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Transthyretin Stabilization by AG10 in Symptomatic Transthyretin Amyloid Cardiomyopathy

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ABSTRACT

BACKGROUND Transthyretin (TTR) amyloidosis is an underdiagnosed disease caused by destabilization of TTR due to pathogenic mutations or aging. Both pathogenic and protective mutations illuminate mechanisms of disease and potential interventions. AG10 is a selective, oral TTR stabilizer under development for transthyretin amyloidosis cardiomyopathy (ATTR-CM) that mimics a protective TTR mutation.

OBJECTIVES This randomized, double-blind, placebo-controlled study evaluated safety, tolerability, pharmacokinetics, and pharmacodynamics of AG10 in ATTR-CM patients with symptomatic, chronic heart failure.

METHODS ATTR-CM, New York Heart Association functional class II to III subjects (n = 49, mutant or wild-type) were randomized 1:1:1 to AG10 400 mg, AG10 800 mg, or placebo twice daily for 28 days. Safety and tolerability were assessed by clinical and laboratory criteria. AG10 plasma levels were measured. TTR stability was assessed by changes in serum TTR, and 2 established ex vivo assays (fluorescent probe exclusion and Western blot).

RESULTS AG10 treatment was well-tolerated, achieved target plasma concentrations and demonstrated near-complete stabilization of TTR. TTR stabilization was more complete and less variable at the higher dose with stabilization by fluorescent probe exclusion of 92 ± 10% (mean ± SD) at trough and 96 ± 9% at peak (both $p < 10^{-12}$ vs. placebo). Average serum TTR increased by 36 ± 21% and 51 ± 38% at 400 and 800 mg, respectively (both $p < 0.0001$ vs. placebo). Baseline serum TTR in treated subjects was below normal in 80% of mutant and 33% of wild-type subjects. AG10 treatment restored serum TTR to the normal range in all subjects.

CONCLUSIONS AG10 has the potential to be a safe and effective treatment for patients with ATTR-CM. A phase 3 trial is ongoing. (Study of AG10 in Amyloid Cardiomyopathy; NCT03458130) (J Am Coll Cardiol 2019;74:285–95)

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ABBREVIATIONS AND ACRONYMS

ATTR-CM = transthyretin amyloid cardiomyopathy

FPE = fluorescent probe exclusion

NT-proBNP = N-terminal pro-brain type natriuretic peptide

TTR = transthyretin

Transthyretin amyloidosis (ATTR) is a progressive, fatal disease in which deposition of amyloid derived from either mutant or wild-type transthyretin (TTR) causes severe organ damage. Clinically, the ATTR phenotypes responsible for the greatest morbidity and mortality are recognized as transthyretin amyloid cardiomyopathy (ATTR-CM) or transthyretin amyloid polyneuropathy (ATTR-PN); familial (mutant) ATTR frequently presents with a mixed phenotype.

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ATTR-CM is an infiltrative, restrictive cardiomyopathy characterized by right and left heart failure, usually with preserved left ventricular ejection fraction, and is associated with a high risk of both heart block and atrial fibrillation. At the time of writing, there were no therapies specifically approved for the treatment of ATTR-CM (1), although a positive phase 3 study of tafamidis, another TTR stabilizer, has been reported (2). In that study, over a 30-month treatment duration, active treatment was associated with lower all-cause mortality (29.5% vs. 42.9%) and lower frequency of cardiovascular-related hospitalizations (0.48/year vs. 0.70/year) compared with placebo. This result demonstrates the therapeutic potential of TTR stabilization for the treatment of ATTR-CM. While encouraging, the high residual morbidity and mortality observed in the active treatment arms demonstrates a clear unmet need.

Familial ATTR (ATTRm, or mutant ATTR) syndromes are driven by pathogenic point mutations in the TTR gene that display tissue tropism according to genotype. The V122I mutation carried by 3.4% of African Americans (3) is predominantly cardiomyopathic. Conversely, the V30M mutation, endemic in certain geographies, is predominantly polyneuropathic with an early age of onset in these regions (4), although cardiomyopathy is also evident in about one-third of V30M carriers (5) and is more prevalent in late-onset disease.

Older individuals may develop ATTR derived from wild-type TTR (ATTRwt). The major clinical manifestation is ATTR-CM, although the pathologically related preceding presentations of carpal tunnel syndrome, spinal stenosis, or tendon involvement can potentially lead to earlier diagnosis (6–8).

Destabilization and dissociation of the native TTR tetramer is the initiating event in the disease mechanism of ATTR, and it is informed by human genetics. Pathogenic TTR mutations destabilize the native tetramer. The more destabilizing the mutation, the more penetrant and severe the phenotype (9). The prevalent V122I variant dissociates approximately twice as rapidly as wild-type TTR, is associated with more aggressive ATTR-CM compared with wild type, and is associated with lower circulating TTR levels (10–13).

