MAYO CLINIC

ISA
INTERNATIONAL SOCIETY OF AMYLOIDOSIS

XIX INTERNATIONAL SYMPOSIUM ON AMYLOIDOSIS

MAY 26-30, 2024 – ROCHESTER, MN

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DISCLOSURE OF RELEVANT FINANCIAL RELATIONSHIP(S) WITH INDUSTRY

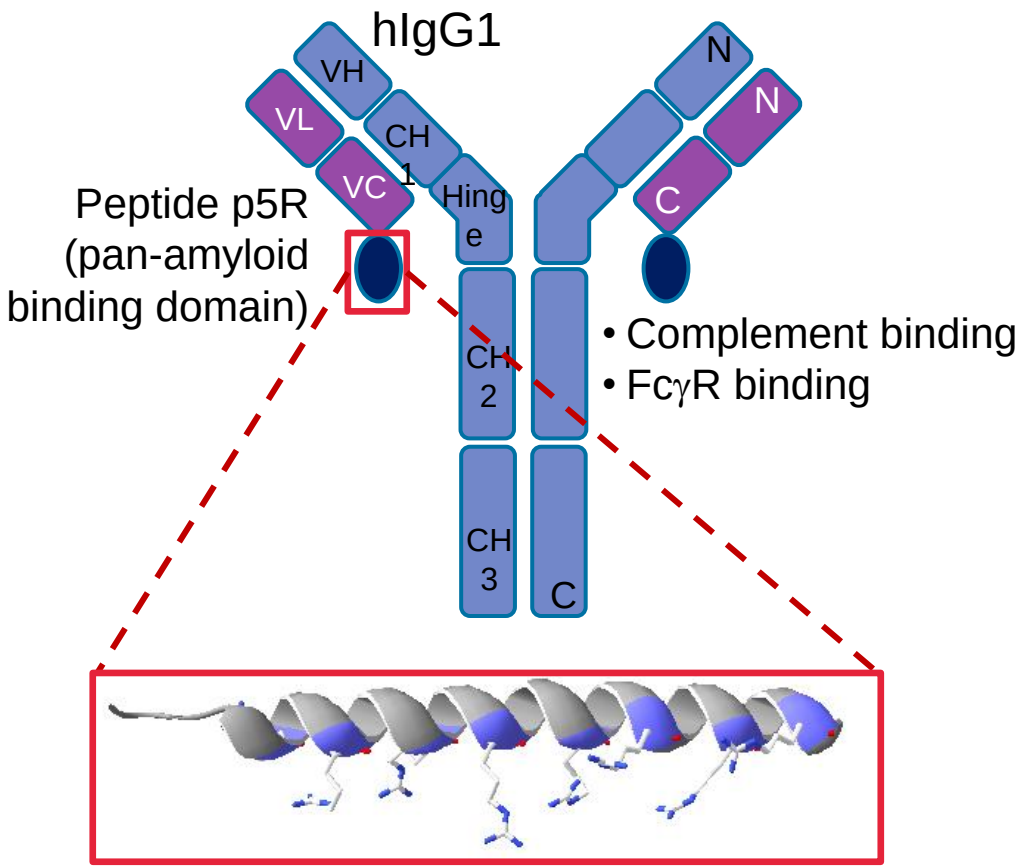
- Founding shareholder and interim CSO of Attralus Inc.
- Research funding from Attralus and the NIH.
- AT-02 was provided by Attralus.
- Inventor on IP related to amyloid-reactive antibodies, peptide-antibody fusions, and amyloid imaging peptides.

