

# Preclinical characterization of AT-02, a pan-amyloid-binding immunoglobulin-peptide fusion protein capable of enhancing phagocytosis and facilitating reduction of amyloid

Jonathan Wall, Michael L. Klein, Suganya Selvarajah, Spencer Guthrie, Gregory Bell, Christophe Sirac, Roussine Codo, Steve J. Foster, Angela Williams, Tine Richey, Manasi Balachandran, Joseph Jackson, Alan Stuckey, Emily Martin, Sallie Macy, Craig Wooliver, R. Eric Heidel, Stephen Kennel

# Disclosure Information

*International Society of Amyloidosis Annual Meeting  
September 4-8, 2022*

*Jonathan Wall, University of Tennessee Graduate School of Medicine*

---

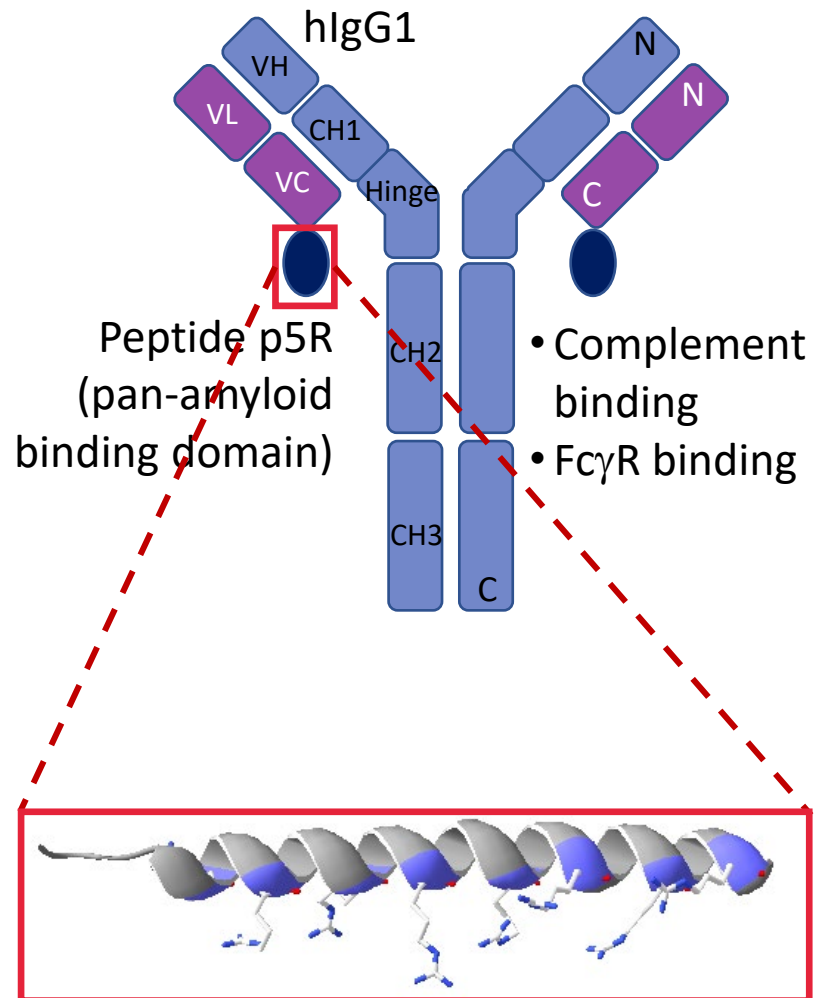
*I have the following financial relationships to disclose:*

Grant support:	NIH and Pharmaceutical Industry
Support from:	Attralus Inc.
Founder, Shareholder, CSO:	Attralus Inc.
Patent rights in:	Amyloid-reactive antibodies and peptides

---

I will not discuss off-label use in my presentation

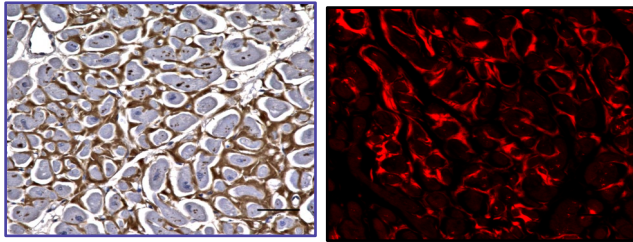
# AT-02: A Humanized IgG1-peptide Fusion with Pan-Amyloid Reactivity



