

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

Paul W Wong, BS¹, Alan X Ji, PhD¹, Jonathan Fox, MD, PhD¹, John L Berk, MD², Uma Sinha, PhD¹
¹ Eidos Therapeutics Inc, San Francisco, CA. ² Boston Univ School of Medicine and Boston Medical Center, Boston, MA

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}

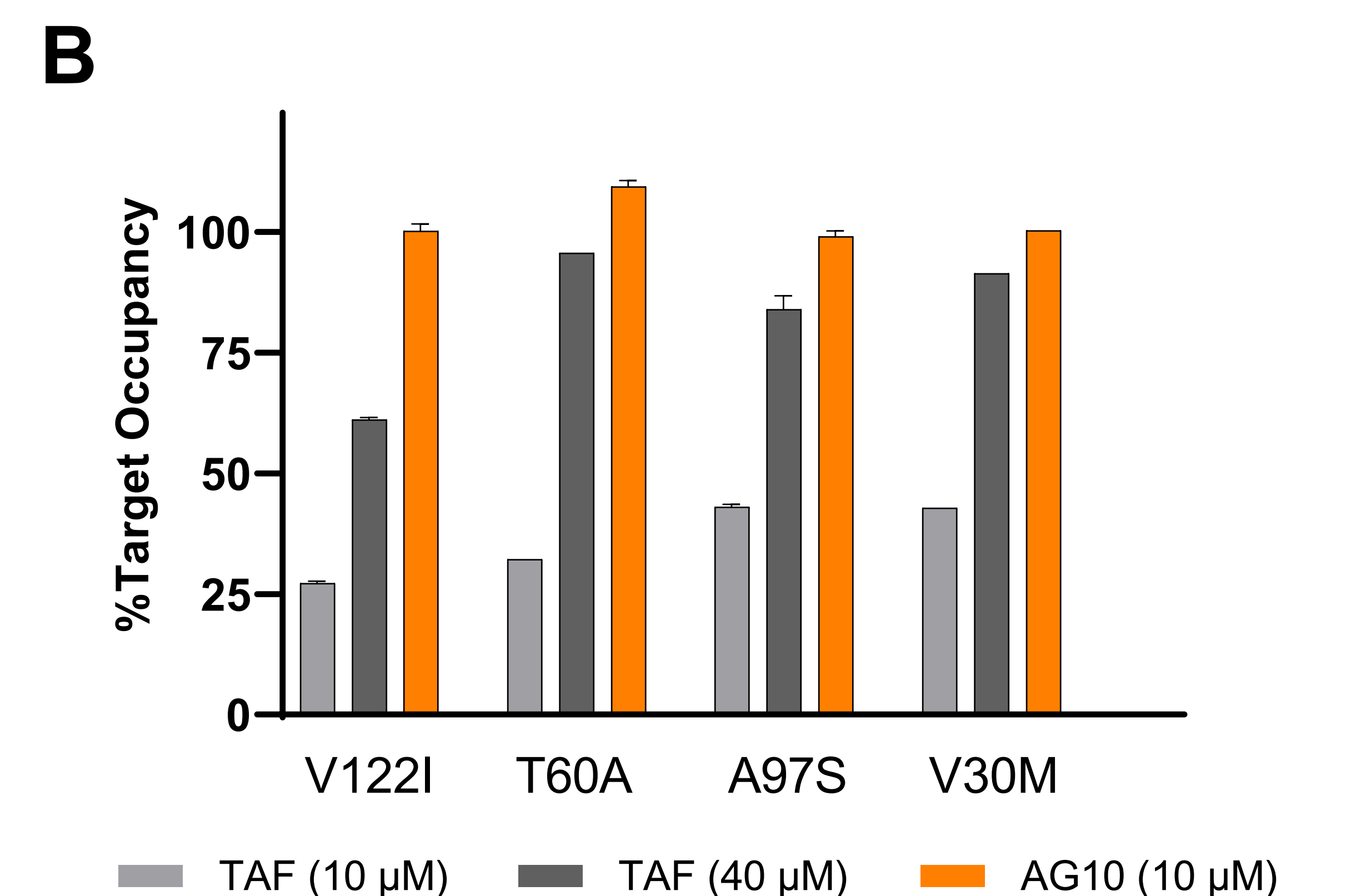
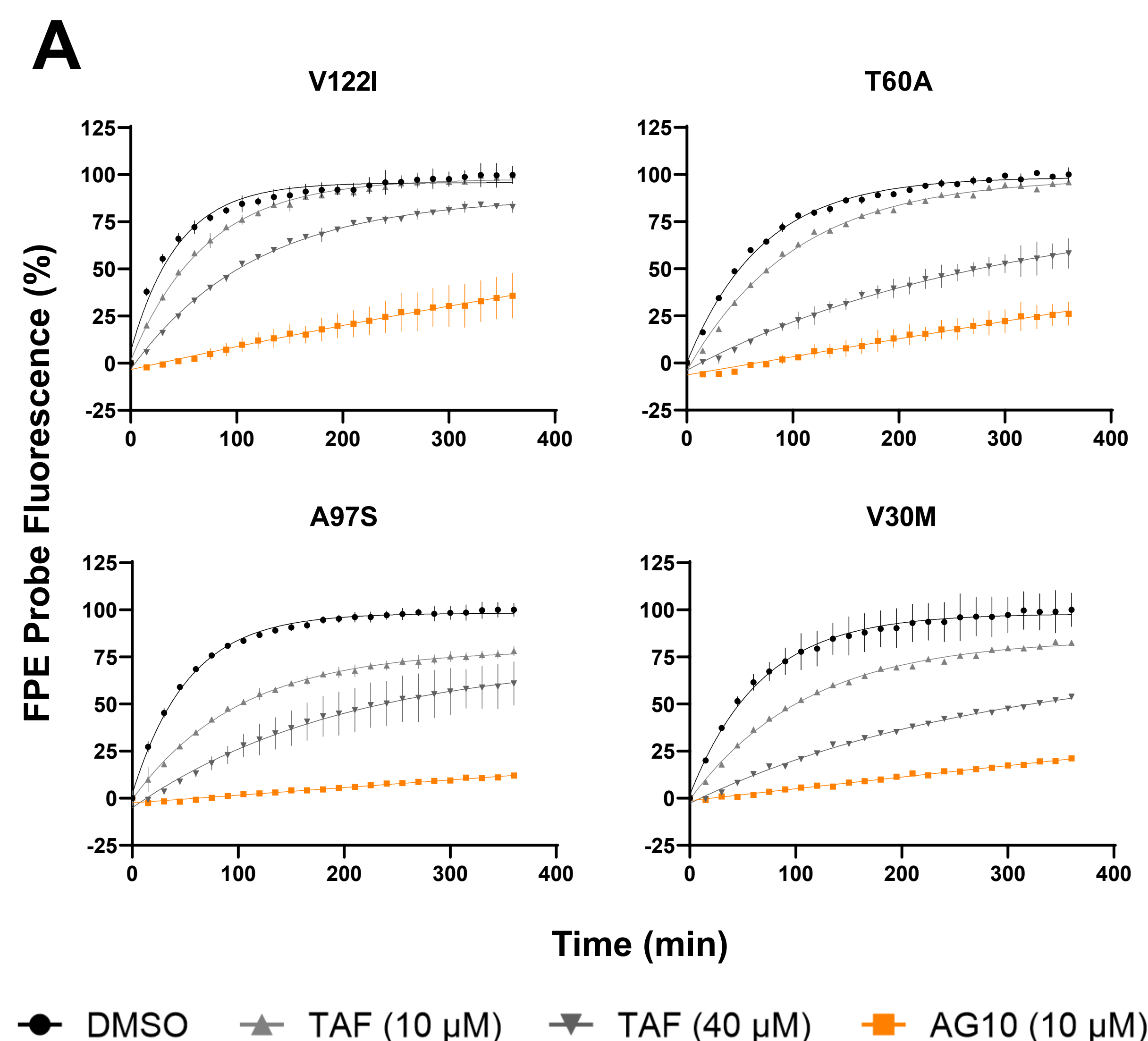
Hypothesis

