Background

- Transthyretin (TTR) amyloidosis (ATTR) is a progressive, fatal disease wherein deposition of either mutant or wild-type TTR amyloid can cause severe organ damage and dysfunction.
- ATTR cardiomyopathy (ATTR-CM) results in a high burden of morbidity and mortality from progressive heart failure with few therapeutic options.

Hypothesis

- AG10 displays differential TTR binding, kinetic stability, and a higher degree of stabilization compared to other TTR stabilizers.
- In vitro, AG10 achieves near-complete stabilization of TTR at clinical concentrations.

Materials & Methods

- Commercially available tafamidismeglumine (TAF) was used in this study. Purified human TTR was purchased from Athens Research and AlexoTech. Pooled human serum and plasma was from Innovative Research.
- Thermodynamic stability (Kd) of TTR interaction was determined by microscale thermophoresis (MST) using a Nanotemper Monolith NT.115 instrument with red maleimide labeled TTR. Data was processed using PALMIST software.
- Kinetic stability was assessed by surface plasmon resonance (SPR) using a GE Biacoat T200 instrument. Purified TTR was immobilized on CM5 chips via NHS/EDC coupling. Data was processed using GE T200 Evaluation software.
- The ability of each stabilizer to prevent accelerated tetramer dissociation over 72 hrs at pH 3.8 alone or in combination was measured by Western Blot.

Summary

- The affinity of AG10 for purified TTR, as measured by MST, is 3X greater than that of tafamidis. Kinetic stability by SPR reveals over 4X longer residence time for AG10 bound to TTR as compared to tafamidis.
- When evaluated at therapeutic plasma concentrations, in vitro incubation of tafamidis alone does not completely stabilize tetrameric TTR.
- In vitro addition of AG10 to tafamidis results in complete stabilization of tetrameric TTR.
- Therapeutic concentrations of tafamidis in serum do not fully occupy the TTR binding site. Addition of AG10 to tafamidis treated samples results in complete binding site occupancy.

Conclusions

- The extended residence time of AG10 compared to tafamidis results in improved TTR binding site occupancy and stabilization.
- In vitro, AG10 completely stabilized TTR in plasma samples with or without therapeutic concentrations of tafamidis.
- These findings support further development of AG10 as a disease-modifying treatment for patients with ATTR cardiomyopathy.

References