AG10 Stabilizes Pathogenic TTR Variants With High Potency – Potential for an Effective Treatment for ATTR Cardiomyopathy
ATTR-Cardiomyopathy (CM) and ATTR-Polyneuropathy (PN) are caused by aggregation of misfolded TTR monomers.
TTR amyloidosis is a systemic disorder

**ATTR cardiomyopathy (ATTR-CM)**
- Deposition of mutant (i.e. V122I) or wild-type TTR amyloid in the heart
  - Leads to predominantly diastolic heart failure
  - Afib/stroke and heart block frequently seen
- Affects > 200,000 patients worldwide
- Late onset (50-60+), death within 4-6 years
- No FDA approved treatment

**ATTR polyneuropathy (ATTR-PN)**
- Affects ~10,000 patients worldwide, mostly EU & Japan
- Deposition of mutant TTR (i.e. V30M) amyloid in peripheral nerves
  - Autosomal dominant with variable penetrance
  - Leads to sensorimotor & autonomic deficits
- No FDA-approved treatments

Does AG10 Stabilize a Broad Range of Pathogenic TTR Variants?

<table>
<thead>
<tr>
<th>TTR Variant</th>
<th>Disease</th>
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<tbody>
<tr>
<td>V122I</td>
<td>Cardiomyopathy</td>
</tr>
<tr>
<td>T60A</td>
<td>Cardiomyopathy &amp; polyneuropathy</td>
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<tr>
<td>P24S</td>
<td>Cardiomyopathy &amp; polyneuropathy</td>
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<tr>
<td>D38A</td>
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<tr>
<td>L58H</td>
<td>Cardiomyopathy &amp; polyneuropathy</td>
</tr>
<tr>
<td>F64L</td>
<td>Polyneuropathy</td>
</tr>
<tr>
<td>Y114C</td>
<td>Polyneuropathy with leptomeningeal complications</td>
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</tbody>
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AG10 Stabilizes Mutant TTR from V122I ATTR Patients

**FPE Assay:** Change in fluorescence due to modification of TTR in human serum by a covalent probe, which becomes fluorescent following binding to TTR. The lower the binding of the probe/fluorescence the higher the binding selectivity and affinity of the ligand to TTR.

AG10 was used at 10 μM
AG10 Stabilizes Mutant TTR from T60A ATTR Patients

![Graph showing relative fluorescence over time with different treatments.](image)

![Image of gel electrophoresis showing time points and treatments.](image)

- **FPE probe**
- **10 µM AG10**

**Notes:**
- Tetramer + RBP tetramer
AG10 Stabilizes Mutant TTR from P24S ATTR Patients
AG10 Stabilizes Mutant TTR from D38A ATTR Patients

Graph showing relative fluorescence over time for FPE probe and 10 μM AG10.

Western blot images showing samples at different time points with annotations for tetramer + RBP tetramer and human IgG light chain.
AG10 Stabilizes Mutant TTR from L58H ATTR Patients
AG10 Stabilizes Mutant TTR from F64L ATTR Patients
AG10 Stabilizes Mutant TTR from Y114C ATTR Patients

**Graph:**
- **Y-axis:** Relative fluorescence
- **X-axis:** Time (mins)

- **Lines:**
  - Black: FPE probe
  - Red: 10 μM AG10

**Image:**
- Expression of tetramer and RBP tetramer
- Loading of human IgG light chain
Does AG10 Stabilize a Broad Range of Pathogenic TTR Variants? - YES
AG10 has a unique binding mode, which mimics the effect of the TTR trans-suppressor mutation - T119M

The naturally occurring trans-suppressor mutation T119M super-stabilizes TTR

AG10 binding to TTR mimics the stabilizing interactions of T119M variant to S117

- The T119M polymorphism creates H-bonds within the complex that super-stabilize the TTR tetramer and functions as a trans-suppressor mutation in V30M carriers.

- T119M heterozygotes have a 5-10 year longer life-span and significantly lower risk of cerebrovascular disease

- AG10 mimics the structural effects of T119M.

- Stabilization of TTR by AG10 may mimic the clinical effect and lead to improved outcomes

Miller et al, unpublished data
Coworkers and Collaborators

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> 100 mutations in the TTR gene have been found to cause TTR amyloidosis (ATTR).

Most of these alter the TTR structure, resulting in either kinetic or thermodynamic destabilization.

The most common ATTR mutations are V122I (3.4% of African Americans) and V30M.
Discovery and Development of AG10

- HTS of 130,000 identified 32 compounds with IC50 < 1 μM
- Crystal structure of top novel ligands

- The crystal structure of Ligand 7 was used as a starting point for SAR studies.

- AG10 was the most potent analogue with the best physicochemical properties
- AG10 was selected for ADME and Toxicity studies
- IND was filed in August 2017 and Phase 1 clinical studies started in September 2017

AG10 binds with high affinity and high selectivity to human serum TTR

No or low affinity TTR ligand: covalent probe forms an amide bond with Lys-15 of TTR, creating a fluorescent conjugate.

High affinity/selectivity TTR ligand competes effectively with the FPE probe and prevents the formation of a fluorescent conjugate.