- 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 method⁵. 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 Blots¹. Tetrameric TTR bands were quantified using Li-Cor Image Studio software.

Occupancy of Mutant TTR by AG10 and Tafamidis by FPE Assay



C

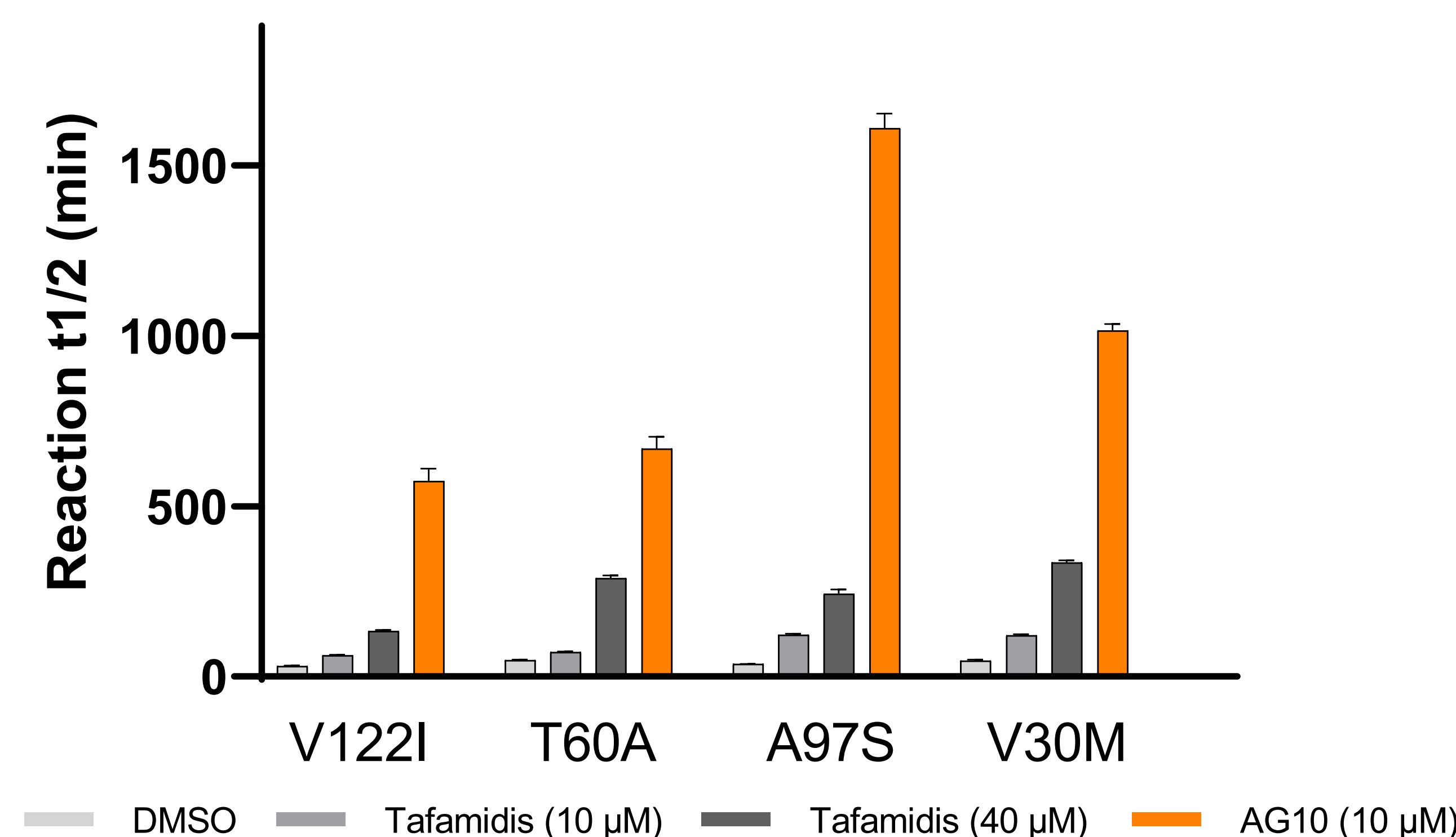
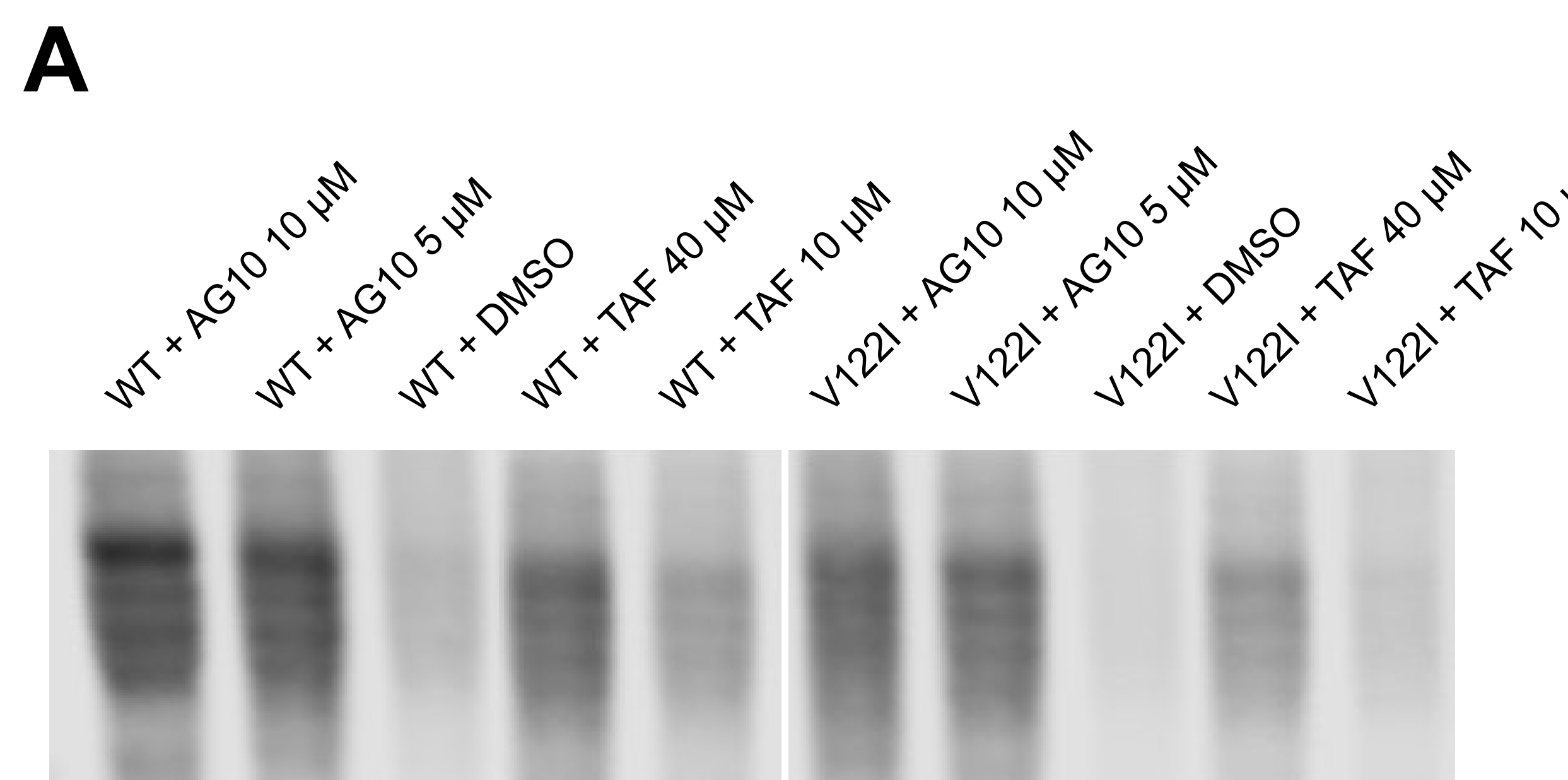