REFERENCES TO OFF-LABEL USAGE(S) OF PHARMACEUTICALS OR INSTRUMENTS

- Nothing to disclose

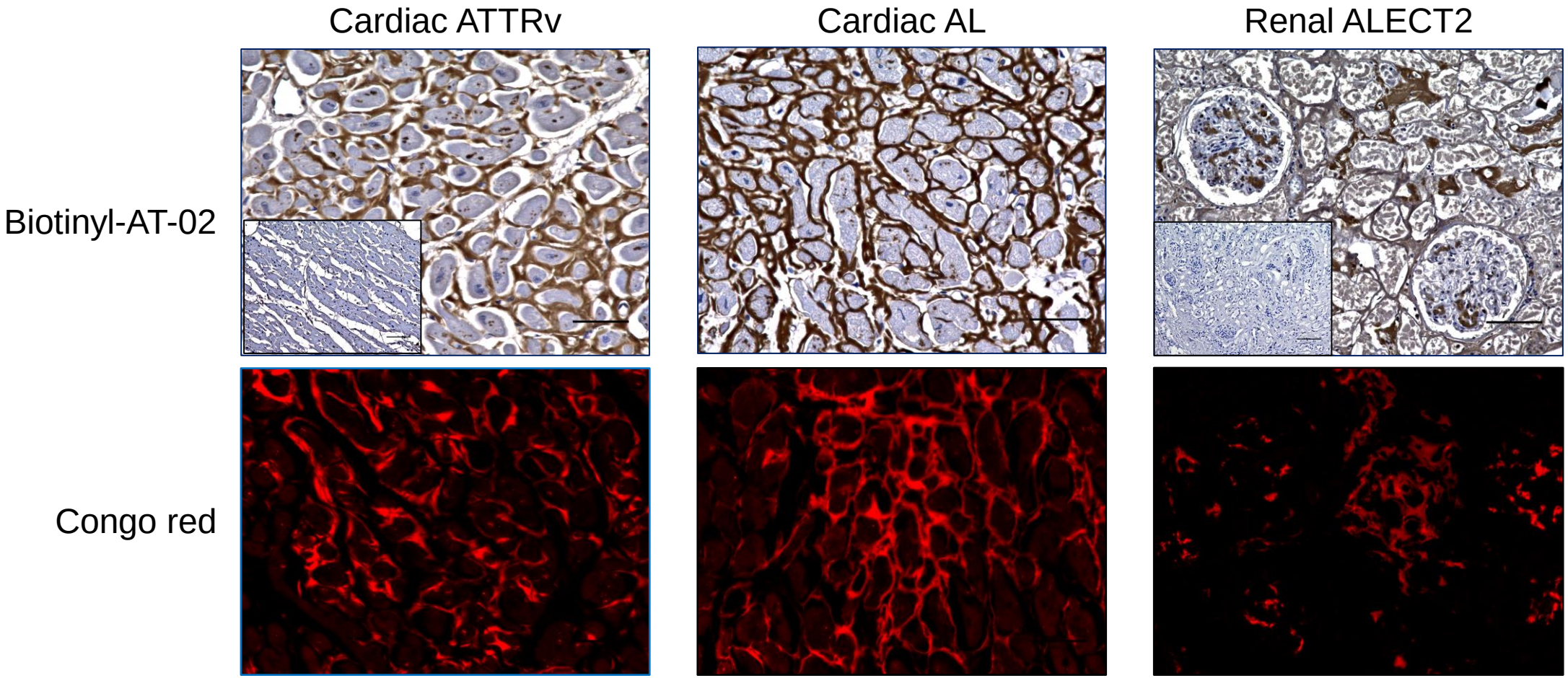
CHARACTERIZATION OF THE PEPTIDE-ANTIBODY FUSION AT-02

STUDIES TO SUPPORT ITS USE AS AN IMMUNOTHERAPY IN PATIENTS WITH AMYLOIDOSIS



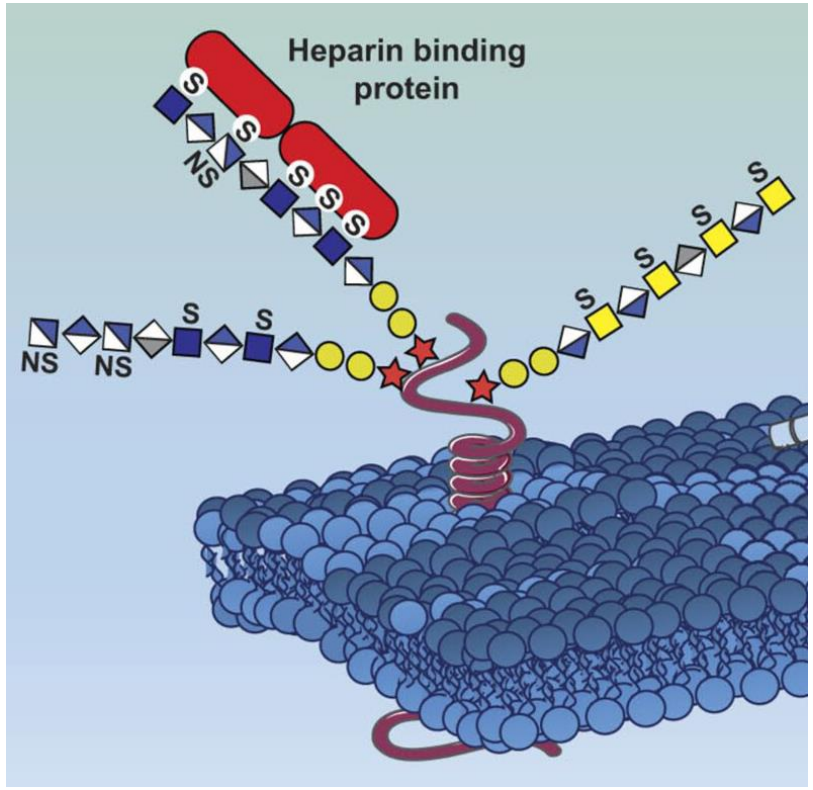
- AT-02 is a humanized IgG1-peptide fusion reagent.
- The pan-amyloid reactive peptide p5R is fused to the C-terminal of the light chain.
- Peptide p5R binds fibrils and hypersulfated glycosaminoglycans via electrostatic interactions.
  - Same peptide technology as the ^{124}I-AT-01 and ^{99m}Tc-AT-05 imaging agents which has shown to bind in key organs in patients with many types of amyloid.
- AT-02 was designed to be capable of:
  1. Targeting amyloid deposits *in vivo*.
  2. Binding to many types of amyloid.
  3. Opsonizing the deposits and promoting macrophage-mediated phagocytosis.

