come from double-stranded RNA cleaved by a protein complex containing Dicer nuclease. siRNAs are the resulting fragments of approximately 23 nucleotide pairs. One strand of the siRNA is cleaved by Argonaute and discarded. The remaining strand is assembled with a set of proteins to form an RNA-induced silencing complex (RISC). The complex will seeks out its target RNAs which, in the case of siRNAs, mostly come from virus or transposable element. (Alberts et al. 2015, 429–31)

Patisiran is a double strand siRNA. The sequence (figure 1) targets the 3' untranslated region (3'UTR) of the TTR mRNAs (Coelho et al. 2013). The siRNA sequence is independent of the *ttr* mutations and will target mutated and wild-type *ttr* genes.

Patisiran use lipid nanoparticules (LNP) as the delivery system for the siRNAs. LNP is among the most commonly used method to transfect the cells with siRNA. It facilitates encasing and enfoldind siRNAs, and help administer to the body intravenously. (Rizk and Tüzmen 2017)

During the phase 2 multi-dose study, the effect of Patisiran on the TTR proteins

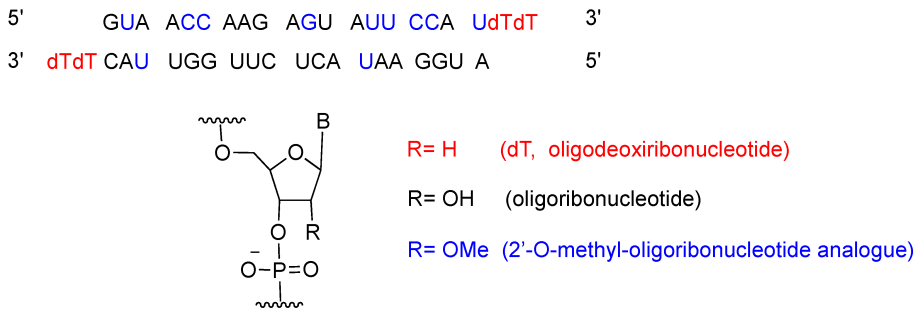

Figure 1: Patisiran sequence and chemical composition. (From Al Shaer et al. (2019), CC BY 4.0)

can clearly be seen (Figure 2). The increase in dose will increase the knockdown effect. The ideal dose is 0.30 mg/kg every 3 weeks. Multiple doses of patisiran were shown to be generally safe and well tolerated (Suhr et al. 2015). The majority of adverse effects were mild or moderate in severity and no dose-limiting toxicities were observed (Suhr et al. 2015).

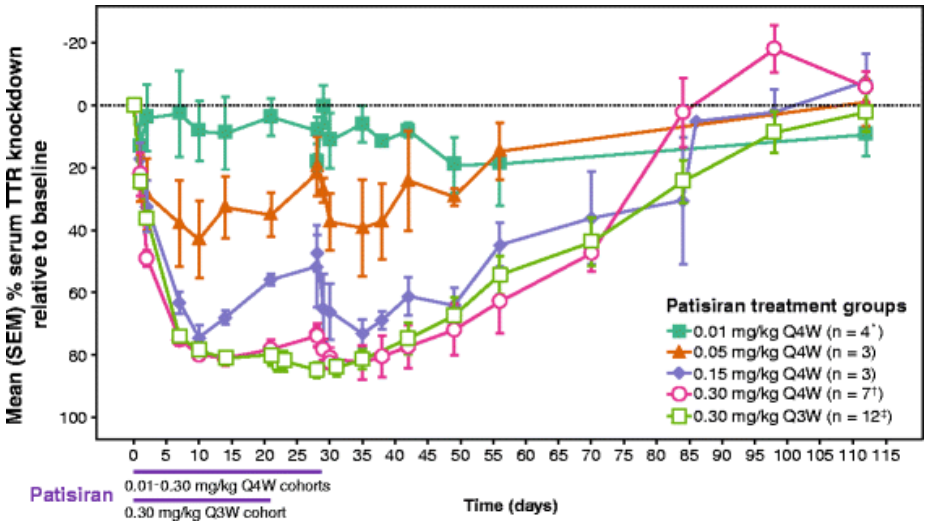

Figure 2: Dose response and duration of TTR knockdown. Mean (with SEM) of percentage of baseline serum concentration-time profile. Q3W: every 3 weeks; Q4W: every 4 weeks. (From Suhr et al. (2015), CC BY 4.0)

The delivery method of the siRNAs is one of the most impressive aspect of patisiran. Delivery of naked siRNA is possible, but the nonspecific uptake and the unknown escape routes will be big challenges for an siRNA like patisiran, which has hepatocyte cells as targets (Setten, Rossi, and Han 2019). Patisiran

needs an excipient. The use of nanomedicines like LNP have several advantages. The size and surface characteristics can be manipulated; it is possible to control and sustain the release of the payload, siRNAs, at the target site; and site-specific targeting can be achieved by attaching targeting ligands to the surface (Wahlich et al. 2019). The balance between the benefits of the active pharmaceutical ingredient, siRNAs, with the coasts and potential side effects of the excipient, LNP, must be checked, but it can be balanced with sufficiently high drug loading (Wahlich et al. 2019).