A super-stabilizing mutation (T119M) has been identified that protects carriers from the disease. The T119M variant reduces the dissociation rate of tetrameric TTR by over 33-fold compared with wild type (9). Studies have shown that its enhanced stability is likely due to the formation of hydrogen bonds between neighboring serine residues at position 117 of each monomer. This feature has not been observed for either wild-type or any other variant TTR (14–19). Compound heterozygotes carrying both T119M and the ATTR-PN-associated V30M mutation develop few if any manifestations of the disease (20). A large observational study also reported that T119M carriers have on average 20% higher serum TTR levels, are at lower risk of cerebrovascular events, and live 5 to 10 years longer compared with the general population (21).

All known stabilizers bind to the thyroxine binding site of tetrameric TTR and prevent or slow dissociation of the tetramer into amyloidogenic monomers, the initiating event in amyloidogenesis (17,22–24). By preventing dissociation of the tetramer, stabilizers are predicted to reduce the rate of generation of

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unstable monomers, thereby slowing or halting ATTR disease progression.

The rationale for regarding serum TTR as an in vivo biomarker of TTR stability is based on several lines of evidence:

- In healthy adults, serum TTR turns over with a half-life of about 2 days and hepatic secretion has a "set point" reflecting nutritional status uninfluenced by intrinsic stability.
- Destabilizing mutations drive below normal serum TTR concentrations in ATTRm reflecting lower tetrameric stability; ATTRwt patients also tend to have low-normal to below normal levels (13).
- Serum TTR is an independent predictor of survival in ATTRwt-CM (25).
- Heterozygous T119M carriers have 20% higher TTR concentrations compared with the general population (21).
- Treatment with a TTR stabilizer increases circulating TTR concentrations in patients with ATTR-CM (25,26).

AG10 is a potent, highly selective TTR stabilizer that was designed to mimic the structural influence of the protective T119M mutation. Compared with other known stabilizers, AG10 is unique in its capacity to form hydrogen bonds with the same serine residues at position 117 that stabilize the T119M variant.

The current investigation (NCT03458130) was a phase 2, safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) study in ATTR-CM patients with symptomatic chronic heart failure (New York Heart Association [NYHA] functional class II to III). The primary objective of this randomized, double-blind, placebo-controlled study was to establish overall safety and tolerability of AG10 at oral doses of either 400 or 800 mg administered twice daily for 28 days. We also sought to determine if chronic oral administration of AG10 achieved similar plasma levels and evidence of TTR stabilizing activity, including effects on serum TTR as an in vivo reflection of TTR stabilization, in both wild-type or mutant ATTR-CM, as has been observed in healthy adult volunteers (27).

METHODS

STUDY DESIGN. Patients age 18 to 90 years with an established diagnosis of ATTR-CM and with NYHA functional class II to III symptoms were eligible for the study. The diagnosis of ATTR-CM could have been established by either positive endomyocardial biopsy or positive ^{99m}Tc-pyrophosphate scan with appropriate exclusionary testing for immunoglobulin light chain amyloidosis (see the Online Appendix for complete eligibility criteria). Subjects were randomized in a 1:1:1 ratio to, 400 or 800 mg AG10 or matching placebo, administered twice daily. The primary objective of the study was to evaluate the safety and tolerability of AG10 compared with placebo. Secondary endpoints included PK (AG10 plasma concentrations), change from baseline in serum TTR concentration, and 2 distinct ex vivo measures of TTR stabilization.

The study was approved by the institutional review boards at the respective participating investigative sites. All participants provided written informed consent. The trial was conducted at experienced ATTR-CM centers according to the Good Clinical Practice guidelines of the International Conference on Harmonisation and the World Health Organization Declaration of Helsinki.

INVESTIGATIONAL MEDICINAL PRODUCT. AG10 (and matching placebo) was provided as 200-mg film-coated tablets to be administered orally.

SAFETY EVALUATION. Safety evaluations included vital signs (blood pressure, heart rate), physical examination, clinical laboratory tests (hematology, clinical chemistry, urinalysis), electrocardiography, and assessment of adverse events (AEs). Biomarkers of ATTR-CM disease activity or heart failure severity (N-terminal pro-brain type natriuretic peptide [NT-proBNP] and troponin I) were also analyzed.

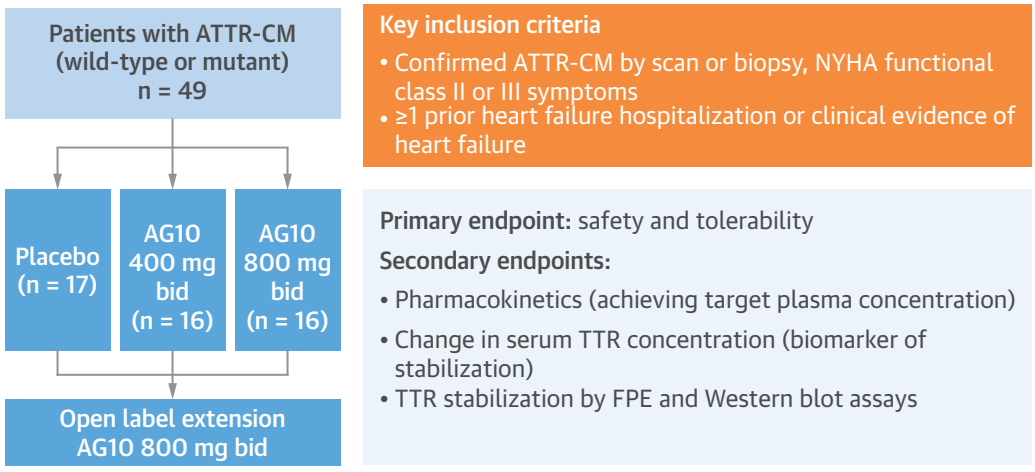
SAMPLE COLLECTION. Serial blood samples were collected at baseline (prior to first dose of AG10 or placebo), at a 14-day visit, and at study end (28-day visit). At each visit, samples were obtained pre-dose (baseline on day 1; trough on day 14 or 28), and at 0.5, 1, and 2 h post-dose (covering estimated T_{max}). Samples were analyzed for PK and PD assays as described in the following text.

