Preclinical Characterization of AT-04, a Pan–Amyloid-Binding Fc Domain-Peptide Fusion, to Serve as an Opsonin for Macrophage-Mediated Clearance of Amyloid Deposits

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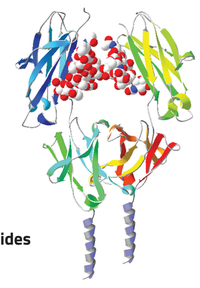
Background

- Systemic amyloidosis is a severe, progressive, and often fatal protein misfolding and deposition disorder[1]
- Deposition of insoluble amyloid fibrils in organs including the heart, kidneys, liver, and nerves leads to organ dysfunction and is associated with significant morbidity and mortality[2]
- Current therapeutic approaches are focused on reducing or stabilizing the precursor protein and do not directly address clearance of toxic amyloid[3]
- Because there are significant amyloid deposits at the time of patient diagnosis, there is an urgent unmet need for therapies that promote amyloid removal and restore organ function[3]
- Evolving approaches for amyloid removal involves engaging monocytes and macrophages and inducing Fc-receptor mediated phagocytosis[4]
  - Given the diverse types of amyloid, a pan-amyloid reactive macrophage-activating agent could have widespread clinical utility
- We have previously described a murine Fc-peptide fusion (peptibody) that demonstrated pan-amyloid reactivity and Fc-mediated phagocytosis[5]
- A next-generation human peptibody, comprising an immunoglobulin G1 (IgG1) Fc and the amyloid-reactive peptide p5R,[6] has now been generated (AT-04) and it has the potential to serve as an opsonin for clearance of diverse types of amyloid

AT-04: An Amyloid Removal Fusion Protein

- AT-04 is a fusion protein (peptibody) that consists of a human IgG1 Fc domain with the amyloid-reactive p5R peptide fused to the Fc C-terminal (**Figure 1**)
  - The peptide domain binds specifically to heparan sulfate and protein fibrils ubiquitously found in all types of amyloid
  - When bound, the IgG1-Fc domain can actively engage the immune system to signal macrophages to engulf and remove amyloid through phagocytosis

Figure 1. Schematic of AT-04 peptide-Fc fusion



Molecular weight of AT-04 is 59.33 kD (approximately 2.5× smaller than a typical monoclonal antibody).

Objective

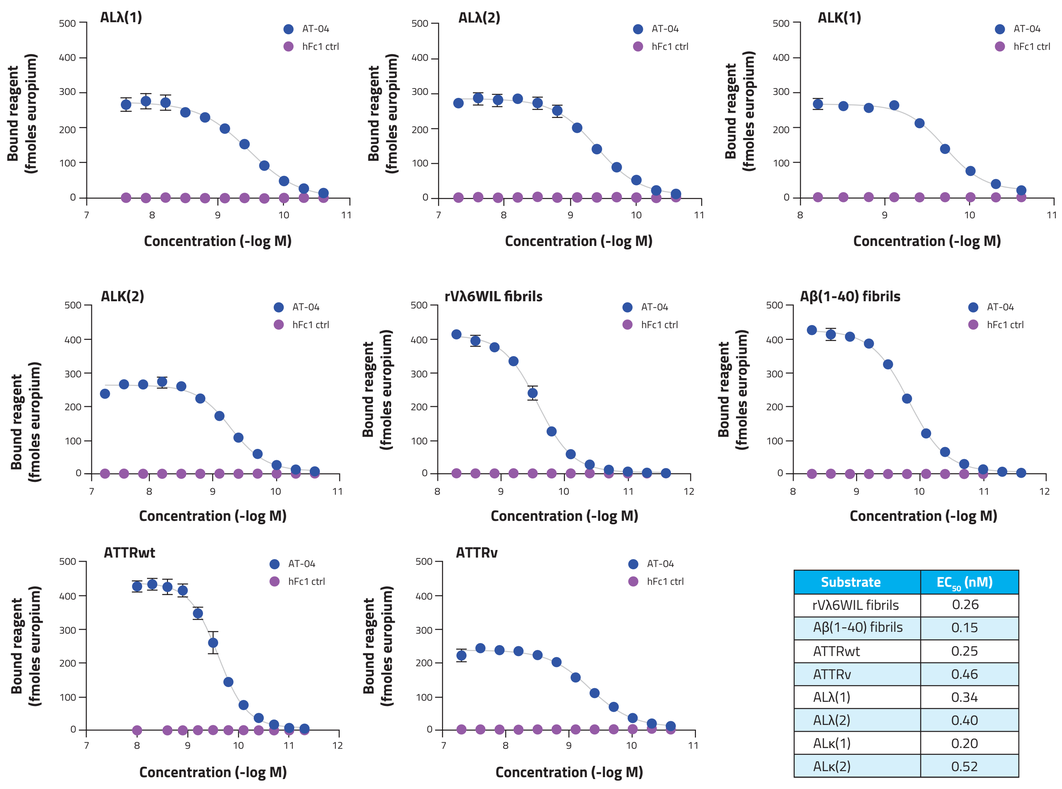
- To characterize the activity of the novel AT-04 peptibody, including its ability to bind amyloid of diverse types and induce Fc-mediated phagocytosis of amyloid-related substrates