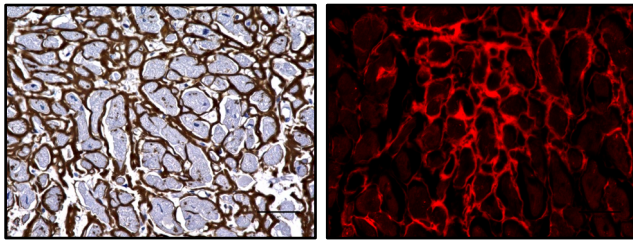
- AT-02 is a humanized IgG1-peptide fusion reagent
- The pan-amyloid reactive peptide p5R is fused to the C-terminal of the light chain
- Peptide p5R binds the ubiquitous hypersulfated glycosaminoglycans and fibrils in amyloid via electrostatic interactions
  - Same peptide technology as the  $^{124}\text{I}$ -AT-01 imaging agent which has shown uptake in key organs in multiple amyloid types in Ph 1/2 clinical trials
- AT-02 was designed to be capable of:
  1. Binding to all types of amyloid deposits
  2. Opsonizing the deposits and promoting macrophage-mediated amyloid clearance
  3. Binding complement to enhance phagocytosis of amyloid

# Pan-Amyloid Reactivity and Potent Binding of AT-02

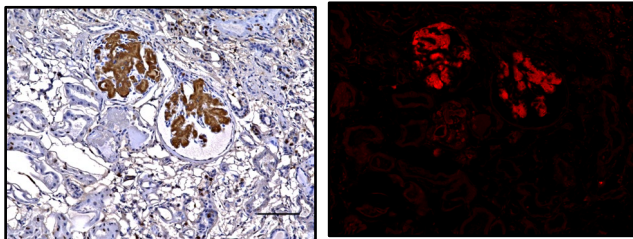
Human ATTRv (T60A) - heart



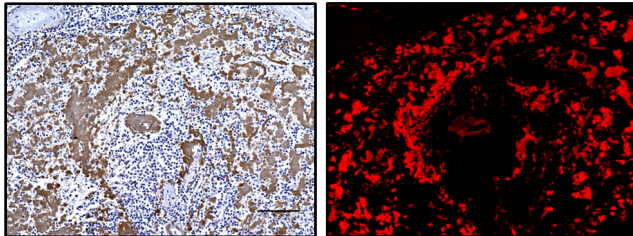
Human AL $\lambda$  - heart



Human AL $\lambda$  - kidney

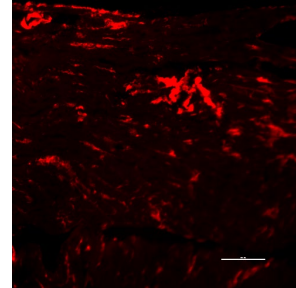


Human ALECT2 - spleen

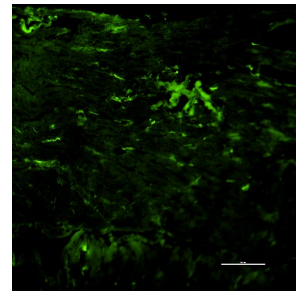


AL Mouse Model  
Heart

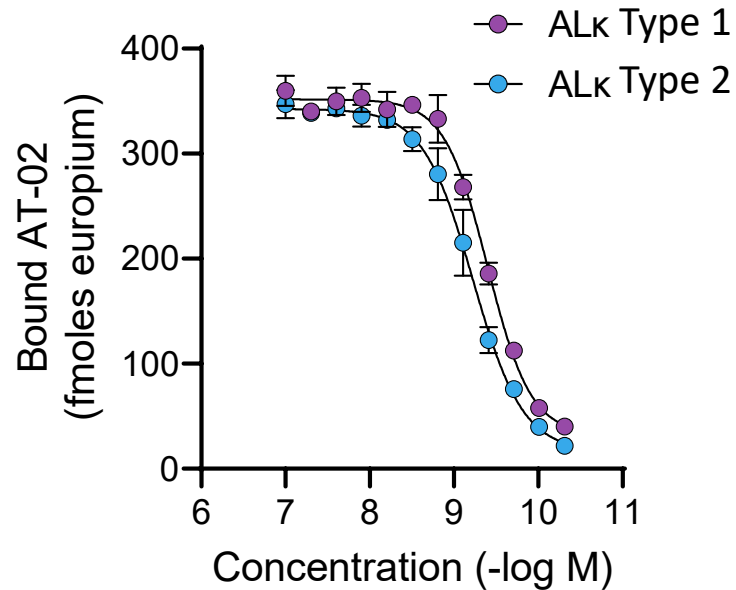
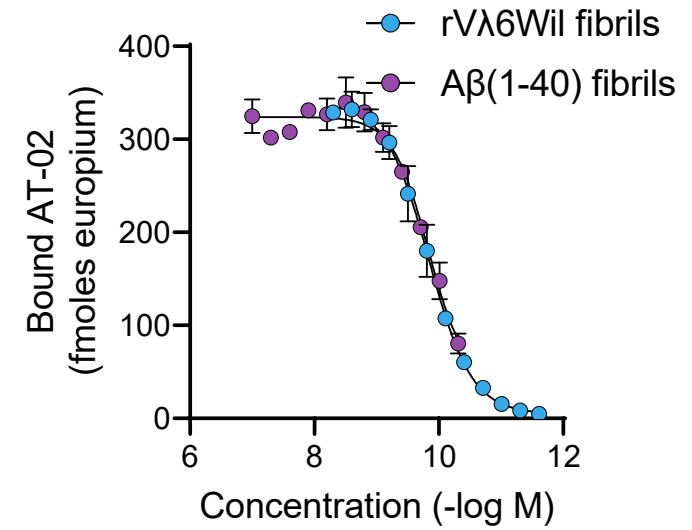
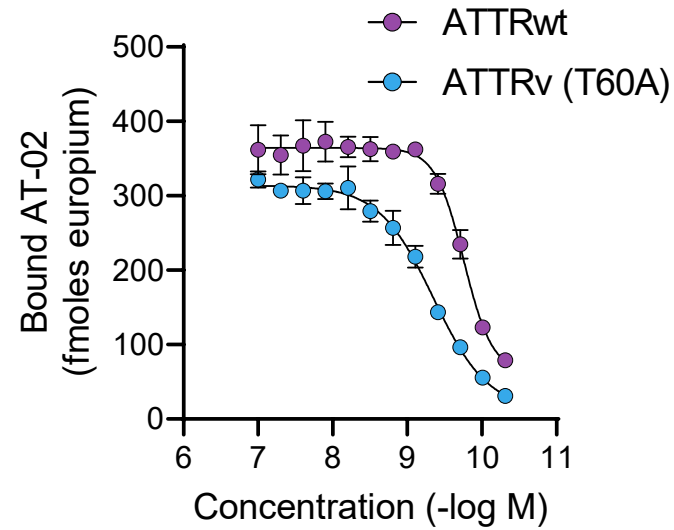
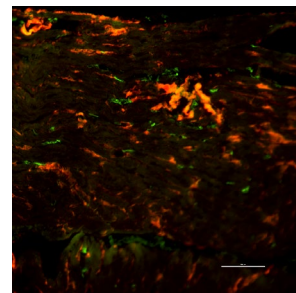
Congo red (flu)