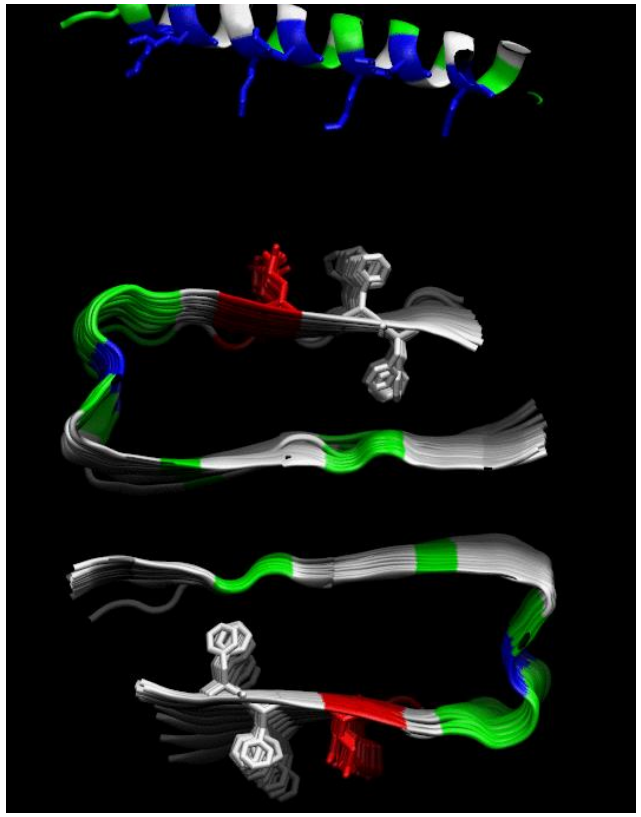
AT-02 SPECIFICALLY BINDS AMYLOID IN TISSUE SECTIONS



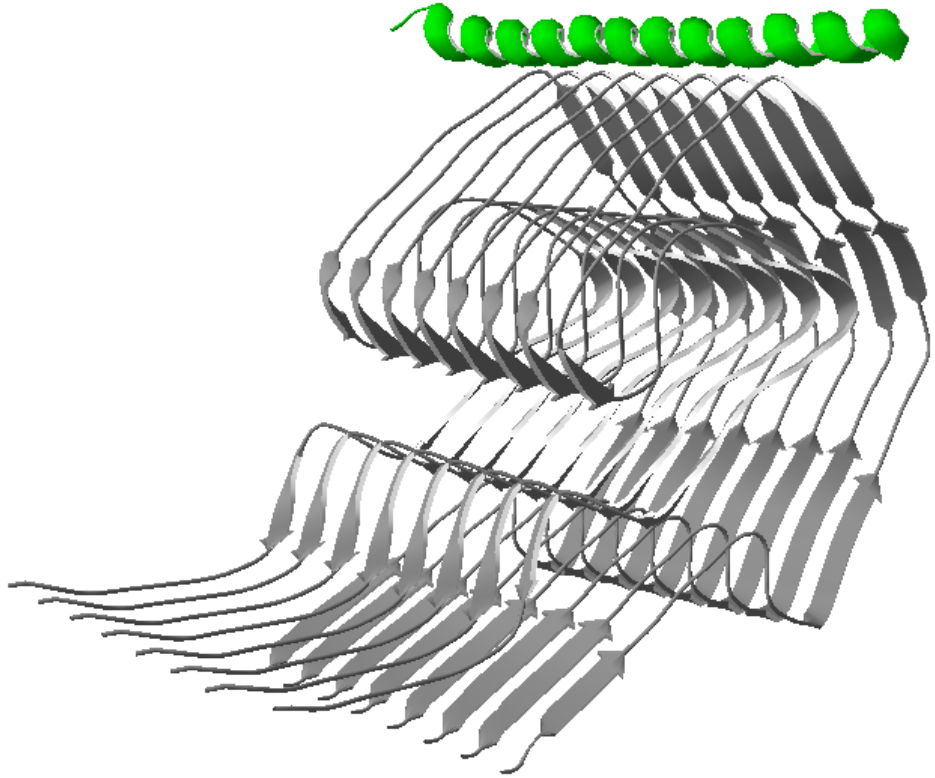
PEPTIDE p5R - LEVERAGE MULTIPLE BINDING SITES WITHIN THE AMYLOID

Charged amino acid side chains exposed on the amyloid fibril array



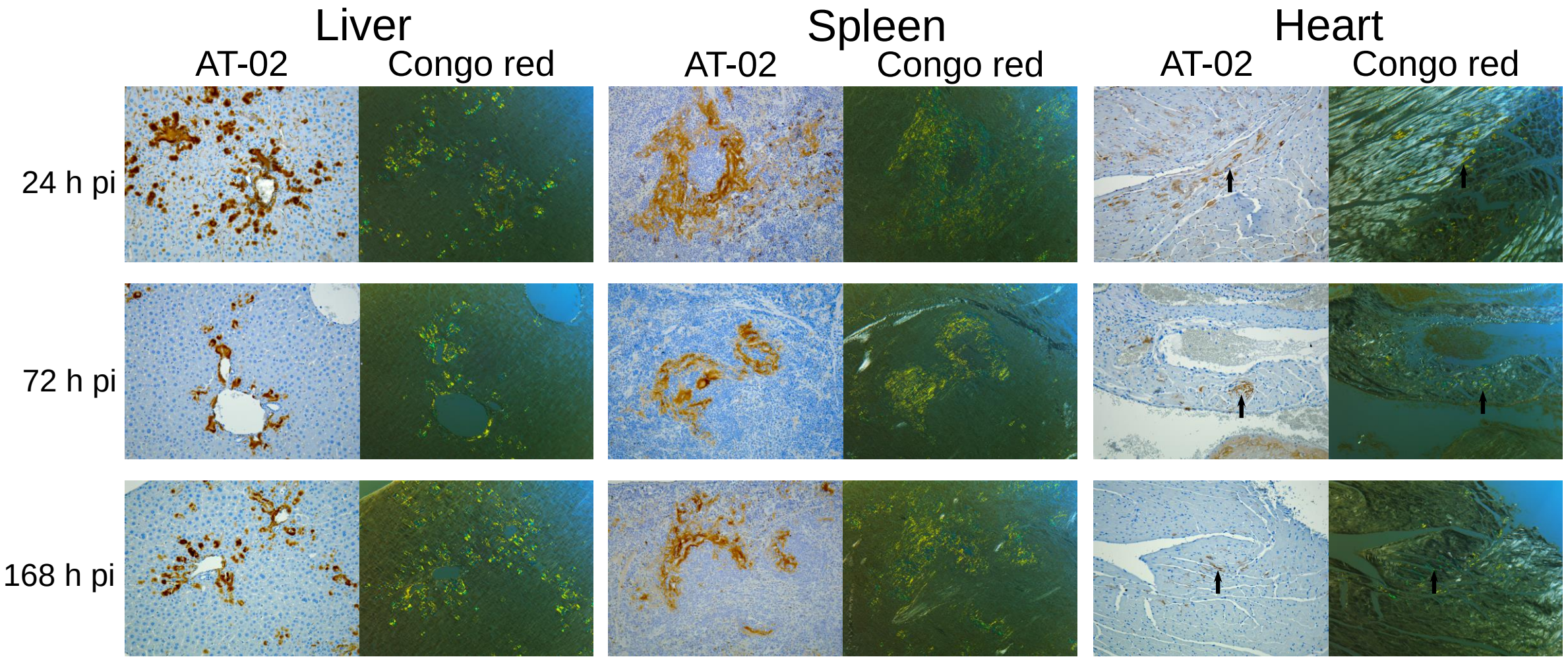


Current hypothesis of peptide-fibril interaction

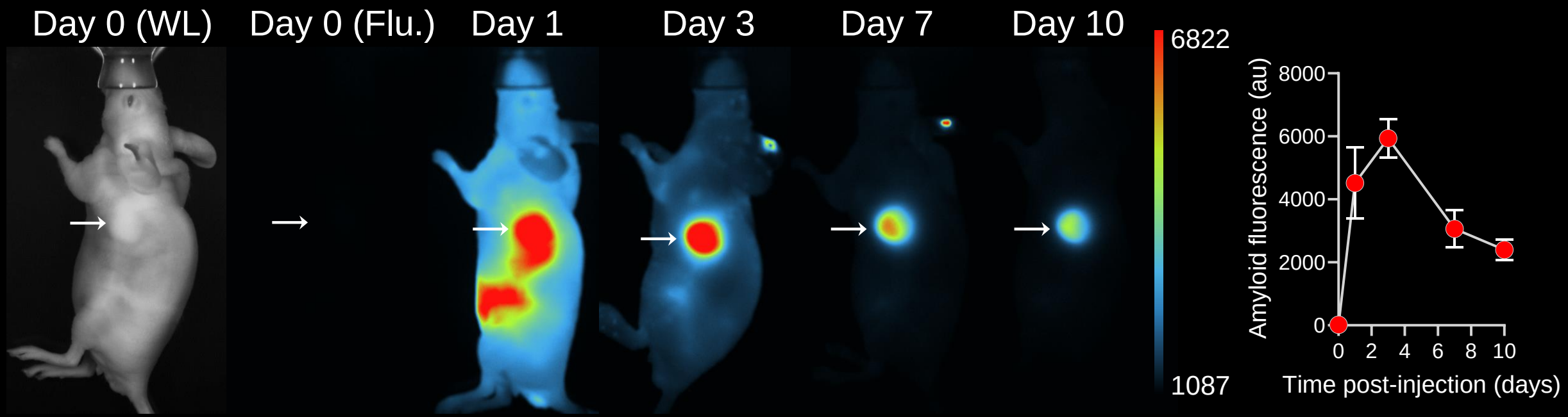


https://doi.org/10.1369/0022155420988661

AT-02 (400 µg INJECTED IV) SPECIFICALLY BINDS AA AMYLOID *IN VIVO* – AT-02 CAN BE DETECTED IN AMYLOID 7 DAYS pi