PK ENDPOINTS. Plasma samples were analyzed for AG10 by a validated method (range 10.0 to 10,000 ng/ml) (Worldwide Clinical Trials, Austin, Texas).

PD ENDPOINTS. PD of AG10 were assessed ex vivo in samples obtained before and after administration of AG10 by established assays of TTR stabilization (Fluorescent Probe Exclusion [FPE] and Western blot). The former is a competitive binding assay measuring occupancy at the thyroxine binding site of TTR; the latter is a measure of a bound ligand's ability to prevent the accelerated dissociation of tetrameric TTR under denaturing conditions.

The FPE assay was performed according to an established method (28). The probe emits a fluorescent signal only when covalently bound to the thyroxine binding site of TTR. The time-dependent

FIGURE 1 AG10-201 Study Design, Main Entry Criteria and Endpoints



A total of 49 subjects were randomized 1:1:1 to AG10 400 mg twice daily (bid), 800 mg bid, or matching placebo. All subjects completed the study (NCT03458130). ATTR-CM = transthyretin amyloid cardiomyopathy; FPE = fluorescent probe exclusion; NYHA = New York Heart Association; TTR = transthyretin.

development of the fluorescence signal is reduced in direct proportion to the percentage binding occupancy by a competing ligand. Relative fluorescent units (RFUs) at t = 60 min were normalized to t = 60 min RFU from individual pre-dose samples to determine % target occupancy by the competing ligand. Complete (100%) occupancy is defined as zero fluorescence; 0% occupancy is defined as fluorescence of individual pre-dose samples.

The Western blot assay was performed as previously described (17). Plasma samples were acidified to pH 4.0 and incubated for 72 h prior to crosslinking with glutaraldehyde. "Time zero" replicates from each sample were acidified immediately prior to crosslinking. Replicates were subjected to denaturing gel electrophoresis and immunoblotting using anti-TTR antiserum (DAKO-A0002, DAKO, Carpinteria, California). Immunoblots were quantified using a ChemiDoc MP Imaging System (Biorad Laboratories, Richmond, California). Band intensities for tetrameric-TTR were compared between 0 and 72 h denaturation for each sample to determine percent stabilization. Complete stabilization is defined as 100% retention of tetrameric-TTR after 72 h; 0% stabilization is defined as no residual tetrameric-TTR.

Serum TTR (prealbumin) concentrations were determined in a central clinical laboratory (ARUP Laboratories, Salt Lake City, Utah). The reference range in this laboratory is 20 to 40 mg/dl.

STATISTICAL ANALYSIS. The analysis set for safety analyses was all subjects dosed. Statistical comparison of PK and PD (serum TTR [prealbumin], FPE, and Western blot) data between dose groups and placebo were prespecified. Differences in TTR stabilization by FPE were calculated using 2-tailed, heteroscedastic *t*-test. Difference in proportion of patients below and above normal TTR between baseline and day 28 was performed using McNemar's test in a post hoc analysis. All p values are 2-tailed, and $p < 0.05$ was considered statistically significant for the pre-specified analyses.

RESULTS

SUBJECT DISPOSITION. The study design is illustrated in Figure 1. The study enrolled 49 subjects, of which 14 (29%) had known mutant ATTR-CM (V122I, n = 11; T60A, n = 2; V30M, n = 1). Subjects ranged in age from 60 to 86 years, with a mean of 74.1 years, and 92% were male. All subjects had symptomatic, chronic heart failure due to ATTR-CM with NYHA functional class II or III symptoms; approximately 30% of subjects were enrolled with NYHA functional class III symptoms. All subjects had relatively high baseline NT-proBNP concentrations (median 2,677 pg/ml [interquartile range: 1,205 to 4,217 pg/ml]) and were positive for troponin I (>0.02 ng/ml) at entry and throughout the study. Importantly, on average, subjects had relatively low TTR at baseline (22.0 ± 5.4 mg/dl; laboratory reference range 20 to 40 mg/dl) (Table 1). Baseline characteristics were generally consistent across groups aside from a larger

proportion of ATTRm-CM patients in the 2 active treatment groups compared with the placebo group. All subjects completed the study.