Anti-AT-02

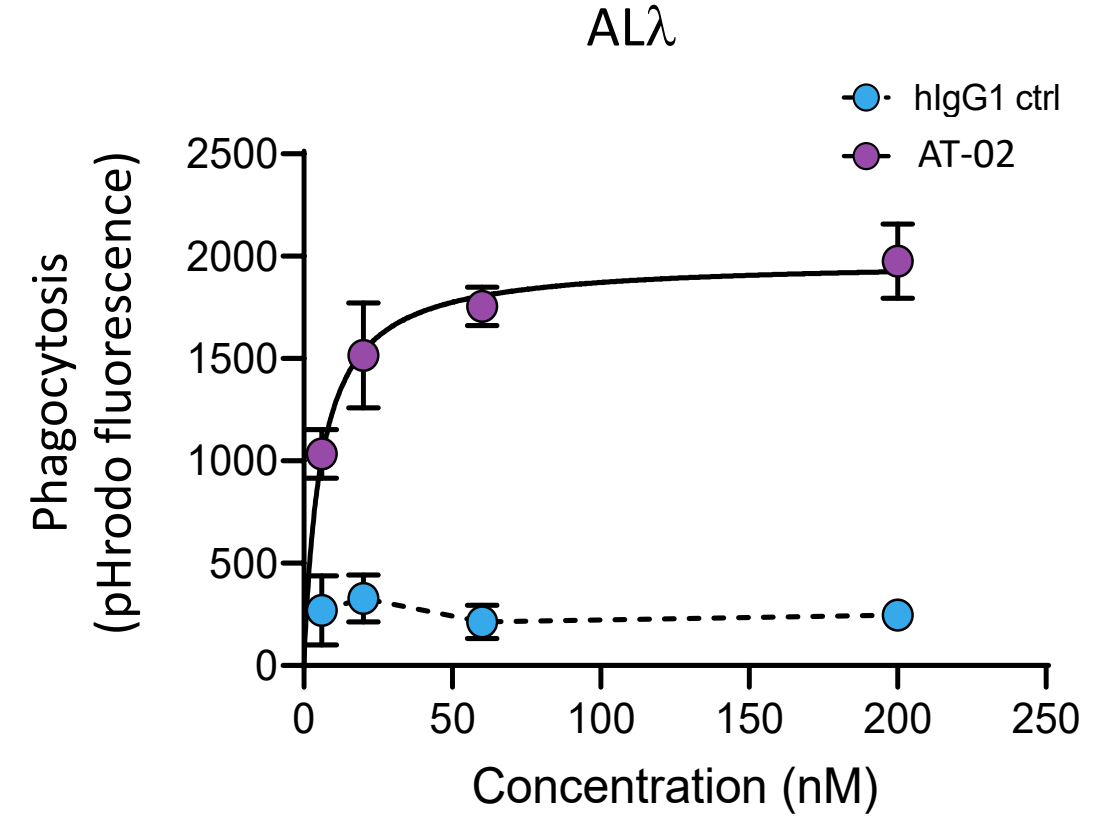