Figure 1. Fluorescent probe exclusion assay (FPE) characterization of TTR binding site occupancy. **A)** Normalized average fluorescence vs. time plots of patient serum samples. **B)** Average target occupancy at 1 hr. **C)** FPE reaction half-times were derived for each patient sample. Mean and standard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



B

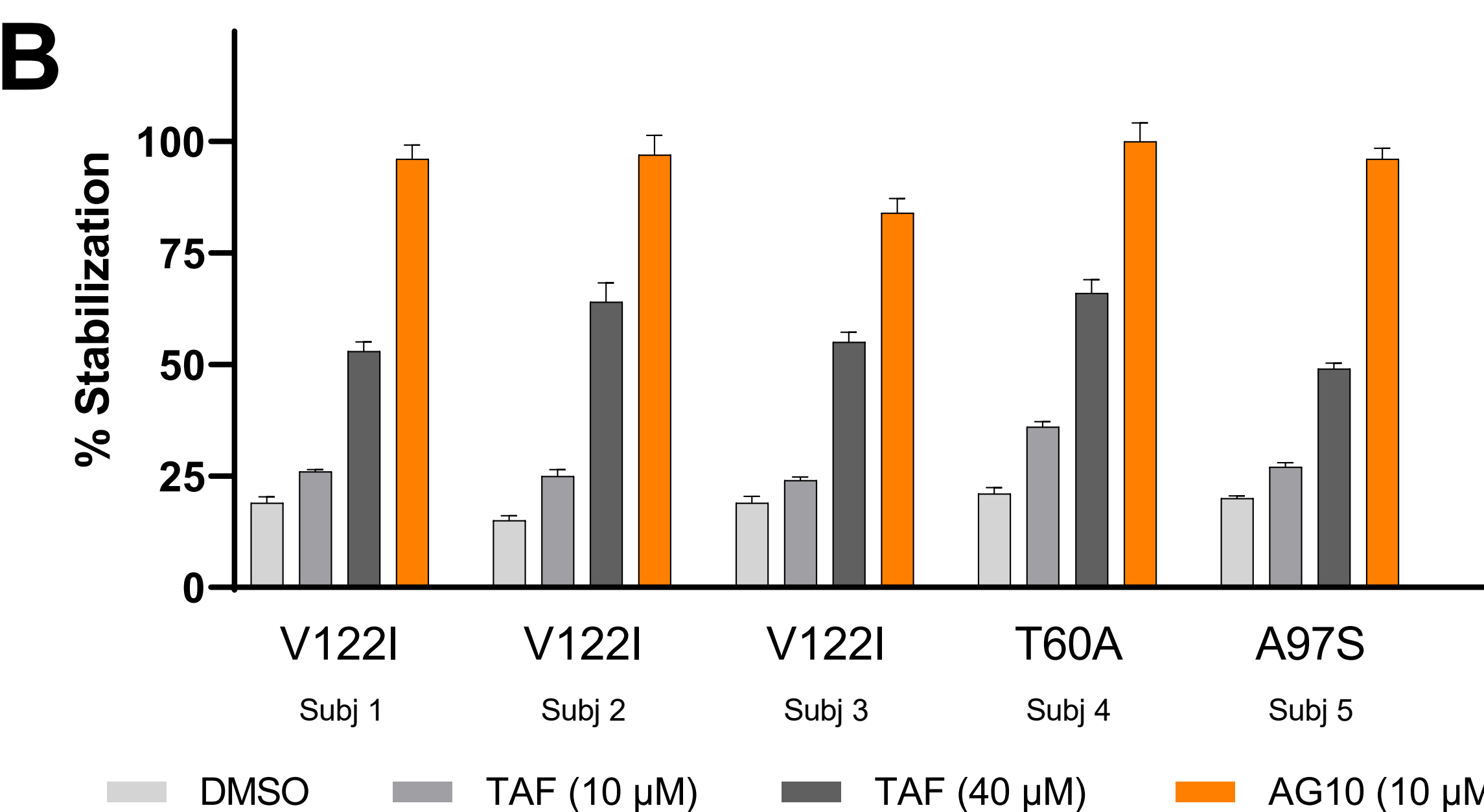


Figure 2. Western Blot quantitation of tetrameric TTR in plasma samples subjected to low pH conditions. **A)** Representative Western Blot image with tetrameric TTR stabilization at 72 hr. WT depicts pooled normal human plasma. **B)** Percent stabilization of tetrameric TTR after 72 hr acidification. 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 half-time, 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

- Fox JC *et al.* First-in-Human Study of AG10, a Novel, Oral, Specific, Selective, and Potent Transthyretin Stabilizer for the Treatment of Transthyretin Amyloidosis: A Phase 1 Safety, Tolerability, Pharmacokinetic, and Pharmacodynamic Study in Healthy Adult Volunteers. *Clin Pharmacol Drug Dev.* 2019 Jun 6.
- Judge DP *et al.* Transthyretin Stabilization by AG10 in Symptomatic Transthyretin Amyloid Cardiomyopathy. *J Am Coll Cardiol.* 2019 Jul 23;74(3):285-295.
- Summary Review for Regulatory Action – NDA 211996/NDA212161 (tafamidis meglumine/free acid). May 2, 2019: 12.
- Clinical Review, NDA 211996 Vyndaqel (Tafamidis meglumine). Nov. 2, 2018: 37.
- Choi S, Kelly JW. A competition assay to identify amyloidogenesis inhibitors by monitoring the fluorescence emitted by the covalent attachment of a stilbene derivative to transthyretin. *Bioorganic Med Chem* 2011;19:1505–14.