Results

AT-04 Demonstrates Binding to AL, ATTR, and Aβ Amyloid Substrates with High Potency

- AT-04 bound synthetic amyloid-like fibrils, rVλ6Wil and Aβ(1-40), and human immunoglobulin light chain-associated amyloidosis (AL) and transthyretin-associated amyloidosis (ATTR) amyloid extracts with high potency (half-maximal concentration [EC_{50}] values of 0.15 - 0.52 nM) (**Figure 2**)

Figure 2. AT-04 binding to AL, ATTR, Aβ amyloid substrates. Binding potency (EC_{50}) was assessed by ELISA



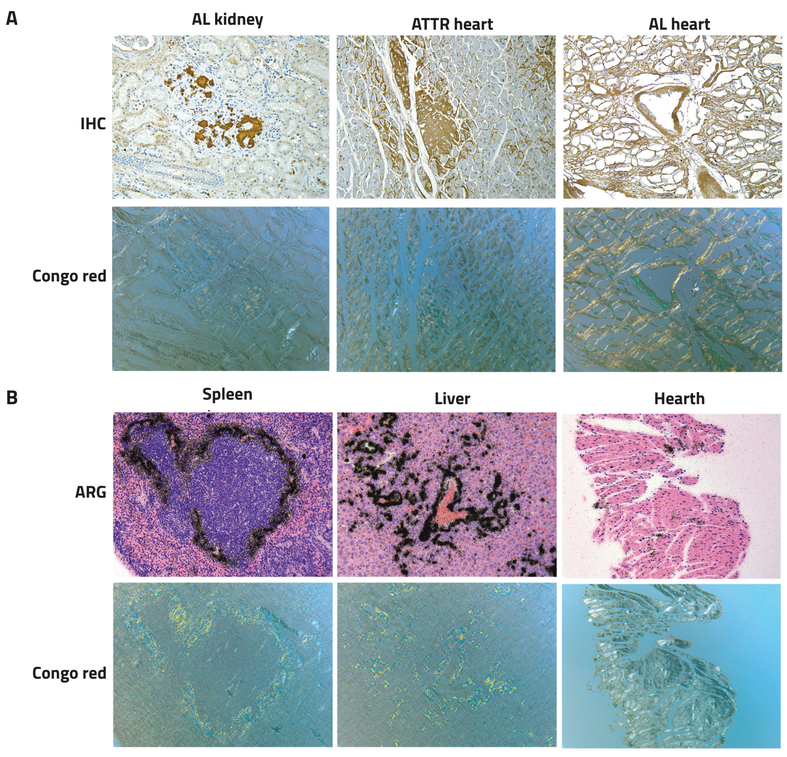
Substrate	EC_{50} (nM)
rVλ6WIL fibrils	0.26
Aβ(1-40) fibrils	0.15
ATTRwt	0.25
ATTRv	0.46
ALλ(1)	0.34
ALλ(2)	0.40
ALκ(1)	0.20
ALκ(2)	0.52

AL, immunoglobulin light-chain associated amyloidosis; ATTR, transthyretin-associated amyloidosis; ATTRv, variant transthyretin-associated amyloidosis; ATTRwt, wild-type transthyretin-associated amyloidosis; ctrl, control; EC_{50}, half-maximal concentration; ELISA, enzyme-linked immunosorbent assay.

