Differential Ex Vivo Stabilization Of Transthyretin By AG10 And Tafamidis In Samples From Patients With Moderately Or Severely Destabilizing Mutations

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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.
- Formation of TTR amyloid is initiated by dissociation of destabilized tetrameric TTR into its constituent monomers and subsequent misfolding, aggregation, and tissue deposition as amyloid fibrils.
- AG10, an investigational molecule, is a highly selective and potent stabilizer of TTR that mimics the T119M rescue mutation and has been studied in Phase 1 and 2 clinical studies.[1, 2]

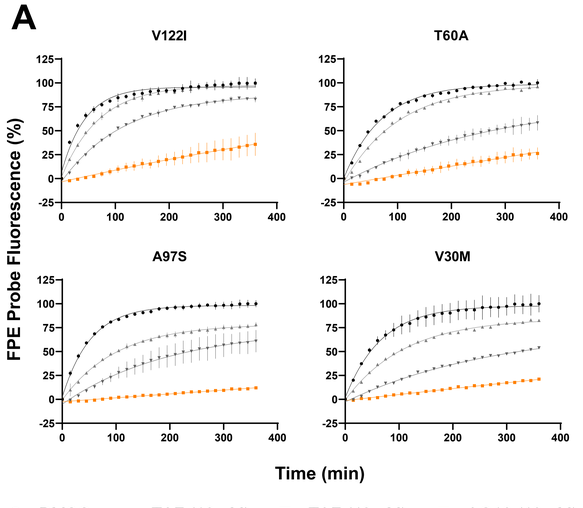
Hypothesis

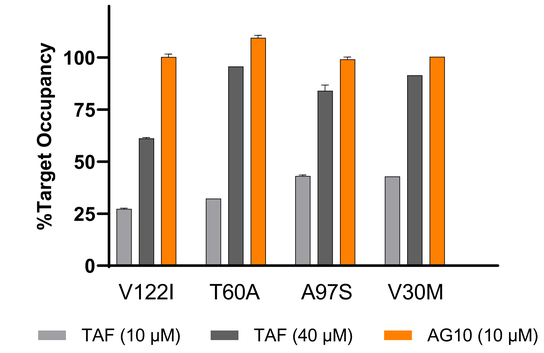
- Pathogenic TTR variants with varied intrinsic instability display differential stabilization by AG10 or tafamidis.
- In vitro, AG10 achieves near-complete stabilization of TTR at clinical concentrations.

Materials and Methods

- Two in vitro assays were used to assess TTR stabilization in patient samples by AG10 or tafamidis (TAF): Fluorescent Probe Exclusion assay (FPE) and Western Blot (WB). Commercially available TAF was used in this study. Patient samples were obtained from AG10 clinical trials.
- Individual patient samples representing a spectrum of intrinsic instability and clinical phenotypes (V122I, T60A, A97S) were assayed following in vitro addition of AG10 or TAF at concentrations spanning their therapeutic ranges[3, 4]. N=1-2 for FPE assay, N=4-8 for Western Blot.
- The binding site occupancy of TTR in serum was measured by FPE according to an established method[5]. Rate constants were calculated using a one-phase association fit in GraphPad Prism:
  - $RFU=RFU_0 + (Plateau-RFU_0)*(1-e^{-K*minutes})$
  - Plateau constraint: Global RFU_{max} from untreated sample
- 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 Blots[1]. Tetrameric TTR bands were quantified using Li-Cor Image Studio software.

Occupancy of Mutant TTR by AG10 and Tafamidis by FPE Assay





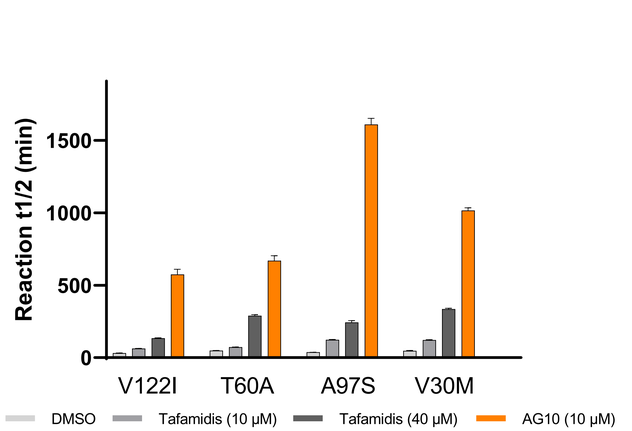
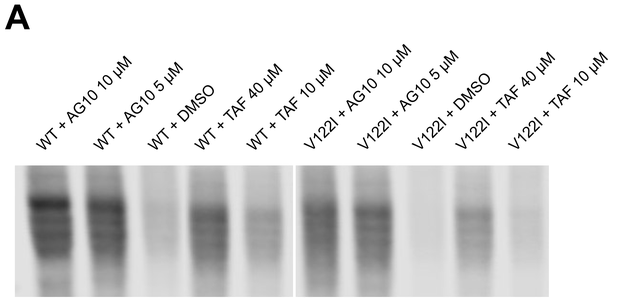


Figure 1. *Fluorescent probe exclusion assay (FPE) characterization of TTR binding site occupancy.* ***A)*** *Normalized average fluorescence vs. time plots of patient serum samples.* ***B)*** *Average target occupancy at 1 hr.* ***C)*** *FPE reaction halftimes were derived for each patient sample. Mean and standard deviation shown for* ***A*** *and* ***B,*** *mean and standard error shown for* ***C****.*

Stabilization of WT and V122I TTR by AG10 and Tafamidis by Western Blot



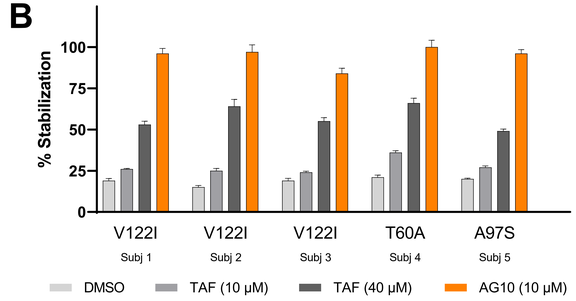


Figure 2. *Western Blot quantitation of tetrameric TTR in plasma samples subjected to low pH conditions.* ***A)*** *Representative Western Blot image with tetrameric TTR stabilization at 72 hr. WT depicts pooled normal human plasma.* ***B)*** *Percent stabilization of tetrameric TTR after 72 hr acidification. Results from five individuals with destabilizing TTR mutations are shown. Mean and standard error shown.*

Summary

- At concentrations spanning their reported therapeutic ranges, AG10 bound serum TTR to a greater extent than tafamidis.
- FPE reaction halftime, a measure of TTR binding efficiency, is 2-6 fold longer for 10 µM AG10 than for 40 µM tafamidis.
- In vitro addition of AG10 resulted in consistently greater and more durable TTR tetramer stabilization than adding tafamidis in all individual patient plasma samples tested.

Conclusions

- At therapeutic concentrations, AG10 more completely stabilizes variant TTR samples representing a range of destabilizing mutations and clinical phenotypes than does tafamidis.
- AG10 has the potential to demonstrate clinical benefit in patients with a variety of genotypes associated with both TTR cardiomyopathy and polyneuropathy.
- These findings support further development of AG10 as a disease-modifying treatment for patients with hereditary ATTR.

References

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