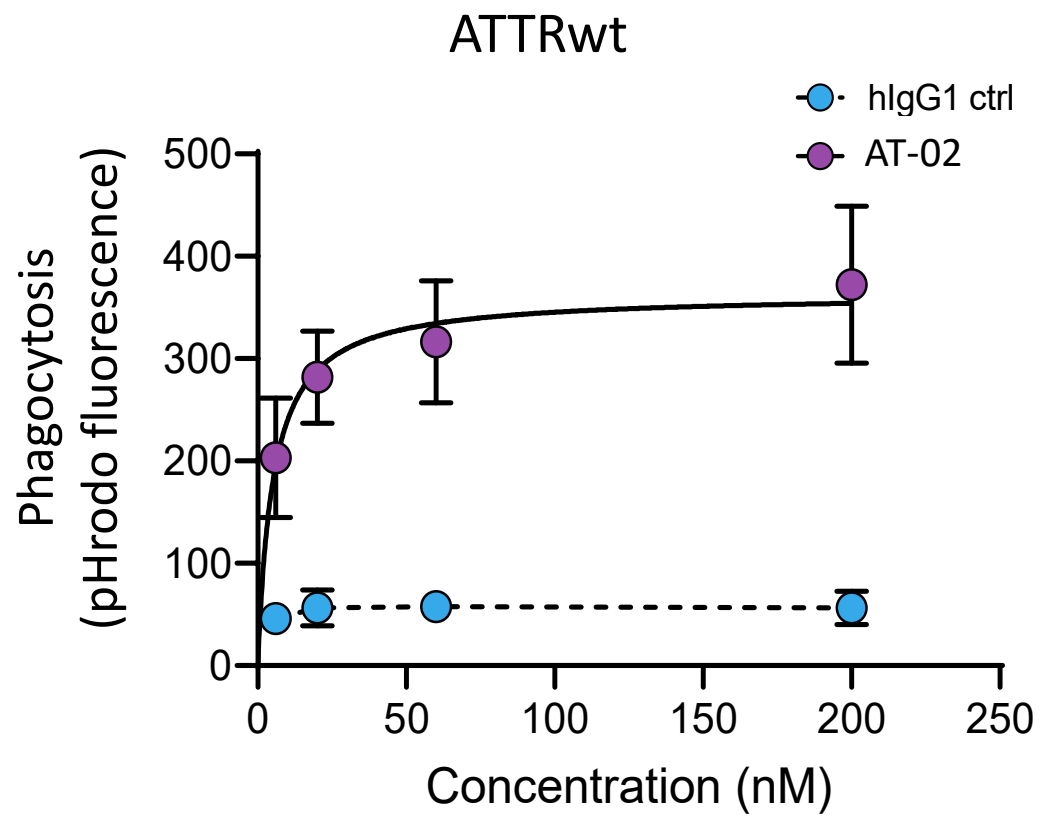
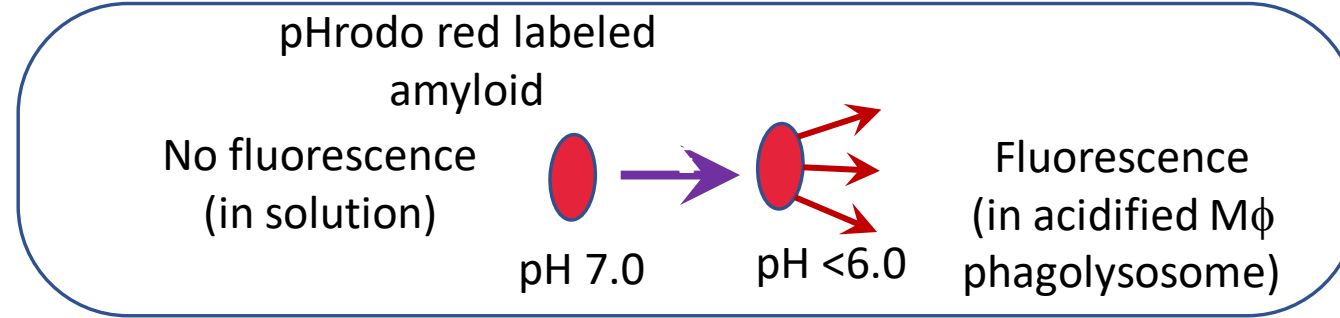