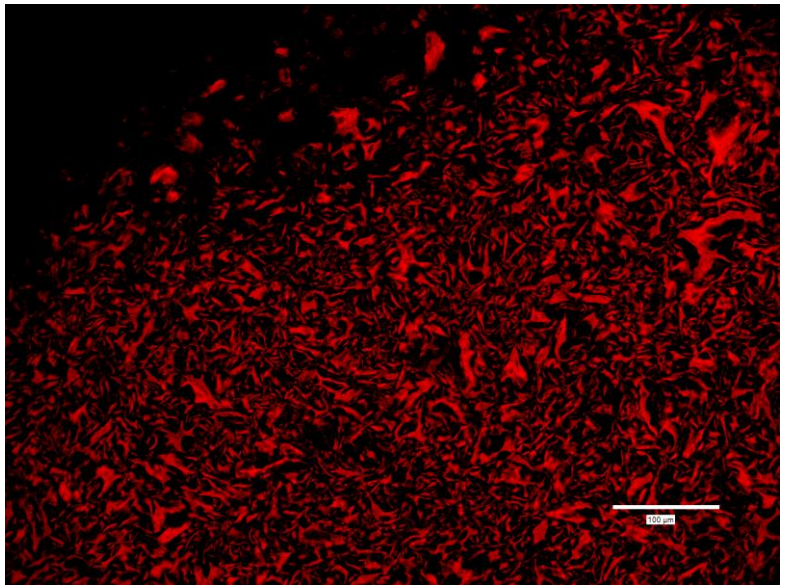
FLUOROPHORE-LABELED AT-02 (700 µg INJECTED IP) RAPIDLY BINDS HUMAN AL AMYLOID *IN VIVO*



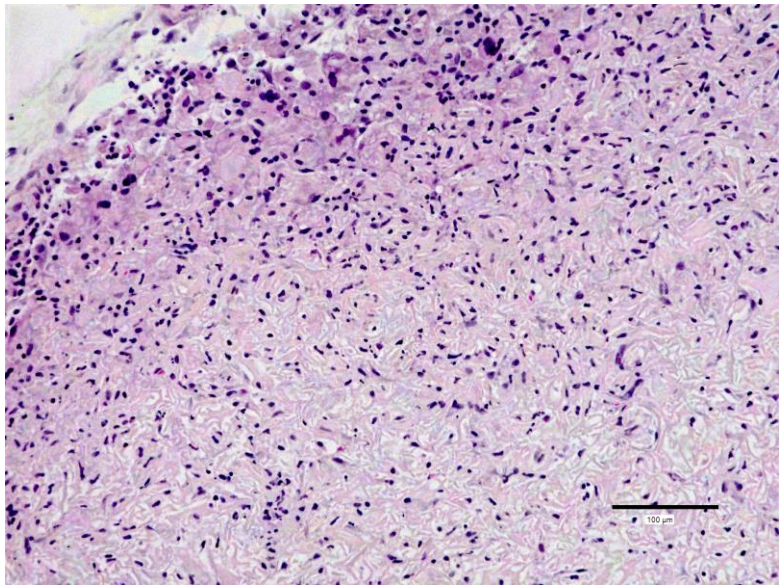
Dylight800-labeled AT-02, injected intraperitoneally (700 µg) colocalizes with human AL amyloid in a murine model of AL amyloidoma. Uptake in the amyloid was assessed by vital optical imaging. Images were quantified using background correction for distribution in non-amyloid regions.

FLUOROPHORE-LABELED AT-02 (700 µg INJECTED IP) BINDS HUMAN AL AMYLOID *IN VIVO*

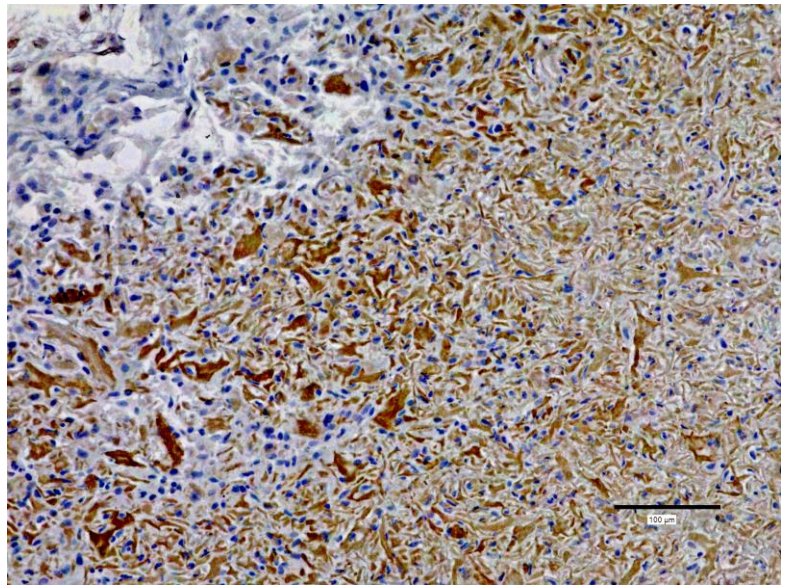
Congo red (flu.)



