AG10, an orally available, novel transthyretin (TTR) stabilizer: integrated preclinical evaluation predicts a highly effective treatment for TTR amyloid cardiomyopathy

Mark Miller[1], Arindom Pal[1], Wabel Albusairi[1], Isabella Graef[2], Uma Sinha[3], Jonathan Fox[3], Robert Zamboni[3], Neil Kumar[3], Mamoun Alhamadsheh[1]

[1]Department of Pharmaceutics and Medicinal Chemistry, University of the Pacific, Stockton, California, USA
[2]Department of Pathology, Stanford University School of Medicine, Stanford, California, USA
[3]Eidos Therapeutics, Inc., Palo Alto, California, USA

Introduction

TTR amyloid cardiomyopathy (ATTR-CM) is a degenerative disease caused by TTR tetramer dissociation, monomer misfolding, aggregation and myocardial deposition of toxic TTR amyloid aggregates. Missense mutations in TTR resulting in less stable tetramers cause an autosomal dominant form of the disease (ATTRm-CM) that is generally more aggressive than that associated with wild-type TTR (ATTRwt-CM). Individuals carrying the trans-suppressor T119M variant together with the polyneuropathy (ATTR-PN) associated V30M-TTR mutation, present a more benign evolution of ATTR-PN or no disease compared to heterozygote kindred carrying the V30M-TTR mutation alone. Genetic, structural and clinical evidence (diflunisal and tafamidis trials in ATTR-PN) suggests that TTR stabilization may be an effective disease-modifying strategy for ATTR-CM. No therapies are currently approved to treat ATTR-CM.

Purpose

To document the therapeutic potential of AG10[1,2] for treating ATTR-CM based on *in vitro* and *in vivo* demonstration of TTR stabilizing activity.

Methods

Due to the lack of animal models that faithfully reproduce the pathology of human ATTR-CM, new approaches to test the efficacy of TTR kinetic stabilizers are greatly needed. We took a comprehensive structural and biochemical approach *in vitro* and *in vivo* (in dogs) to characterize the TTR-stabilizing activity of AG10. Structural studies were used to document and characterize the nature of binding between AG10 and TTR at the thyroxin binding sites. Following oral dosing of AG10 to dogs, a combination of *ex vivo* fluorescence and western blot assays characterized the ability of AG10 to occupy and stabilize the TTR tetramer, preventing dissociation and amyloidogenesis.

References: (1) Alhamadsheh MM, et al. Potent kinetic stabilizers that prevent transthyretin-mediated cardiomyocyte proteotoxicity. *Sci Transl Med. 2011;3(97):97ra81.* (2) . Penchala S, et al. AG10 inhibits amyloidogenesis and cellular toxicity of the familial amyloid cardiomyopathy associated V122I transthyretin. *Proc Natl Acad Sci U S A. 2013,110(24):9992-7*

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Results

Figure 1. AG10 stabilizes TTR in human serum *in vitro* more effectively than other known stabilizers

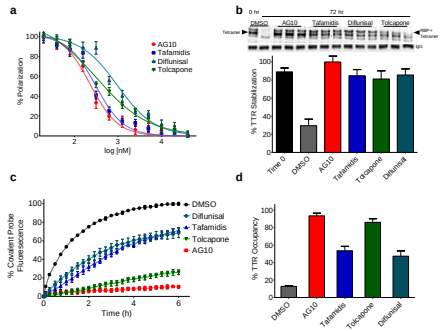

Figure 1. (**a**) Binding affinity of stabilizers to TTR in buffer. (**b**) %TTR stabilization in serum by stabilizers using Western blot; AG10 (10 μM), tafamidis (20 μM), tolcapone (20 μM), diflunisal (200 μM). (**c**,**d**) %TTR occupancy in human serum by fluorescence probe exclusion (FPE) assay.

Figure 3. AG10 effectively stabilizes TTR in beagle dog serum *in vitro*

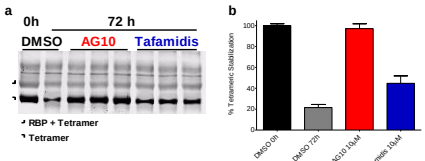

Figure 3. (**a**,**b**) Western bot analysis of the *in vitro* stabilization of TTR in dog serum by AG10 (10 μM) and tafamidis (10 μM).

Figure 2. AG10 stabilizes TTR by mimicking the disease suppressing T119M variant

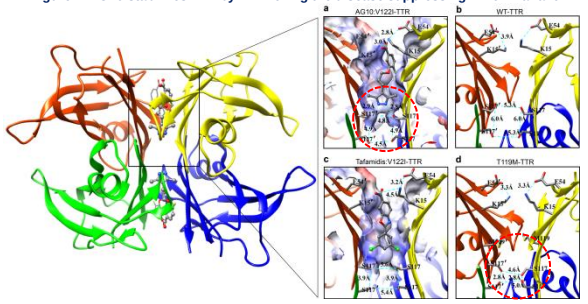

Figure 2. Crystal structures highlighting the similar interactions caused by the trans-suppressor stabilizing T119M mutation and binding of AG10 to TTR. Quaternary structure of (**a**) AG10 bound to V122I-TTR (PDB: 4HIQ). (**b**) WT-TTR (PDB: 3CFM). (**c**) tafamidis bound to V122I-TTR (PDB: 4HIS). (**d**) T119M-TTR variant (PDB: 1FHN) with dashed lines and red circles highlighting key interactions between the hydroxyl groups of S117/S117'.

Figure 4. Orally administered AG10 effectively stabilizes TTR in beagle dogs serum *ex vivo*

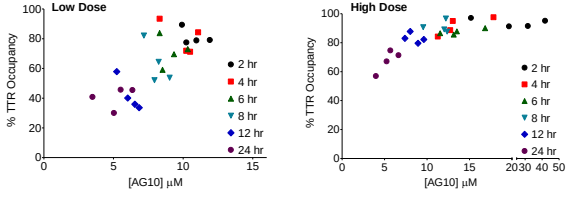

Figure 4. PK-PD analysis of AG10 in dogs receiving single oral doses of AG10 at (**a**) low dose and (**b**) high dose. Scatterplot of concentration [AG10] vs. %TTR occupancy of serum samples obtained from dogs at various time points (n=4, 2 males/2 females per dosing group).

Conclusion

AG10 participates in potent, specific, unique and unprecedented binding interactions with TTR that mimic the protective T119M mutation, as reflected in structural, biochemical, and *in vivo* studies. Despite the limitations of available animal models, the healthy dog presents an informative nonclinical model for exploring the PK-PD relationships of orally available, small molecule TTR stabilizers like AG10.