Merge



	EC50 (nM)
rVλ6Wil	0.15
Aβ(1-40)	0.15
ATTRwt	0.18
ATTRv	0.45
ALλ1	0.32
ALλ2	0.36
ALk1	0.40
ALk2	0.60

# AT-02 Promotes Phagocytosis of ATTR and AL Amyloid *In Vitro*

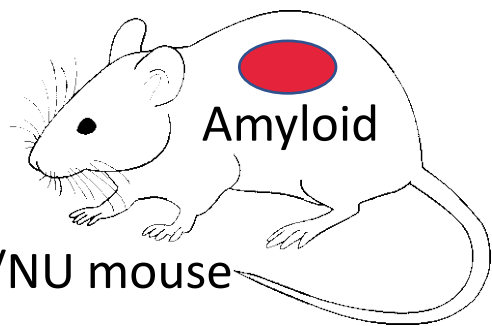


# AT-02 Promotes Phagocytosis of Amyloid *In Vivo*

Amyloid labeled with pHrodo red

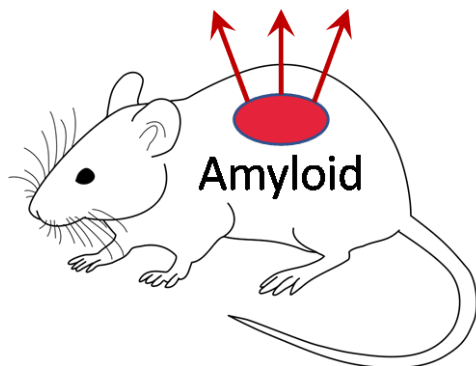
Pretreat 500  $\mu$ g AT-02

Implanted 2 mg SQ



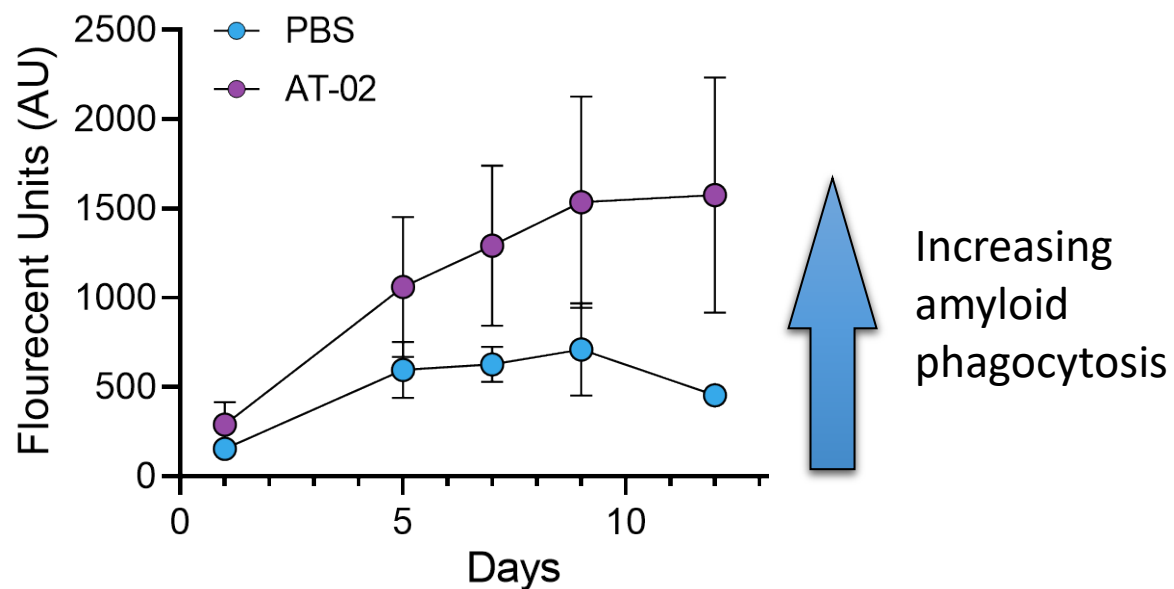
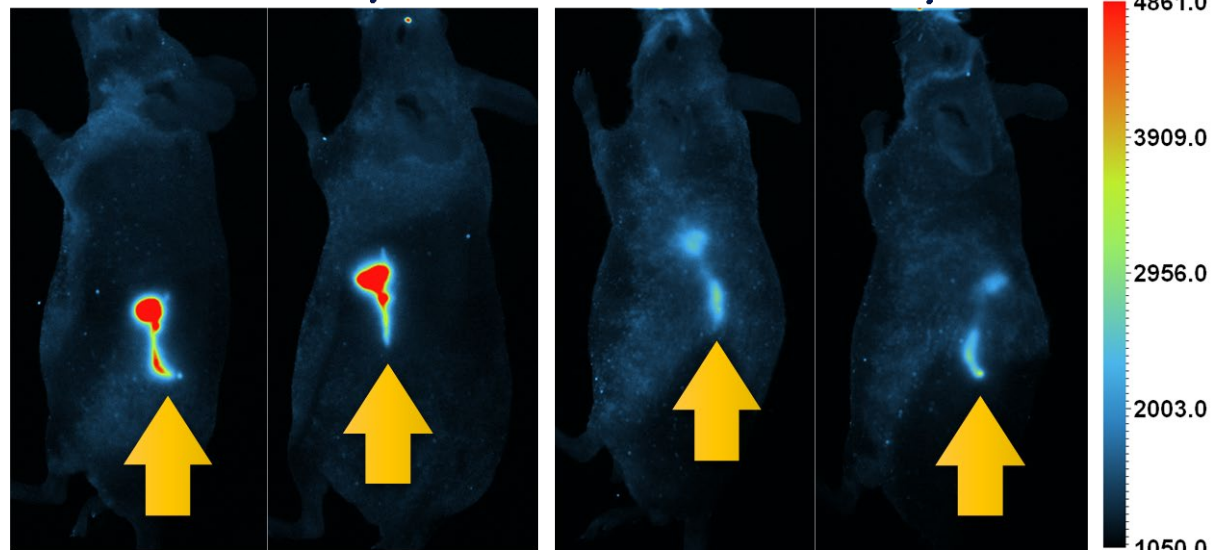
Phagocytosis of amyloid *in vivo*