H&E

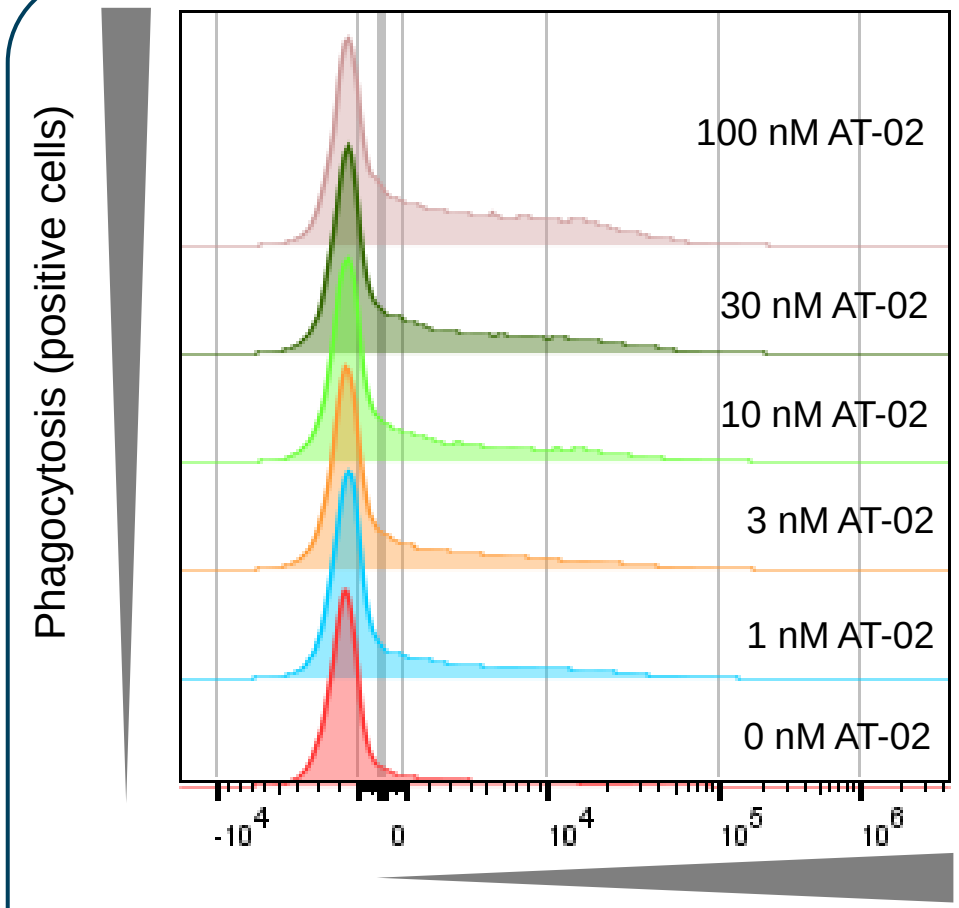


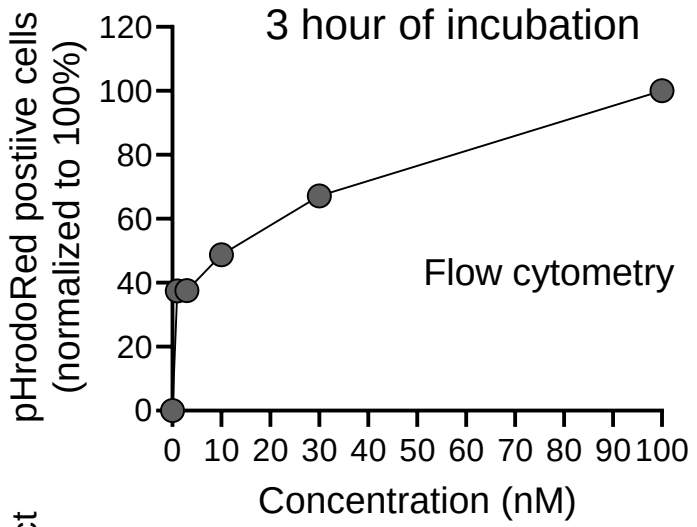
Anti-human Fc

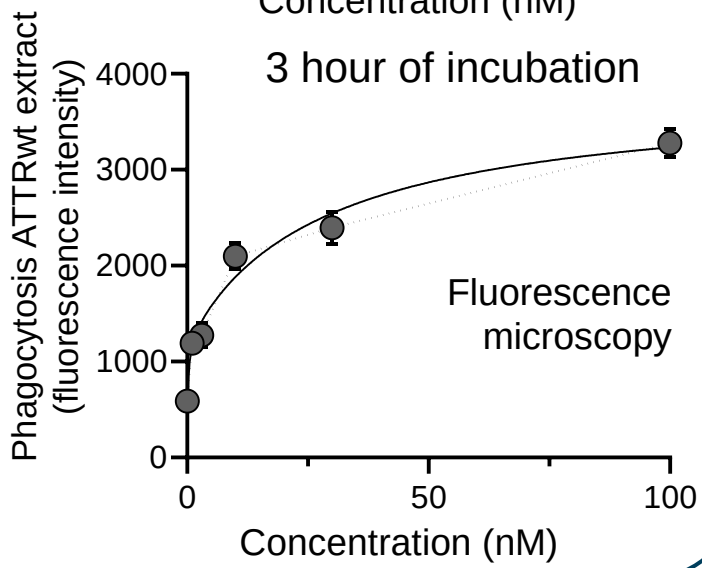


AT-02 INDUCES PHAGOCYTOSIS OF AMYLOID

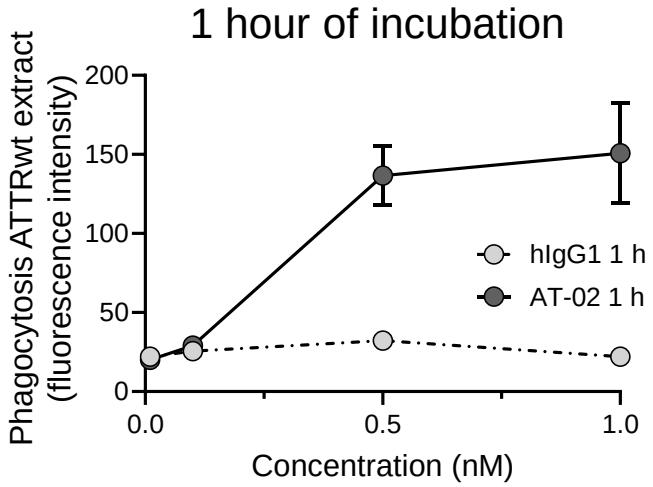
Human ATTRwt amyloid (30 µg) presented in solid phase (bound to collagen on a plate) using human THP-1 monocytes

