# AT-03 Demonstrated Pan-Amyloid Binding and Stimulated the Removal of Amyloid Deposits Through Macrophage-Mediated Phagocytosis

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## Background

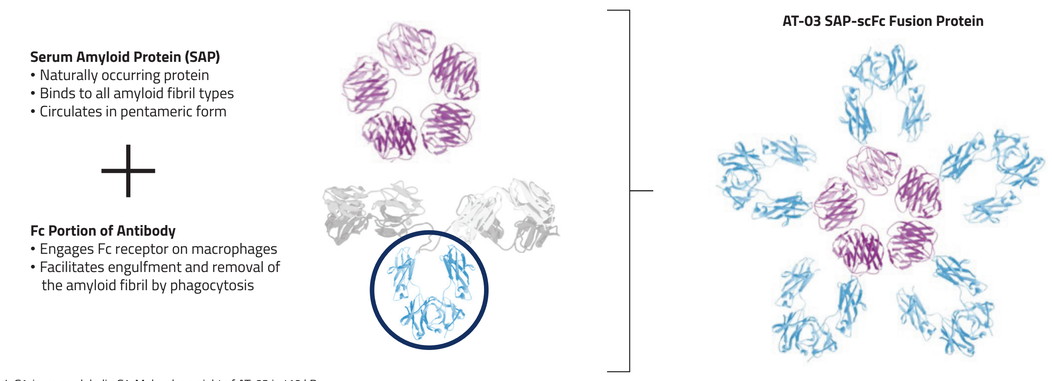
### Systemic Amyloidosis

- Systemic amyloidosis is a severe, progressive, and often fatal protein misfolding and deposition disorder[1]
- There are approximately 17 different types of systemic amyloid diseases, each caused by a specific precursor protein that deposits in the extracellular space as amyloid fibrils[2]
- Deposition of insoluble amyloid fibrils in organs such as the heart, kidneys, liver, and nerves leads to organ dysfunction and is associated with significant morbidity and mortality[3]
- Current therapeutic approaches are focused on reducing or stabilizing the precursor protein and do not directly address toxic amyloid[4]
- Because of significant amyloid deposition at the time of patient diagnosis, there is an urgent unmet need for therapies that promote amyloid removal and restore organ function[4]

### AT-03: An Amyloid Removal Fusion Protein

- AT-03 is a fusion protein comprising serum amyloid protein (SAP) linked to a single-chain human immunoglobulin G1 (IgG1) Fc domain (**Figure 1**)
  - The naturally occurring pentameric serum protein SAP binds to all forms of amyloid
  - An IgG1 single chain Fc (scFc) is linked to the monomeric SAP, which adopts its pentameric structure with 5 Fc domains
  - 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 representation of AT-03 SAP-scFc fusion protein designed to promote opsonization of amyloid. AT-03 pentamer consists of 5 AT-03 monomers. The peripheral blue regions represent human IgG1Fc (CH2 and CH3), and the central purple regions represent SAP




IgG1, immunoglobulin G1; Molecular weight of AT-03 is 410 kD.

## Objective

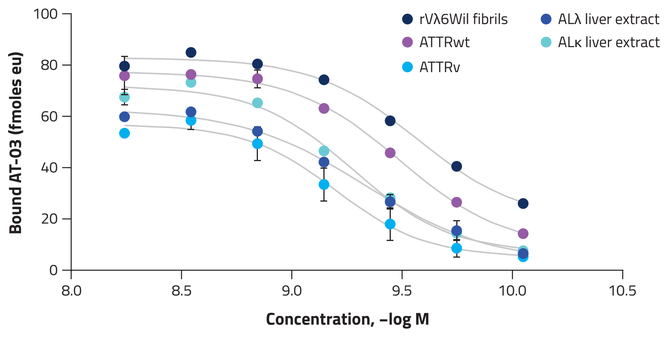
- To characterize the preclinical profile of AT-03, including its binding to amyloid extracts and fibrils, biodistribution in mouse models of amyloidosis, mechanism of action (promotion of macrophage-mediated phagocytosis), and efficacy (amyloid clearance)

## Results

### AT-03 Demonstrates Potent Binding to AL and ATTR Amyloid Substrates

- AT-03–bound human light chain (AL) and transthyretin (ATTR) amyloid extracts with subnanomolar half-maximal concentration ($EC_{50}$) values (**Figure 2**)

Figure 2. AT-03 binding to amyloid extracts and fibrils.




| Test Agent | Synthetic Fibrils | ATTR Extracts ($EC_{50}$) | | AL Extracts ($EC_{50}$) | |
|---|---|---|---|---|---|
| | rVλ6Wil | ATTRwt | ATTRv | ALλ liver | ALκ liver |
| AT-03 | 0.27 nM | 0.32 nM | 0.63 nM | 0.46 nM | 0.53 nM |