Fluorescence emission



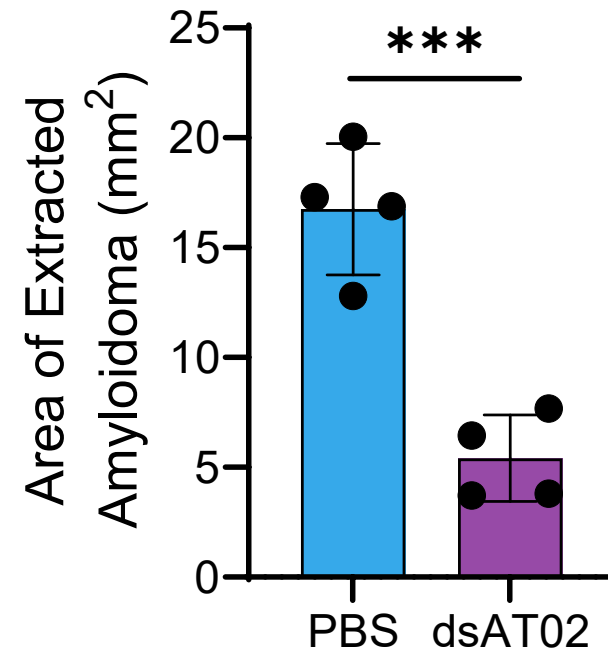
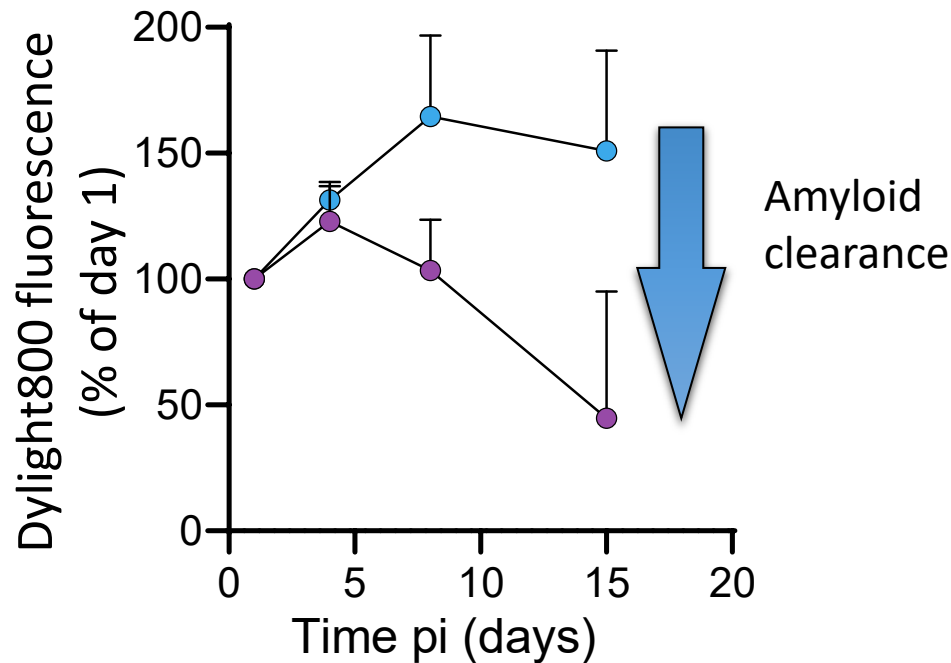
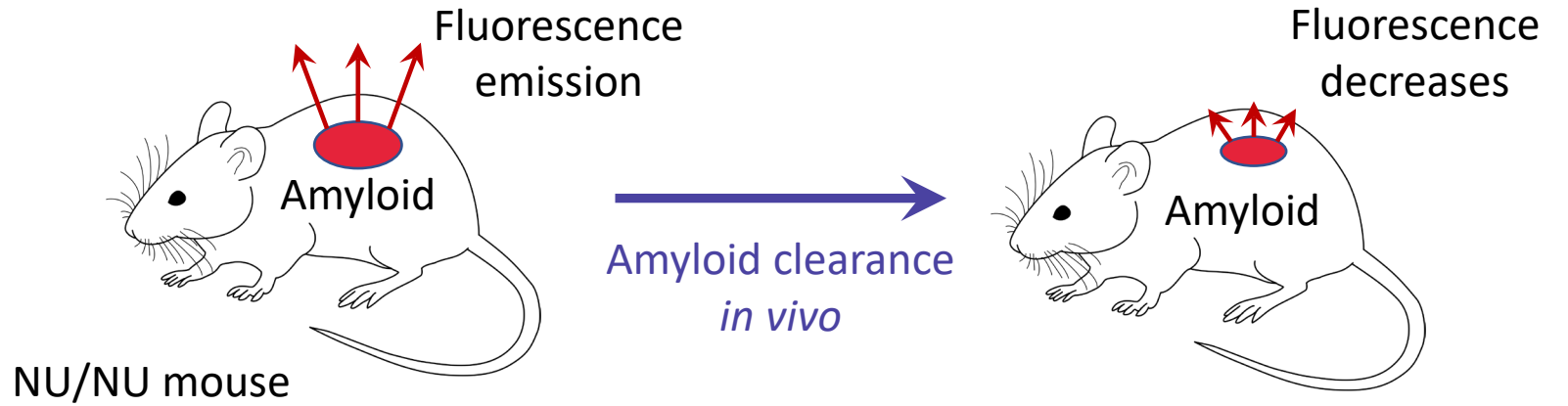
AT-02 – Day 12