SAFETY. AG10 was generally well tolerated. The proportion of subjects who experienced AEs was 88%, 63%, and 69% of subjects administered placebo and 400 and 800 mg AG10, respectively. Most AEs were mild to moderate in severity in both the placebo and active treatment groups (Table 2). The most commonly observed AEs, occurring in 4 or more subjects across all treatment groups including placebo, were atrial fibrillation, constipation, diarrhea, and muscle spasms.

There were 4 serious adverse events (SAEs) reported in the study. One placebo-treated subject experienced 2 SAEs of atrial fibrillation and congestive heart failure. Another placebo-treated subject experienced lower extremity cellulitis. One AG10-treated subject experienced an SAE of dyspnea (attributed to heart failure) requiring hospitalization. There were no deaths in the study.

There were no clinically important changes or trends observed in safety laboratory tests that were inconsistent with the underlying disease (e.g., elevations in NT-proBNP or troponin I) (Table 3), nor were there any clinically important changes from baseline electrocardiography findings in the study.

PHARMACOKINETICS OF AG10. AG10 plasma concentrations were determined at peak following the initial dose, and at peak and trough on days 14 and 28 of the study. Peak plasma concentrations were consistent with those observed in a phase 1 study of AG10 in healthy adult volunteers (29). Trough concentrations of AG10 were similar at days 14 and 28 within each dose group. At the 400 mg twice daily dose, trough plasma concentrations of AG10 were 1,920 ± 837 ng/ml on day 14 and 1,840 ± 488 ng/ml on day 28. In the 800 mg twice daily (bid) group, the trough plasma concentrations were 2,260 ± 983 ng/ml on day 14 and 2,240 ± 900 ng/ml on day 28, consistent with the intersubject variability, less than dose-proportional PK, and terminal elimination half-life observed in the phase 1 study. At the higher dose, the average steady-state trough concentration achieved was predicted by the phase 1 PK-PD relationship to result in near-complete TTR stabilization over the entire dosing interval, an important goal of the AG10 development program that was confirmed by the FPE assay (see the following text).

PHARMACODYNAMICS OF AG10. To explore the in vivo effects of increased TTR stability on its serum concentration, serum TTR (prealbumin) levels were measured in samples collected at baseline and at both the middle and end of study. Subjects in the placebo group experienced a mean reduction of 7 ± 15% in serum TTR concentration by day 28 relative to baseline. Conversely, subjects administered either 400 or 800 mg AG10 bid showed a dose-dependent, mean increase in circulating TTR of 36 ± 21% ($p < 0.0001$ vs. placebo) and 50 ± 38% ($p < 0.0001$ vs. placebo), respectively (Figure 2). There was a greater treatment effect (% increase from baseline, $p < 0.05$) observed in AG10-treated subjects with mutant ATTR-CM (67 ± 42%, hatched columns) compared with subjects with wild-type ATTR-CM (33 ± 20%, solid columns). This

TABLE 1 Baseline Characteristics

	Placebo (n = 17)	AG10 400 mg (n = 16)	AG10 800 mg (n = 16)
Age, yrs	73.2 [60–85]	73.8 [60–83]	75.4 [67–86]
Male	17 (100)	14 (88)	14 (88)
ATTRm*	3 (18)	6 (38)	5 (31)
NYHA functional class III	5 (29)	6 (38)	3 (19)
Race			
White	13 (76)	10 (62)	12 (75)
Black	3 (18)	4 (25)	3 (19)
Other	1 (6)	2 (13)	1 (6)
NT-proBNP, pg/ml†	2,890 (1,119–3,890)	3,084 (1,193–5,517)	2,466 (1,575–3,811)
Troponin I, ng/ml‡	0.09 (0.06–0.13)	0.12 (0.07–0.21)	0.07 (0.06–0.11)
TTR, mg/dl§	23.4 ± 5.5	23.2 ± 5.7	19.5 ± 4.2
Creatinine, mg/dl	1.24 ± 0.25	1.38 ± 0.29	1.31 ± 0.41
LV ejection fraction, %	56.0 ± 13.2	47.8 ± 15.1	55.7 ± 9.0
Interventricular wall thickness, cm	1.75 ± 0.22	1.65 ± 0.34	1.82 ± 0.34

Values are mean [range], n (%), median (interquartile range), or mean ± SD. *ATTRm-CM variants: V122I (n = 11), T60A (n = 2), and V30M (n = 1). †NT-proBNP normal range = 0 to 449 pg/ml. ‡Troponin I normal range = 0 to 0.02 ng/ml. §TTR normal range = 20 to 40 mg/dl.

ATTR-CM = transthyretin amyloid cardiomyopathy; FPE = fluorescent probe exclusion; LV = left ventricular; NT-proBNP = N-terminal pro-brain type natriuretic peptide; NYHA = New York Heart Association; TTR, transthyretin.