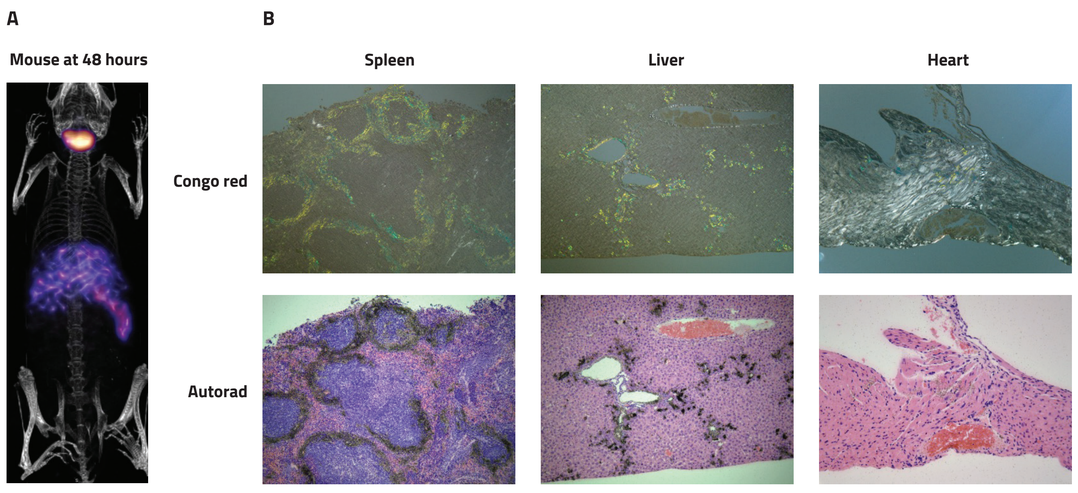
AL, immunoglobulin light-chain associated amyloidosis; ATTR, transthyretin-associated amyloidosis; ATTRv, variant transthyretin-associated amyloidosis; ATTRwt, wild-type transthyretin-associated amyloidosis; $EC_{50}$, half-maximal concentration.

Binding of AT-03 to synthetic amyloid fibrils and human AL and ATTR amyloid extracts was assessed using a europium-linked immunosorbent assay.

### AT-03 Binds Diverse Forms of Amyloid in Preclinical Murine Models

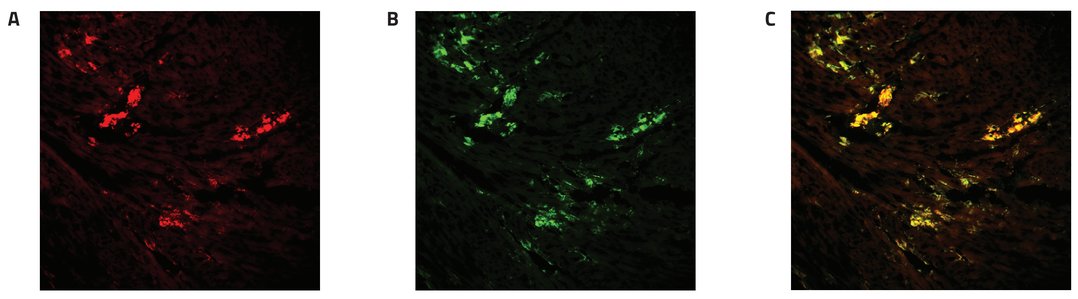
- Intravenous (IV) administration of AT-03 resulted in specific binding to diverse forms of amyloid in the heart, kidney, liver, and spleen in animal models of amyloidosis
  - Small-animal single photon emission computed tomography/computed tomography showed significant retention of $^{125}$I-labeled AT-03 in the liver and spleen of mice with serum amyloid protein A–associated amyloidosis (AA) at 48 hours after injection (**Figure 3**); binding to the hepatosplenic amyloid was observed for at least 192 hours after injection
  - In a novel model of murine AL amyloidosis, AT-03 colocalized with amyloid deposits in the heart of the mouse (**Figure 4**)
  - AT-03 colocalized with amyloid deposits in the heart, liver, and kidney in mice with ApoA2 amyloidosis (**Figure 5**)

Figure 3. (A) SPECT/CT scan of $^{125}$I-AT-03 in AA mice 48 hours after injection. (B) Congo red stain and microautoradiography of spleen, liver, and heart tissue from AA mice injected with $^{125}$I-AT-03.