Control – Day 12



# AT-02 Promotes Clearance of Human AL Amyloid *In Vivo*

Amyloid labeled with **NIR fluorophore, Dylight800**  
Pretreated with 500  $\mu$ g AT-02  
Implanted 2 mg SQ



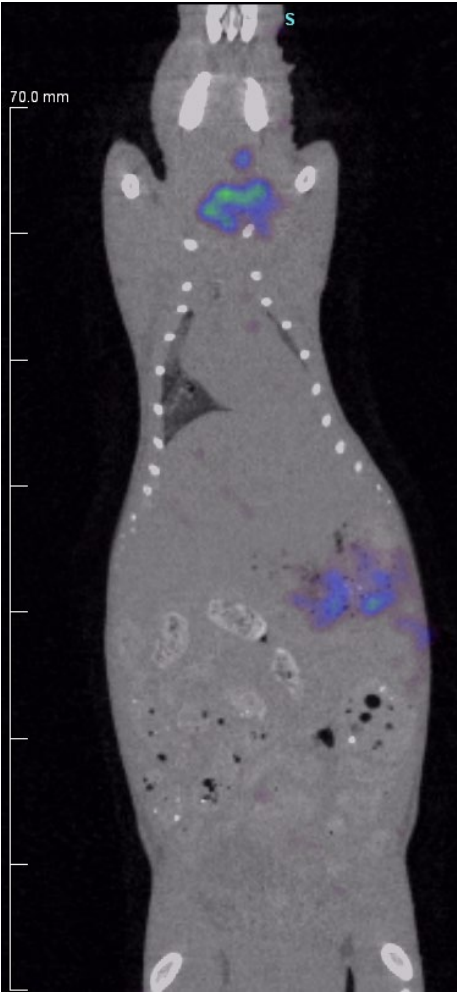
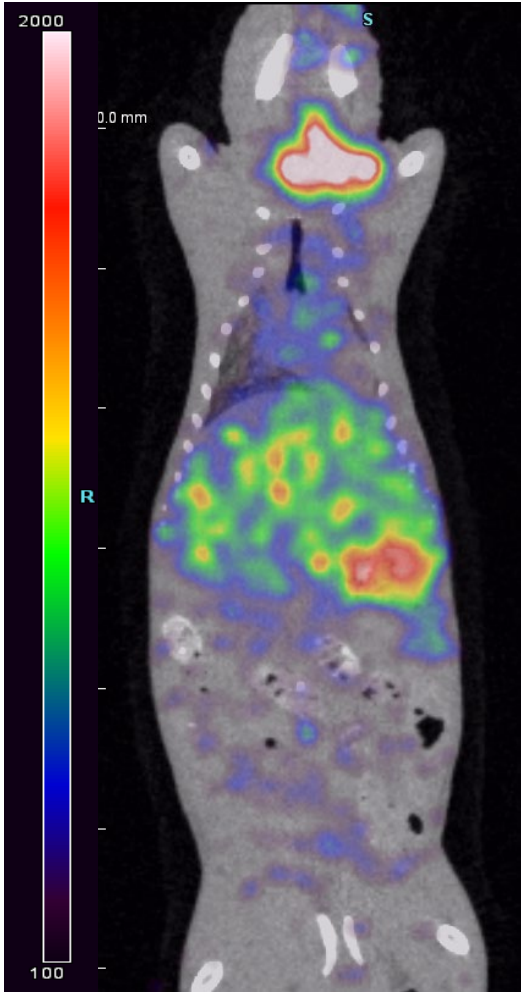
# AT-02 Treatment of hIL-6 Transgenic Mice with Progressive Systemic AA Amyloidosis