TABLE 2 Safety and Tolerability—Summary of Adverse Events*

	Placebo (n = 17)	AG10 400 mg (n = 16)	AG10 800 mg (n = 16)
Any adverse event	15 (88)	10 (63)	11 (69)
Mild	6 (35)	8 (50)	3 (19)
Moderate	8 (47)	2 (13)	7 (44)
Severe	1 (6)	0 (0)	1 (6)
Any serious adverse event†	2 (12)	1 (6)	0 (0)
AF and CHF	1 (6)	0 (0)	0 (0)
Leg cellulitis	1 (6)	0 (0)	0 (0)
Dyspnea	0 (0)	1 (6)	0 (0)

Values are n (%). Most frequent adverse events: (n ≥ 4 subjects): atrial fibrillation (AF), constipation, diarrhea, muscle spasms. *No laboratory safety signals of a potential clinical concern attributed to study drug. †None considered related to study drug.

CHF = congestive heart failure.

TABLE 3 Change From Baseline in NT-proBNP and Troponin I

	NT-proBNP*		Troponin I†	
	Mean	SD	Mean	SD
Placebo	−4	36	−7	15
400 mg	3	33	−4	22
800 mg	−14	16	0	17

Values are %. *Reference range = 0 to 449 pg/ml. †Reference range = 0 to 0.02 ng/ml.
NT-proBNP = N-terminal pro-brain type natriuretic peptide.

might be explained in part by the lower serum TTR levels in mutant ATTR-CM subjects measured at baseline. At baseline, 38% of subjects had serum TTR levels below the normal range. A greater proportion of subjects in the 800 mg AG10 group than the placebo group had below normal TTR levels at baseline. Treatment with AG10 restored serum TTR concentrations to within the normal range in all subjects regardless of baseline value or mutation status, resulting in fewer subjects below the normal range in both 400 and 800 mg relative to placebo (Figure 3) ($p < 0.0001$).

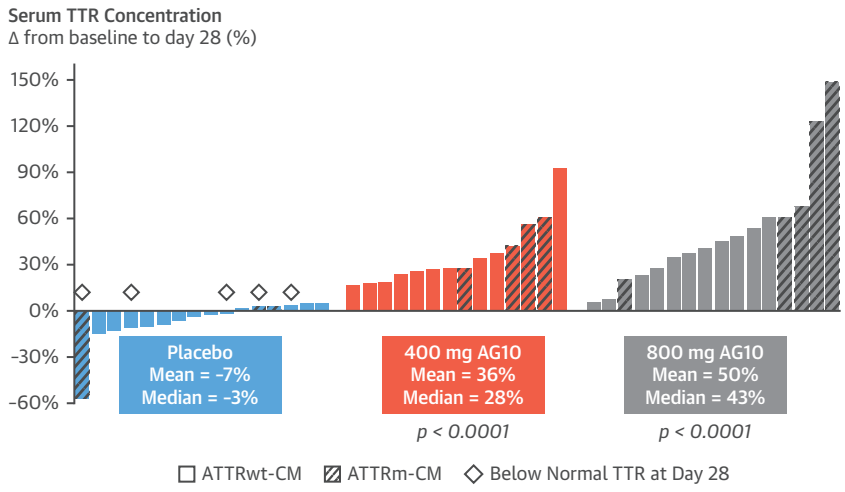
The pharmacological effect in subjects treated with either the 400 or 800 mg bid doses of AG10 was also explored using 2 ex vivo measures of TTR stability. On day 28, mean TTR stabilization by FPE was >90% in both dose groups at peak and trough concentrations and significantly higher than placebo (no stabilization; $p < 10^{-12}$) (Figure 4). Less intersubject variability was observed at the higher dose (SD at trough = 9%) than the lower dose (SD at trough = 18%), despite a similar proportion of mutant TTR subjects in both groups (n = 6, 38% vs. n = 5, 31%, respectively).

To explore whether AG10 treatment was as effective in stabilizing mutant TTR to the same extent as wild-type TTR, we analyzed the serum TTR and FPE results according to mutational status (mutant vs. wild-type). This subgroup analysis, admittedly in a very small sample size (14 mutant, of which 11 were V122I, vs. 35 wild-type), demonstrated that AG10 is fully active in stabilizing mutant TTR-containing tetramers to a similar degree as wild-type (Figure 5).

DISCUSSION

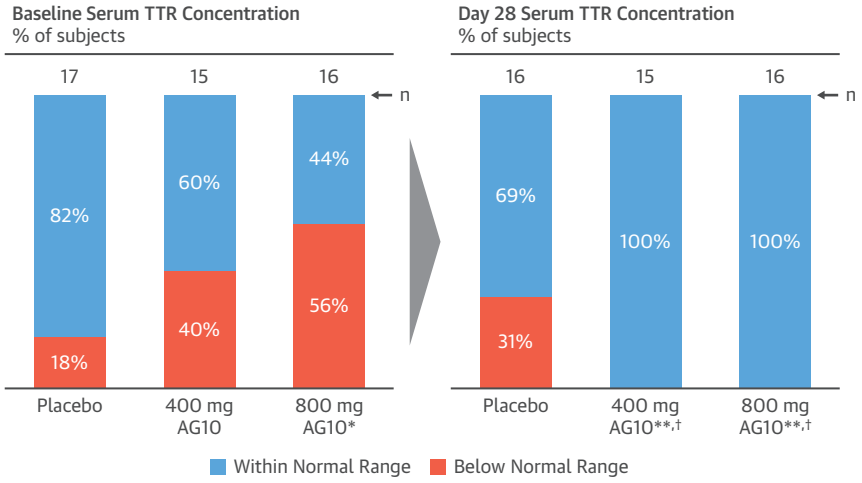
This study represents the first clinical experience with AG10 in the target patient population of ATTR-CM. Administration of AG10 was well tolerated and was not associated with safety signals of potential clinical concern (Central Illustration). The lack of any observed safety signals is consistent with the phase 1 clinical study in healthy adult volunteers and the AG10 toxicology program that has established a No