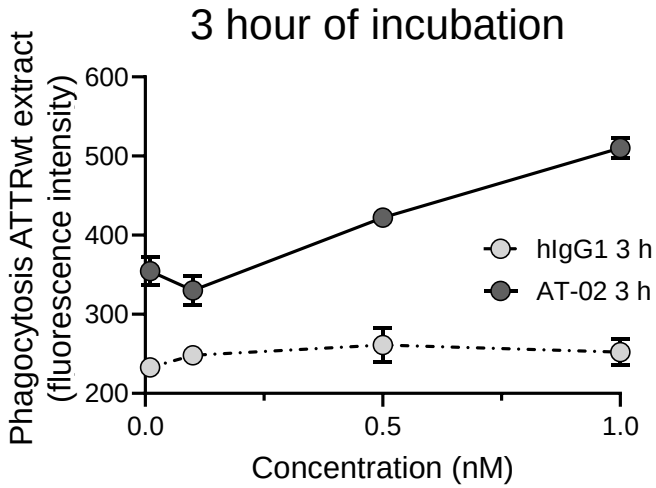






Human ATTRwt amyloid (20 µg) presented in suspension using human THP-1 M0



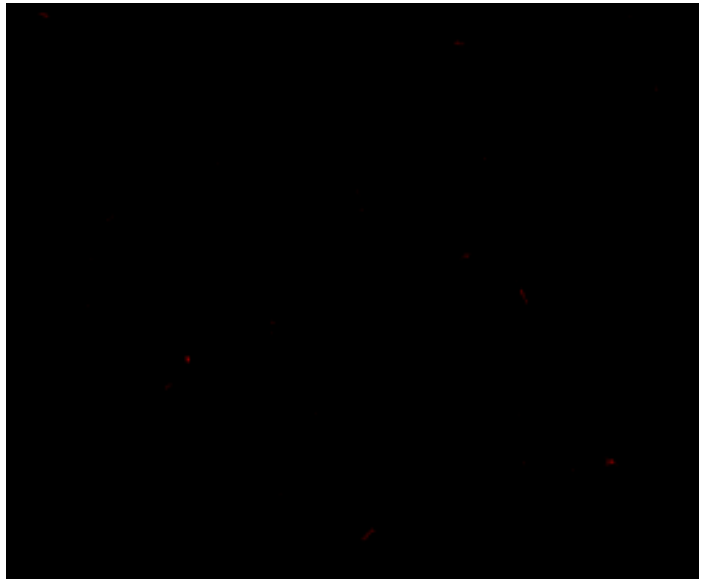


AT-02 INDUCES PHAGOCYTOSIS OF AMYLOID

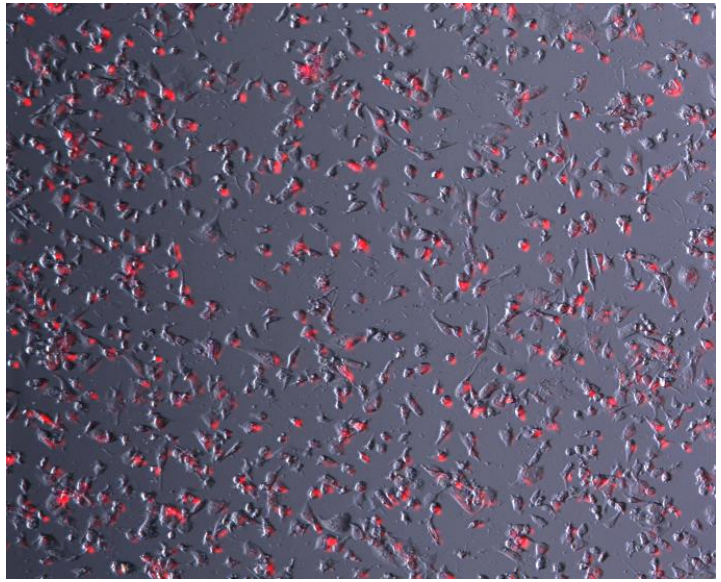
Activated human THP-1 M0 ($1x10^6$) – 90 min incubation