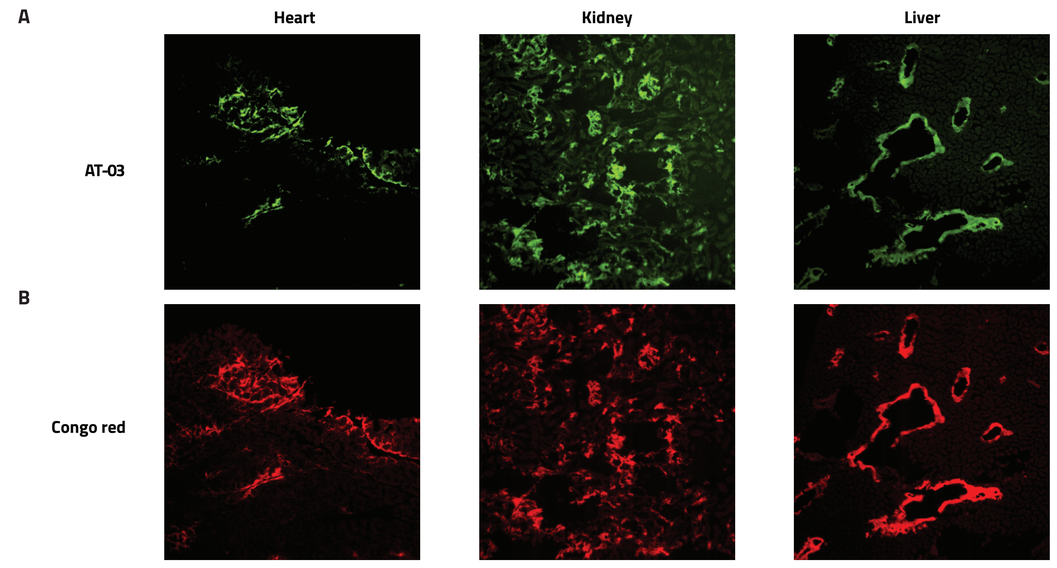

$^{125}$I-AT-03, $^{125}$I-labeled AT-03; AA, protein A–associated amyloidosis; Autorad, microautoradiography; CT, computed tomography; SPECT, single photon emission computed tomography.

Figure 4. AT-03 in vivo biodistribution to cardiac amyloid in a mouse model of AL amyloidosis. AL amyloid mice were injected intravenously in the lateral tail vein with 0.5 mg AT-03. 24 hours after injection, organs were harvested and heart tissue sections was stained with (A) Congo red, (B) anti-hIgG1-FITC to detect AT-03 bound to heart tissue, and (C) merged images of Congo red and AT-03.




AL, immunoglobulin light chain-associated amyloidosis; FITC, fluorescein isothiocyanate; hIgG, human immunoglobulin G.

Figure 5. AT-03 in vivo biodistribution to heart, liver, and kidney in a mouse model of ApoA2 amyloidosis. Mice were injected with 0.5 mg AT-03 and 24 hours after injection, tissue sections were stained with (A) anti-hIgG1-FITC to detect AT-03 bound to tissue, and (B) Congo red.