FIGURE 2 Change in Serum TTR From Baseline (Individual Subject Data)



Laboratory reference range for serum TTR 20 to 40 mg/dl (3.6 to 7.3 μmol/l). Note: Data not available at baseline for 1 400-mg subject and at day 28 for 1 400-mg and 1 placebo subject. Subjects who were carriers of a TTR mutation are represented by the **hatched columns;** the **solid columns** represent wild-type subjects. **Diamonds** identify those subjects whose TTR was below normal at day 28. ATTRm-CM = mutant transthyretin amyloid cardiomyopathy; ATTRwt-CM = wild-type transthyretin amyloid cardiomyopathy; other abbreviations as in Figure 1.

FIGURE 3 AG10-Related Normalization of Serum TTR From Baseline (Responder Analysis)



Laboratory reference range for serum TTR 20 to 40 mg/dl (3.6 to 7.3 μmol/l). Note: Serum TTR concentrations were not available at baseline for 1 400-mg subject and at day 28 for 1 400-mg and 1 placebo subject. TTR concentrations are displayed according whether they were below **(red)** or within **(blue)** the laboratory reference range. *p < 0.05 vs. placebo; **p < 0.0001 vs. placebo; †p < 0.05 vs. baseline. Abbreviations as in Figure 1.

Observed Adverse Effect Level (NOAEL) >10-fold higher than the predicted target therapeutic concentration of 8 μmol/l.

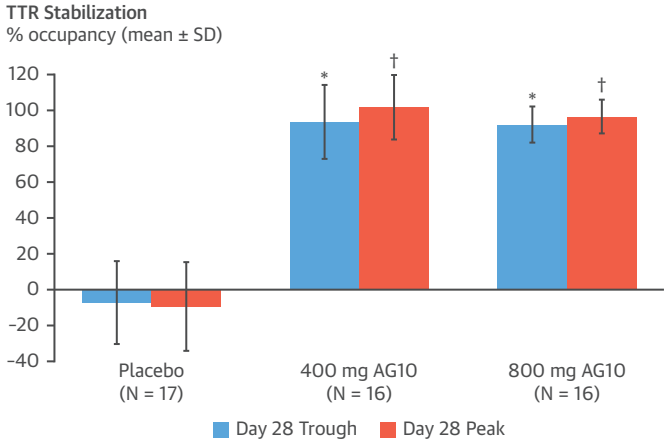
Study subjects achieved target trough plasma concentrations at steady-state resulting in near-complete (>90%) stabilization of TTR at both peak and trough. This finding is consistent with the phase 1 study of AG10 in healthy adult volunteers in which ex vivo stabilization assays (FPE and Western blot) demonstrated near-complete TTR stabilization by AG10, with >90% average tetramer stabilization across the entire dosing interval at the highest dose tested (800 mg every 12 h).

In the present study, AG10 treatment increased serum TTR levels from baseline and brought those levels to within the normal range in all subjects, both mutant and wild type. This included subjects whose baseline levels were markedly below the normal range.

On-treatment increases in serum TTR concentrations in ATTRwt-CM have been reported in 2 small studies for tafamidis 20 mg once daily (17% increase; n = 31) (26) and diflunisal 250 mg bid (25% increase; n = 12) (25). In the present study, treatment with AG10 400 and 800 mg bid resulted in mean increases in serum TTR concentration of 29% and 34% in the subgroup of ATTRwt-CM subjects, respectively, suggesting a potentially larger treatment effect than was observed with the other stabilizers.