hIgG1 control + AL amyloid

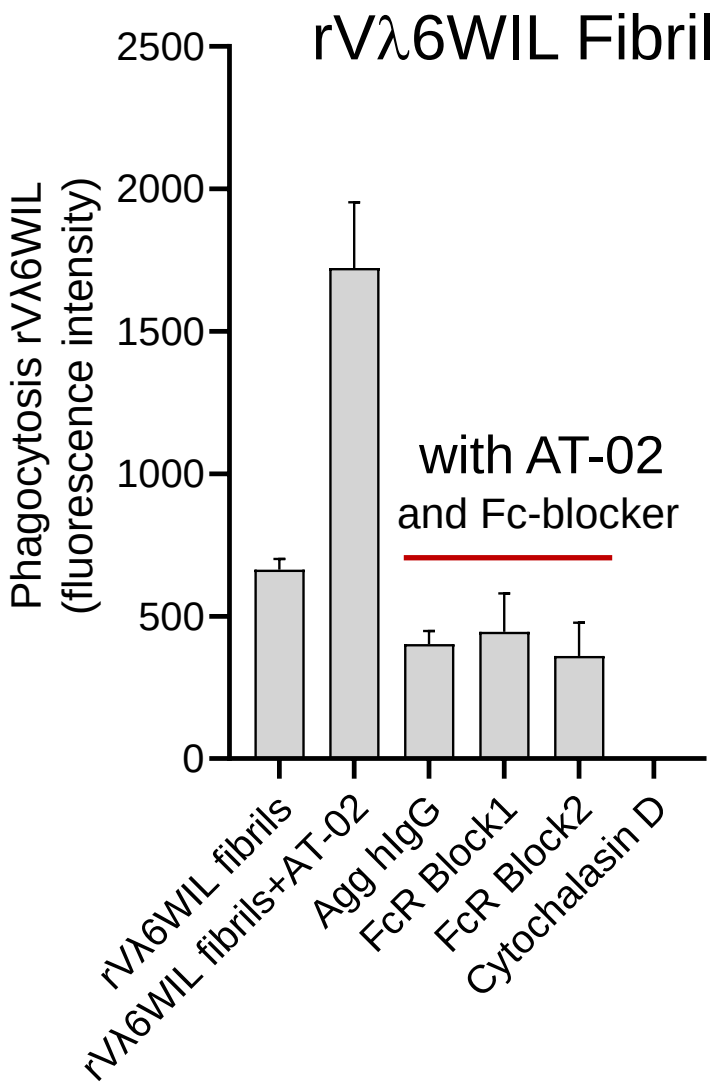
AT-02 + AL amyloid

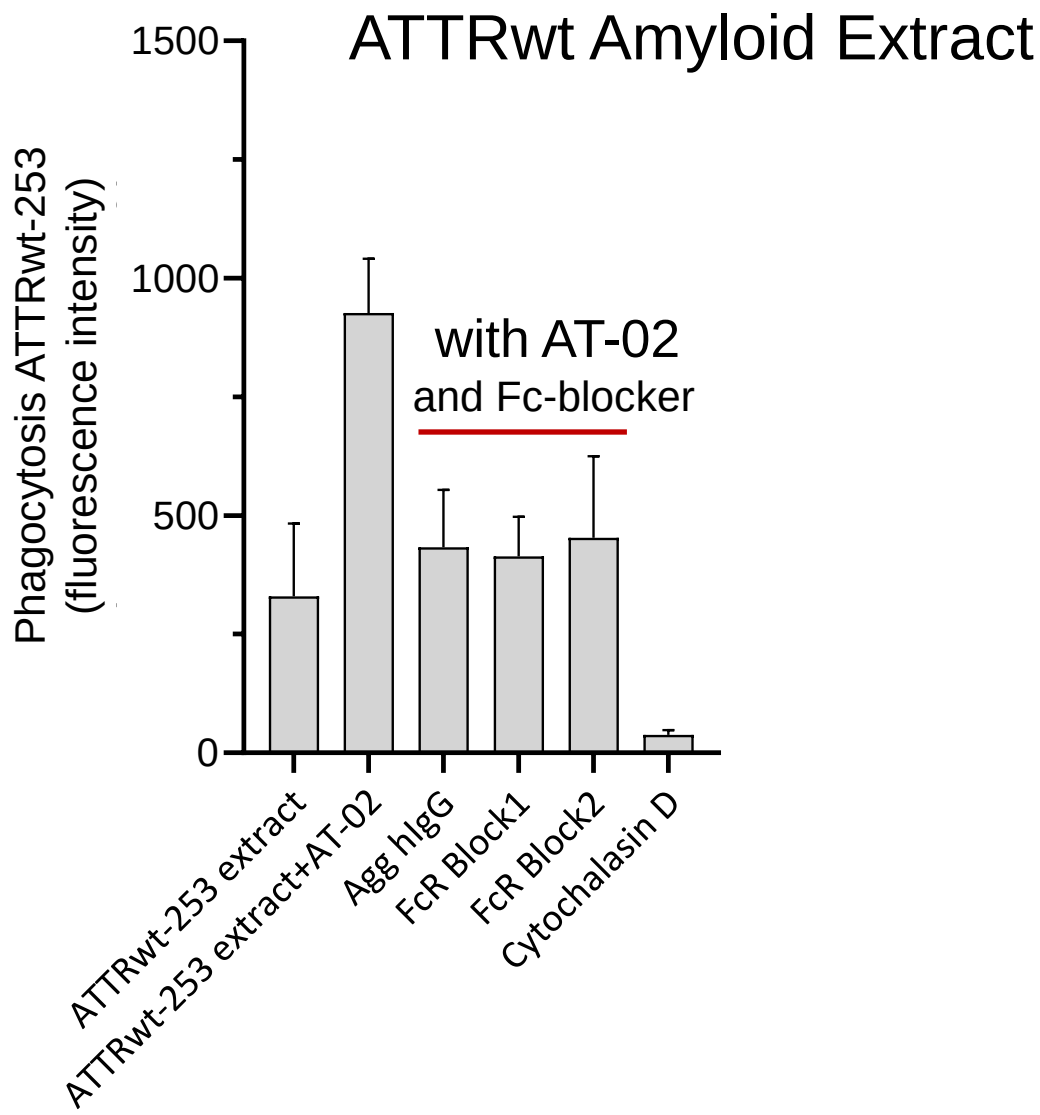


AT-02 + AL amyloid



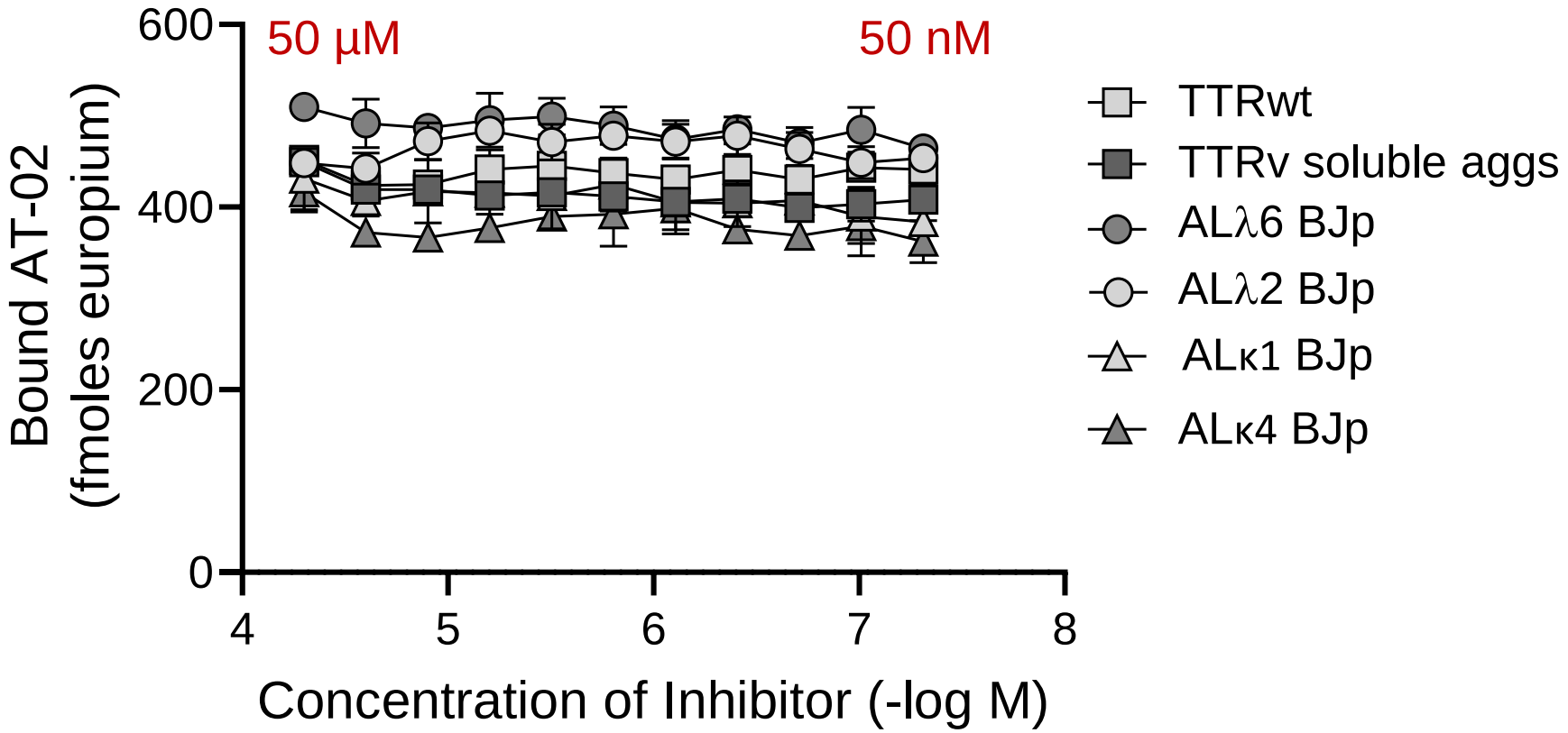
AT-02-INDUCED PHAGOCYTOSIS OF pHrodoRED-AMYLOID IS FcR-DEPENDENT





AT-02 BINDING TO AMYLOID FIBRILS IS NOT INHIBITED BY SOLUBLE PRECURSOR PROTEIN

Precursor proteins (in solution) tested as competitor for AT-02 binding to rVλ6WIL fibrils.
AT-02 (0.7 nM constant) with a 30 min pre-incubation at RT with precursor.
Fibrils (0.83 μM stock) were dried at 37˚C on the plates and the wells blocked with BSA solution.
Binding of AT-02 was assessed by ELISA.