AT-04 Demonstrates Pan-Amyloid Reactivity

- Pan-amyloid reactivity of AT-04 was further evidenced immunohistochemically with specific binding to AL amyloid in the kidney and heart and ATTR amyloid in the heart (**Figure 3A**)
- Radiolabeled AT-04 was also shown to colocalize with amyloid deposits in the spleen, liver, and heart of a murine model of AA amyloidosis (**Figure 3B**)

Figure 3. Pan-amyloid reactivity of AT-04. (A) Reactivity of biotinylated AT-04 with formalin-fixed amyloid laden tissue was assessed immunohistochemically. Specific binding with AL- and ATTR-associated amyloid was evidenced by intense brown (DAB) staining that correlated with the presence of amyloid stained with Congo red in consecutive tissue sections. (B) Microautoradiographs showing in vivo colocalization of ^{125}I-labeled AT-04 (^{125}I-AT-04) with AA Congo red birefringent AA amyloid deposits in a murine model, following intravenous injection. Binding of ^{125}I-AT-04 is evidenced by the presence of black silver grains in the tissue

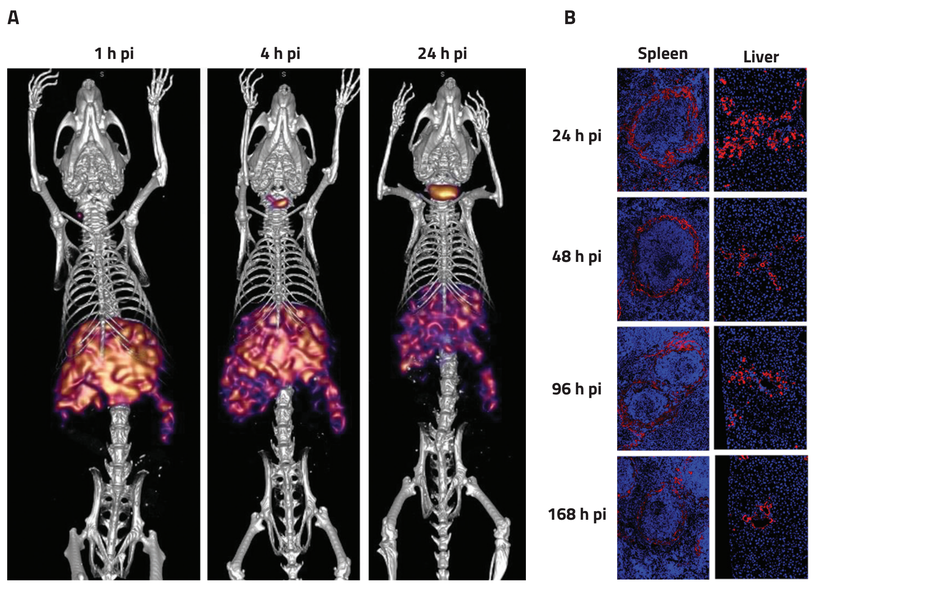


AA, serum amyloid protein A-associated amyloidosis; AL, immunoglobulin light-chain associated amyloidosis; ARG, microautoradiographs; ATTR, transthyretin-associated amyloidosis; IHC, immunohistochemistry.

AT-04 Binds Diverse Forms of Amyloid in Preclinical Murine Models

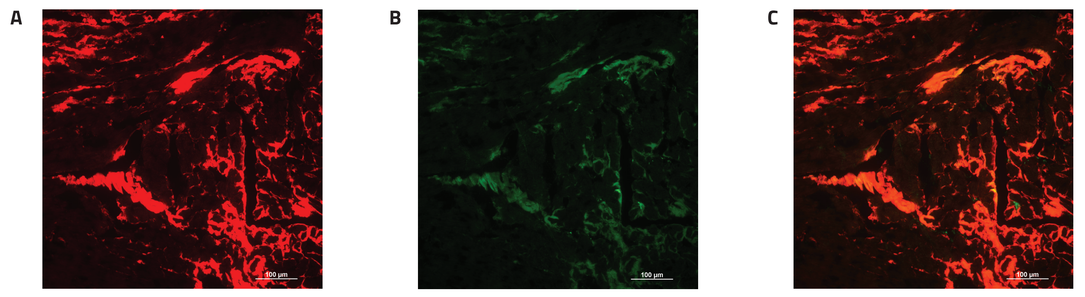
- Intravenous administration of AT-04 resulted in specific binding to AA and AL amyloid in the heart, kidney, liver, and spleen in animal models of systemic amyloidosis
  - Small-animal single photon emission computed tomography/x-ray computed tomography (SPECT/CT) showed significant retention of ^{125}I-AT-04 in the liver and spleen of mice with AA at 1, 4, and 24 hours post injection (pi) (**Figure 4**); binding to the hepatosplenic amyloid was observed for at least 168 hours after injection by immunohistochemical staining in tissue section
    - ^{125}I-AT-04 accumulated rapidly in hepatosplenic (10-20% ID/g), and cardiac (~4% ID/g) amyloid deposits within 1 hour of injection
  - In a novel model of murine AL amyloidosis, AT-04 colocalized with amyloid deposits in the heart of the mouse (**Figure 5**)