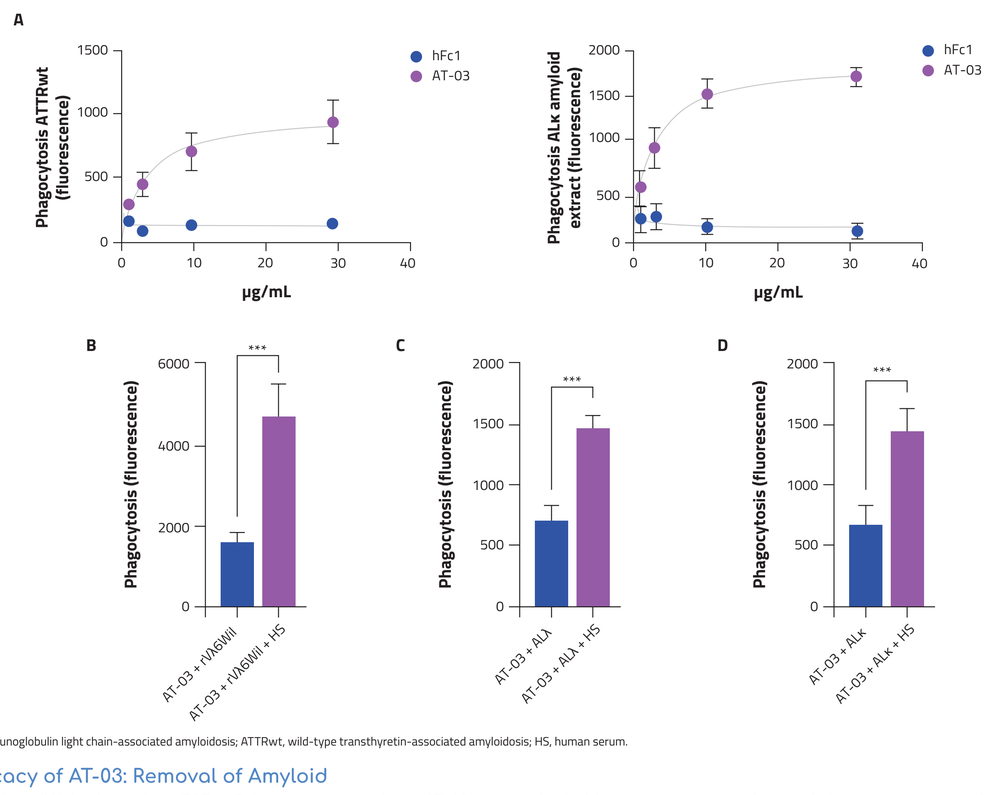



ApoA2, apolipoprotein A-II; FITC, fluorescein isothiocyanate; hIgG1, human immunoglobulin G.

### AT-03 Mechanism of Action

- AT-03 stimulated in vitro phagocytosis of human amyloid extracts (**Figure 6**)

Figure 6. Phagocytosis of AT-03 by PMA-activated THP1 cells. (A) Dose-dependent changes in phagocytosis of ATTRwt extract or AL TAL extracts. Complement-enhanced phagocytosis of (B) rVl6Wil (synthetic ALλ fibrils), (C) ALλ extract, and (D) ALK amyloid extract by PMA-activated THP1 cells following opsonization with 60 nM AT-03. Human serum served as the source of complement.

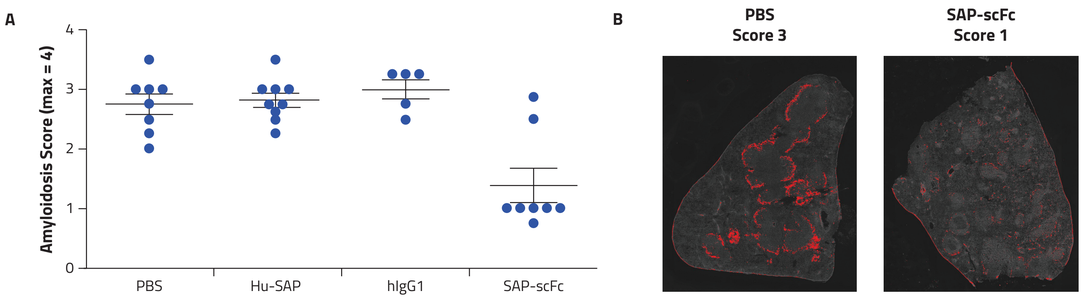



AL, immunoglobulin light chain-associated amyloidosis; ATTRwt, wild-type transthyretin-associated amyloidosis; HS, human serum.

### Efficacy of AT-03: Removal of Amyloid

- A single IV injection of 3 mg SAP-scFc* (a precursor version of AT-03) resulted in significant removal of splenic amyloid in a mouse model of AA amyloidosis (**Figure 7**)

Figure 7. Removal of amyloid by SAP-scFc in a mouse model of AA amyloidosis. (A) SAP-scFc in AA mice amyloid load in the spleen.[a] (B) Congo red staining on the spleen 14 days after a single injection of the indicated proteins.




AA, protein A–associated amyloidosis; Hu, human; IgG1, immunoglobulin G; IV, intravenous; PBS, phosphate-buffered saline; SAP, serum amyloid protein; scFc, single-chain Fc.

[a]Controls (PBS, human SAP or irrelevant human IgG1) are all nonsignificant when compared with each other. Controls (PBS, human SAP, or irrelevant human IgG1) had no effect on amyloid deposition, whereas SAP-scFc (3 mg IV injection) was associated with a significant reduction in amyloid deposition in the spleen. Compared with any of the 3 controls (untreated, human SAP, and human IgG1), SAP-scFc had a *P* value of <0.0001 using 1-way analysis of variance. At least 3 experiments were done (except for irrelevant human IgG1, which had 2 experiments).

*2 amino acids changed between SAP-scFc and AT-03 in order to improve manufacturability. This change had no impact on amyloid binding or Fc effector function.

### Pharmacokinetics of AT-03 in Non-Human Primates

- AT-03 demonstrated a mean half-life of 75.7 hours (>3 days) in a non-human primate study (**Figure 8**)

Figure 8. Pharmacokinetics of AT-03 in a non-human primate model




## Conclusions

- AT-03 is a fusion protein that potently binds AL and ATTR fibrils and extracts and is capable of binding diverse forms of systemic amyloid deposits in mouse models of the disease
- AT-03 enhanced macrophage-mediated phagocytosis of AL and ATTR amyloid extracts in vitro
- AT-03 demonstrated significant splenic amyloid removal in a mouse model of AA amyloidosis
- AT-03 is currently being tested in a phase 1 biodistribution study and could represent a novel therapeutic agent for the removal of systemic amyloid deposits of diverse types

## References

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