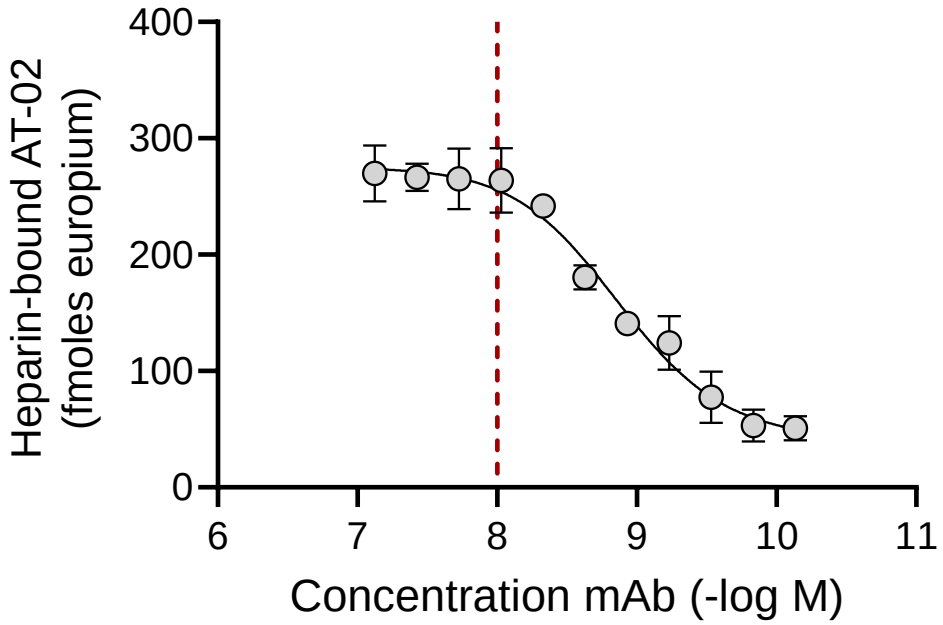
AT-02 RETAINS BIOACTIVITY IN HUMAN SERUM

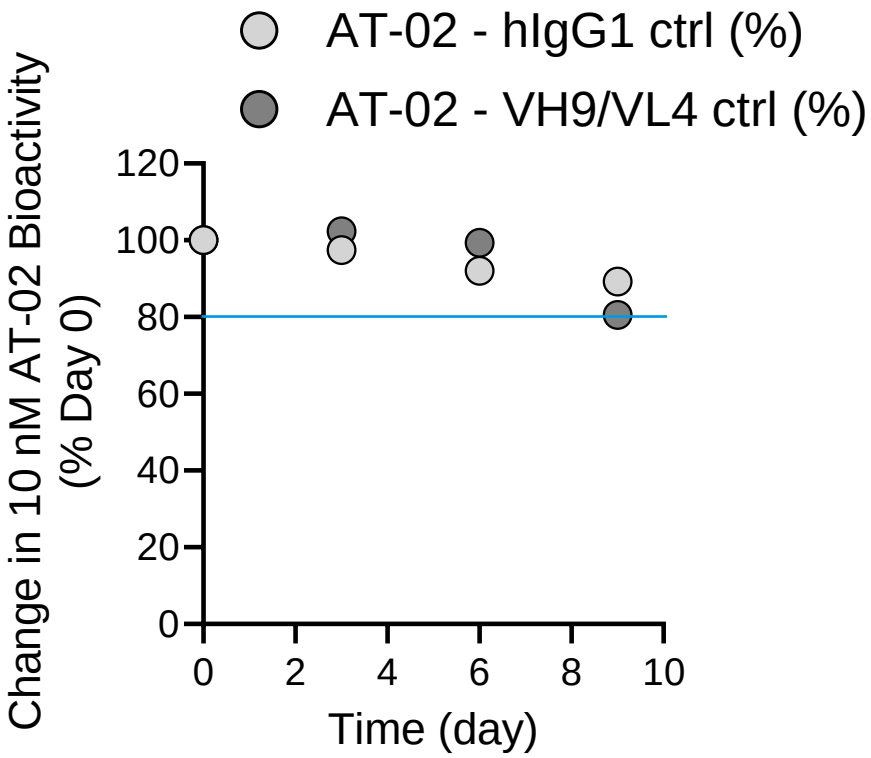
The reagents were incubated in human serum at 300 nM (~50 µg/mL).

Samples were incubated at 37˚C.

Samples were diluted to 10 nM (~1.5 µg/mL) for ELISA.

Heparin binding was assessed (as a surrogate for amyloid HS) – using hIgG1 and the base antibody (VH9/VL4) as the background control.





SUMMARY

- AT-02 is a novel antibody-peptide fusion protein designed to serve as a pan-amyloid clearing therapeutic.
- AT-02 binds many types of amyloid and amyloid-like fibrils with high potency through electrostatic interactions.
- When injected IV, AT-02 colocalized with murine AA amyloid in the liver, spleen and heart and was detected in amyloid deposits 7 days pi.
- When injected IP, Dylight800-labeled AT-02 rapidly colocalized with human AL amyloid implanted sq and was detected in amyloid deposits 10 days pi.
- AT-02 can enhance phagocytosis of ATTRwt amyloid extracts *in vitro* by human THP-1 monocytes and by PMA-activated human THP-1 cells – at doses as low as 0.5 nM.
- AT-02 binding to AL amyloid-like fibrils was not impacted by the presence of amyloid precursor proteins in solution up to 50 µM
- AT-02 showed good stability in human serum at 37˚C for 9 days with only modest reduction in bioactivity at the EC90 concentration.

ACKNOWLEDGEMENTS

UT Graduate School of Medicine
Steve Kennel
Emily Martin
Manasi Balachandran
Joseph Jackson
Trevor J. Hancock
Steve Foster
Angela Williams
Alan Stuckey
Tina Richey
Sallie Macy
Craig Wooliver
Eric Heidel

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attralus

QUESTIONS & ANSWERS