Figure 4. (A) SPECT/CT images of ^{125}I-AT-04 in AA mice 1, 4, and 24 hours after injection. (B) IHC of liver and spleen tissue from AA mice injected with ^{125}I-AT-04. Mice were euthanized at various timepoints following injection and AT-04 was detected using Alexa594-conjugated anti-human Fc (red) and counterstained with Hoescht DNA-staining dye



AA, serum amyloid protein A-associated amyloidosis; IHC, immunohistochemistry; ^{125}I-AT-04, ^{125}I-labeled AT-04; pi, post injection; SPECT/CT, single-photon emission computed tomography/x-ray computed tomography.

Figure 5. AT-04 in vivo binding to cardiac amyloid in a mouse model of AL amyloidosis. Mice were administered 0.5 mg AT-04. 24 hours after injection, heart tissue sections were prepared and were stained with (A) Congo red (fluorescence), (B) anti-hIgG1-FITC (to detect AT-04), and (C) merged Congo red and AT-04 stained sections.

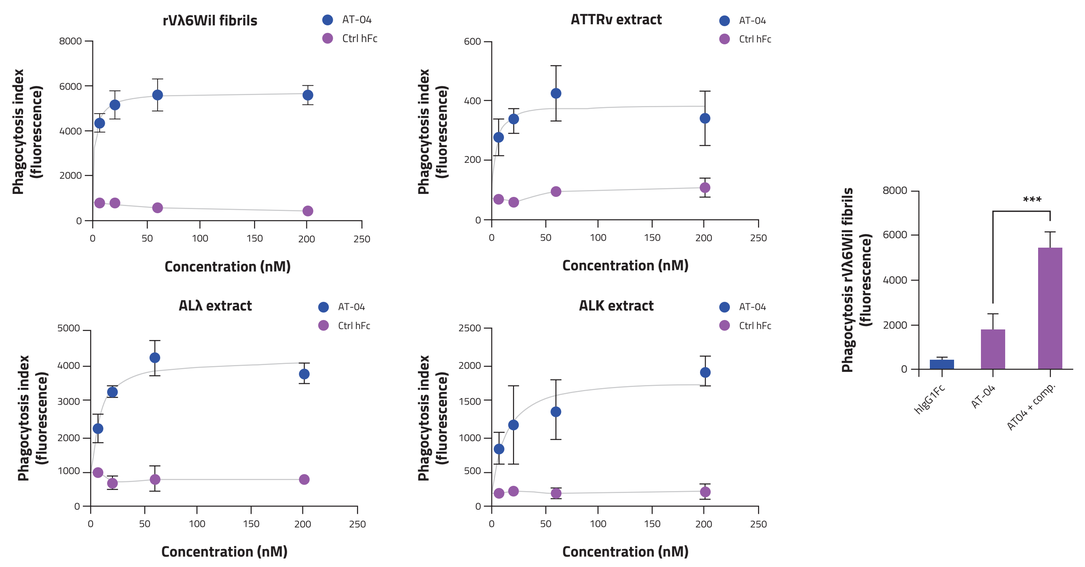


AL, immunoglobulin light-chain associated amyloidosis; FITC, fluorescein isothcyanate; hIgG1,human immunoglobulin G1; mAb, monoclonal antibodies.