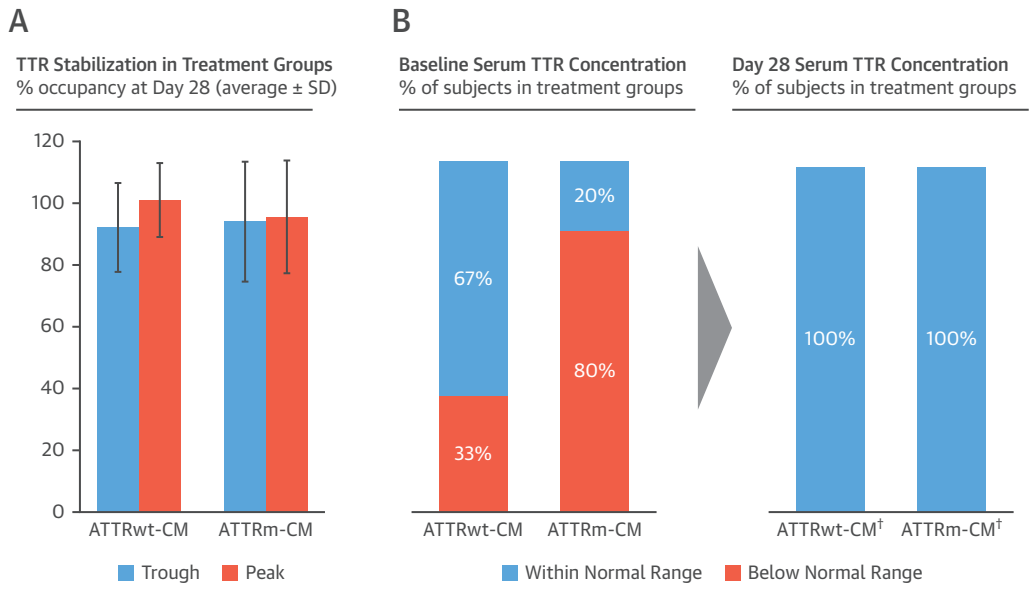
The equivalent levels of stabilization observed in both wild-type and mutant patients in this study has important therapeutic implications given the proportion of all TTR mutation carriers (~50%) with the V122I mutation versus other TTR mutations in the

FIGURE 4 AG10-Mediated Stabilization of Circulating TTR (Fluorescent Probe Exclusion)



TTR stabilization by fluorescent probe exclusion assay, displayed by dose group and whether samples were obtained at peak **(red)** or trough **(blue)** at steady state. *p < 10^{-12} vs. placebo trough, †p < 10^{-12} vs. placebo peak, no significant differences between trough and peak in any group. TTR = transthyretin.

FIGURE 5 AG10-Mediated Stabilization of Circulating TTR According to Mutation Status (Combined Results of FPE and Serum TTR Assays)



(A) Trough and peak TTR stabilization as measured by fluorescent probe exclusion (FPE) at day 28. **(B)** Proportion of AG10-treated patients with serum TTR concentrations within or below normal range (20 to 40 mg/dl) at baseline and day 28. †$p < 0.05$ vs. baseline. Abbreviations as in Figures 1 and 2.

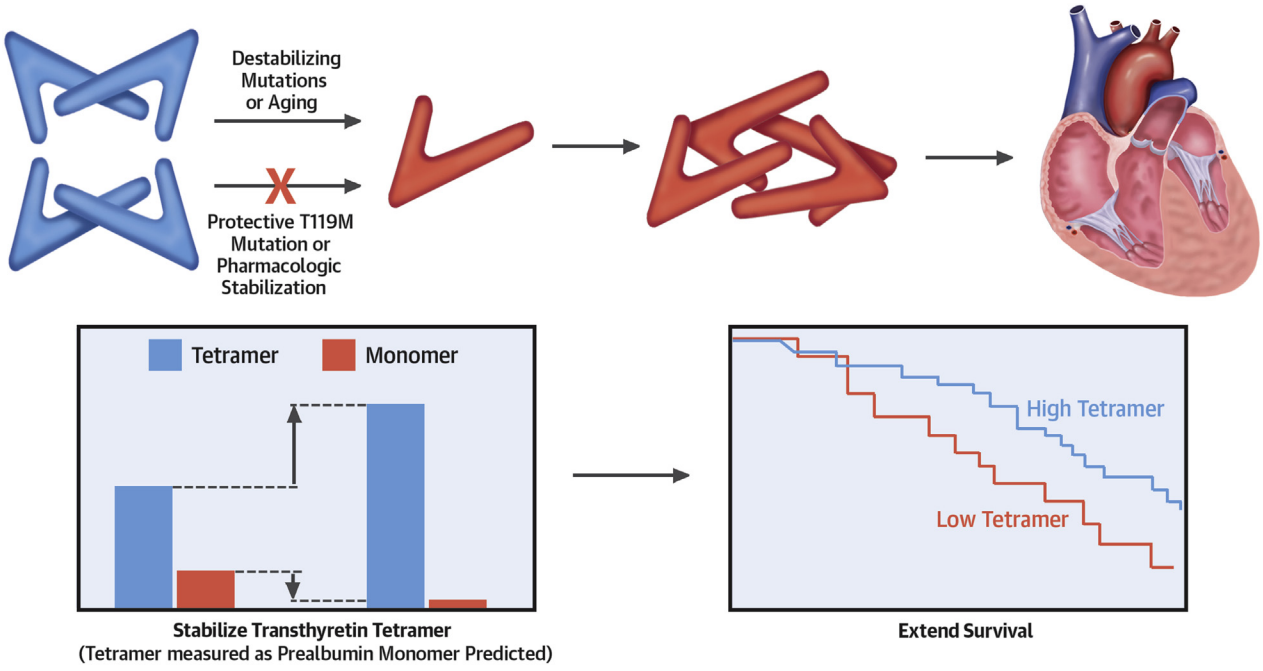
U.S. population, and with similar allele frequencies in other populations of West African descent globally. The ability of AG10 to stabilize a panel of TTR mutations representing the most prevalent mutations worldwide, spanning a broad topological distribution around the 3-dimensional structure of the native protein, and representing the entire spectrum of clinical phenotypes, was previously confirmed in vitro (30).