RNAi-based drugs have four big challenges to overcome: (i) the immunogenic reactions to dsRNA, (ii) the toxicity of excipients, (iii) the unintended RNAi activity, and (iv) the on-target RNAi activity in non-target tissues. The immunogenic reactions to dsRNA is caused from sensing of dsRNAs by PKR, TLR3 and TLR7. This is less of an issue now as recent RNAi-based drugs like patisiran use 2'-O-methyl base modifications (see figure 1) which mitigate this issue. The toxicity of excipients is mitigated by the usage of LNP delivery and pretreatment using corticosteroids and anti-allergy medications has helped to considerably attenuate infusion reactions with patisiran. The unintended RNAi activity is more difficult to mitigate. Off-target RNAi silencing can occur. Screening, primate models and minimizing the administered RNAi dose are the current answer to this problem. The choice of which strand from the siRNAs are loaded onto the RNA-induced silencing complexes has mostly been resolved. But the sequence selection for the antisense strand is the most crucial determinant of pharmacodynamics. Sequence selection has profound effects on strand selectivity, on-target potency and off-target spurious activities. This is not a trivial selection, notably because of the local secondary and tertiary structures of the mRNAs and mRNA-bound proteins or transiting ribosomes. Finally, the on-target RNAi activity in non-target tissues is mitigated by choosing highly-disease-selective genes as the targets for RNAi silencing (as *ttr*) and delivery routes that reduce accumulation in non-target tissue. More development is needed to mitigate this issue. (Setten, Rossi, and Han 2019)

Onpattro^{TM} costs a lot. The price is in the range of a six-digit figure per year for the treatment. It is an important drawback of patisiran. The treatment is calculated to be approximately $450,000 per year at launch (Al Shaer et al. 2019).

Ionis Pharmaceuticals' inotersen is poised to compete with Alnylam's patisiran for the treatment of hATTR amyloidosis. Inotersen is an antisense oligonucleotide (ASO). As the number of oligonucleotide therapeutic approvals increases in the coming years, it is likely that direct competition between ASO and siRNA drugs will only increase. The approval of patisiran and inotersen implies that, for the first time, patients with hATTR amyloidosis have treatment options with considerable probabilities of halting disease progression. Patisiran appears to have better results for safety and efficacy, but the safety and efficacy of both drugs over durations significantly longer than 18 months is unknown. (Setten, Rossi, and Han 2019)

Adams, David, Ole B Suhr, Peter J Dyck, William J Litchy, Raina G Leahy, Jihong Chen, Jared Gollob, and Teresa Coelho. 2017. "Trial Design and Rationale for Apollo, a Phase 3, Placebo-Controlled Study of Patisiran in Patients with Hereditary Attr Amyloidosis with Polyneuropathy." *BMC Neurology* 17 (1): 181. https://doi.org/10.1186/s12883-017-0948-5.

Alberts, Bruce, Alexander Johnson, Julian Lewis, David Morgan, and Martin Raff. 2015. *Molecular Biology of the Cell*. Norton & Company. https://www.ebook.de/de/product/22733166/bruce_alberts_alexander_johnson_julian_lewis_david_morgan_martin_raff_molecular_biology_of_the_cell.html.

Al Shaer, Danah, Othman Al Musaimi, Fernando Albericio, and Beatriz G de la Torre. 2019. "2018 Fda Tides Harvest." *Pharmaceuticals (Basel, Switzerland)* 12 (2). https://doi.org/10.3390/ph12020052.

Coelho, Teresa, David Adams, Ana Silva, Pierre Lozeron, Philip N Hawkins, Timothy Mant, Javier Perez, et al. 2013. "Safety and Efficacy of Rnai Therapy for Transthyretin Amyloidosis." *The New England Journal of Medicine* 369 (9): 819–29. https://doi.org/10.1056/NEJMoa1208760.

Rizk, Malak, and Şükrü Tüzmen. 2017. "Update on the Clinical Utility of an Rna Interference-Based Treatment: Focus on Patisiran." *Pharmacogenomics and Personalized Medicine* 10: 267–78. https://doi.org/10.2147/PGPM.S87945.

Setten, Ryan L, John J Rossi, and Si-Ping Han. 2019. "The Current State and Future Directions of Rnai-Based Therapeutics." *Nature Reviews. Drug Discovery*, March. https://doi.org/10.1038/s41573-019-0017-4.

Suhr, Ole B, Teresa Coelho, Juan Buades, Jean Pouget, Isabel Conceicao, John Berk, Hartmut Schmidt, et al. 2015. "Efficacy and Safety of Patisiran for Familial Amyloidotic Polyneuropathy: A Phase Ii Multi-Dose Study." *Orphanet Journal of Rare Diseases* 10 (September): 109. https://doi.org/10.1186/s13023-015-0326-6.

Wahlich, John, Arpan Desai, Francesca Greco, Kathryn Hill, Arwyn T Jones, Randall J Mrsny, Gianfranco Pasut, et al. 2019. "Nanomedicines for the Delivery of Biologics." *Pharmaceutics* 11 (5). https://doi.org/10.3390/pharmaceutics11050210.