AT-04 Mechanism of Action

- AT-04 stimulated dose-dependent ex vivo phagocytosis of pHrodo red–labeled synthetic rVλ6Wil amyloid-like fibrils and a human ALλ amyloid extract by phorbol myristic acid–activated human THP-1 cells (**Figure 6**)
- Phagocytosis was significantly enhanced in a dose-dependent manner as compared to a control hIgG1 and further increased in the presence of 20% human serum as a source of complement

Figure 6. AT-04 dose-dependent phagocytosis of AL fibrils and extracts

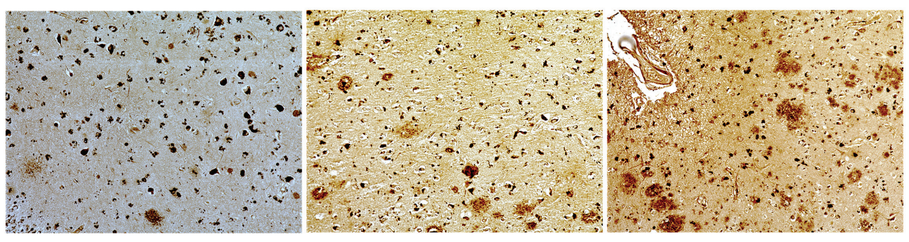


ATTRv, variant transthyretin-associated amyloidosis; Ctrl, control; hIgG1,human immunoglobulin G1.

AT-04 Binding to Aβ Deposits in Brain Sections

- AT-04 binds to Aβ core and diffuse plaques in human brain (**Figure 7**)

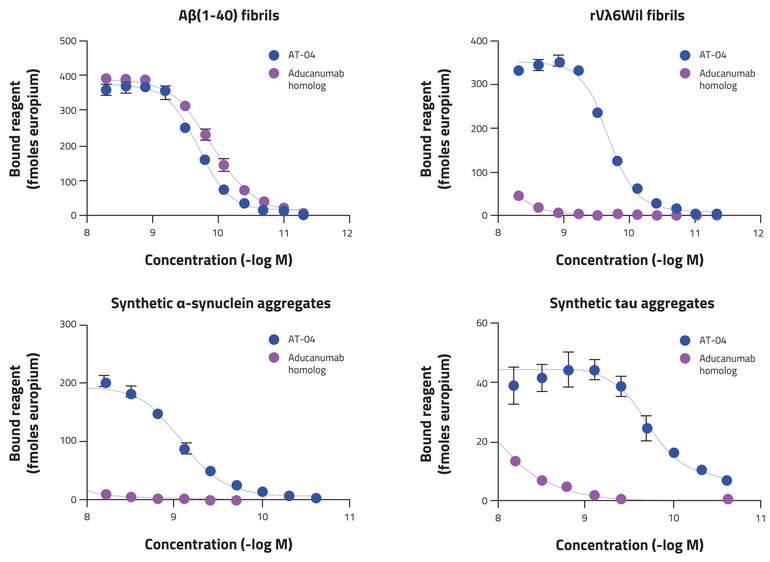
Figure 7. Biotinylated AT-04 staining Aβ core and diffuse plaques in human brain



AT-04 demonstrates binding to tau, α-synuclein, and Aβ aggregates with high potency

- Binding potency of AT-04 to Aβ(1-40) synthetic fibrils was identical to an aducanumab homolog; however, in contrast to the aducanumab reagent, AT-04 also bound synthetic tau, and α-synuclein aggregates with high potency (EC_{50} of 0.5-7 nM) (**Figure 8**)

Figure 8. AT-04 Binding to tau, α-synuclein, and Aβ aggregates as compared to an aducanumab homolog. Binding potency (EC_{50}) was assessed by ELISA



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Disclosures

Foster: Patents & Royalties: Inventor and patent holder. Guthrie: Attralus, Inc.: Current Employment. Klein: Attralus, Inc: Current Employment. Bell: Attralus, Inc: Current Employment. Selvarajah: Attralus, Inc.: Current Employment. Stuckey: Attralus, Inc.: Current holder of stock options in a privately-held company. Martin: Attralus, Inc.: Current holder of stock options in a privately-held company. Kennel: Attralus, Inc.: Current holder of stock options in a privately-held company, Patents & Royalties: Inventor and patent holder. Wall: Attralus, Inc.: Current holder of stock options in a privately-held company, Patents & Royalties: Inventor and patent holder, Research Funding.

Conclusions

- Clearance of amyloid deposits is a significant unmet need
- The human IgG1 peptibody, AT-04, specifically binds systemic and cerebral amyloid with high potency
- This interaction can induce phagocytosis of the material, which can be enhanced by complement
- AT-04 could serve as a potent opsonin to facilitate macrophage-mediated phagocytosis of amyloid
- AT-04 is a relatively small molecule that binds 3 distinct pathologic aggregates associated with neurodegenerative disorders, Aβ, tau, and alpha-synuclein; therefore, it may be an attractive therapeutic candidate for amyloid-related neurodegenerative disorders , like Alzheimer's disease

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