The serum level of TTR, long recognized both as a sensitive index of overall nutritional status and as an acute phase reactant, is becoming more widely appreciated as an independent predictor of survival in ATTR-CM. In 1 recent study of patients with wild-type ATTR-CM, regression analysis suggested that a 1-mg/dl decrement in serum TTR level is associated with a 7% to 11% decrement in survival (25). It will be important if additional observational studies can confirm this finding in ATTRwt-CM and extend it to ATTRm-CM.

Although transthyretin was so named to reflect its capacity to transport thyroxine and retinoic acid, these functions do not appear to be essential for thyroid homeostasis, and levels well below the normal range do not appear to be associated with either hypothyroidism or vitamin A deficiency states. TTR is evolutionarily very well conserved across multiple species. Its relatively high plasma concentration also defines it as a major plasma protein. The metabolic work required to maintain this concentration in the setting of its 2-day half-life suggests that serum TTR may have important functions that are yet to be delineated. Data from a large observational study in almost 70,000 people in Denmark may have yielded some clues: heterozygous carriers of the T119M rescue mutation have on average 20% higher TTR levels compared with noncarriers, have about one-half or less the rates of cerebrovascular clinical events, and live 5 to 10 years longer (21).

The ability of AG10 to uniquely stabilize TTR with a mechanism mimicking the T119M rescue mutation, coupled with the current observations of its ability to increase serum TTR in ATTR-CM patients, has potentially important clinical implications. Reducing the relative potential to generate monomeric TTR that can misfold, aggregate, and be deposited as amyloid is the mechanistic goal shared by TTR stabilizers and gene silencing agents. Clinical benefit has been demonstrated in both ATTR-CM and ATTR-PN based on this principle (31–34). In this context,

CENTRAL ILLUSTRATION **TTR Stabilization Increases Serum TTR Levels and May Correlate With Clinical Benefit in Transthyretin Amyloid Cardiomyopathy**



Judge, D.P. et al. J Am Coll Cardiol. 2019;74(3):285–95.

Circulating, native tetrameric TTR is destabilized by both pathogenic mutations and/or currently undefined, age-related factors. These destabilizing influences are counteracted by either gain-of-function mutation (e.g., T119M) or pharmacological intervention with a stabilizer. When destabilized, the native tetramer more readily dissociates, which is the initiating event in amyloidogenesis. Monomeric TTR readily misfolds, and aggregates are deposited in the myocardium and organize as insoluble fibrils. At the **lower left**, stabilization in vivo leads to slower turnover of tetrameric TTR and increased serum levels. Monomeric circulating TTR is not currently measurable, so their reduction is predicted. The clinical consequences may lead to clinical benefit according to the ability of serum TTR level to predict survival in ATTR-CM, as reported. Image in lower right adapted with permission from Hanson et al. (25). ATTR-CM = transthyretin amyloid cardiomyopathy; TTR = transthyretin.

AG10's properties and the results of the current study highlight its potential as an effective treatment for ATTR-CM.

STUDY LIMITATIONS. The 28-day treatment duration of the present study limits any assessment of clinical benefit. Modest sample size, particularly of TTR mutation carriers, limits generalizability to the broader ATTR-CM population, or to pre-symptomatic mutation carriers. Randomization may have biased the results to favor placebo, due to a higher percentage of ATTRm subjects and lower average serum TTR among those who received either dose of AG10 compared with placebo. The study included 1 subject with the V30M genotype but no clinically apparent polyneuropathy. V30M is more often associated with a predominantly polyneuropathic phenotype, especially in patients with the early age of onset subtype within this genotype. Exclusion of patients with diagnosed ATTR-PN and lack of neurological assessments in this study prevents generalizability to the ATTR-PN population. None of the ex vivo measures of TTR stabilization accurately represent physiological stabilization, although the results reported here are consistent with the observed increases in serum TTR that the authors interpret as evidence of stabilization in vivo.

CONCLUSIONS

In this phase 2 study, AG10 was well tolerated, near-completely stabilized TTR, and restored low TTR levels to normal in all subjects. Combined with the existing preclinical and clinical data on AG10, these data provide a foundation supporting further investigations of the efficacy and safety of AG10 in ATTR with respect to accepted clinical endpoints. AG10 could prove to be an important

option amongst new, disease-modifying treatments, together transforming ATTR from an inevitably progressive, fatal disorder into a treatable chronic disease.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: TTR amyloidosis is caused by destabilization of TTR due to pathogenic mutations or aging. The selective TTR stabilizer AG10 forms hydrogen bonds that mimic a unique protective mutation, and oral administration in patients with heart failure due to TTR amyloid cardiomyopathy restores serum TTR to the normal range.

TRANSLATIONAL OUTLOOK: Randomized clinical trials are needed to evaluate the therapeutic efficacy and safety of AG10 in patients with TTR amyloid cardiomyopathy and/or polyneuropathy.

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KEY WORDS AG10, amyloidosis, ATTR-CM, cardiomyopathy, heart failure, transthyretin

APPENDIX For additional study information, please see the online version of this paper.