# AT-02 Binds AA Amyloid in the hIL-6 Transgenic Mice

$^{125}\text{I}$ -dsAT-02

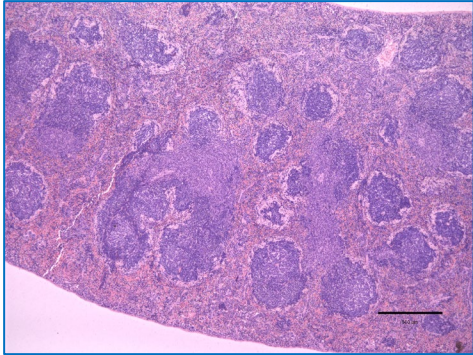
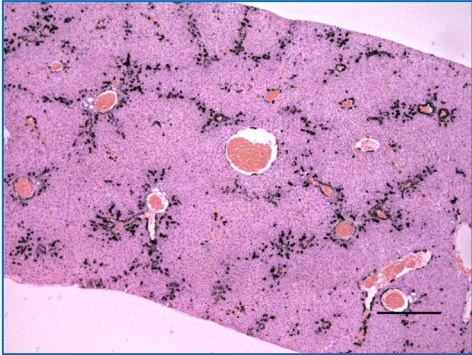
$^{125}\text{I}$ -hIgG1



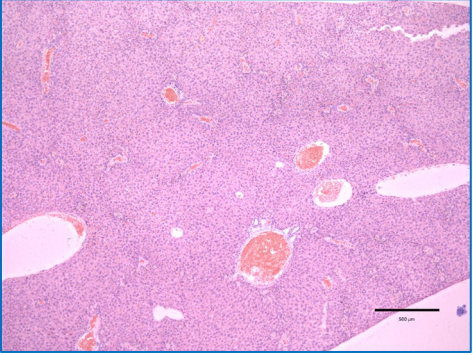
$^{125}\text{I}$ -AT-02

$^{125}\text{I}$ -IgG1

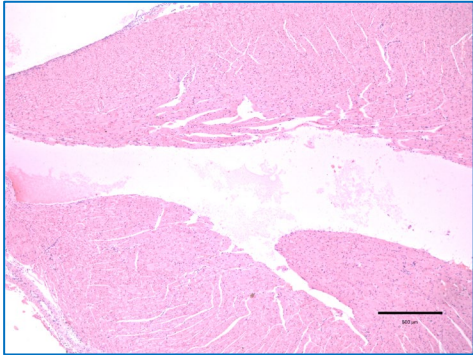
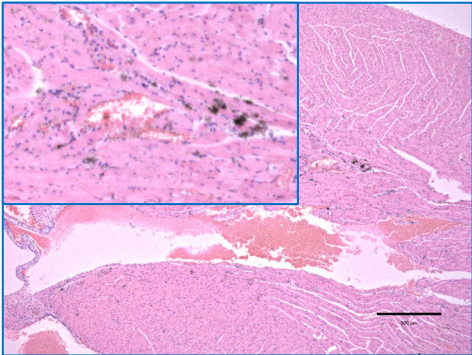
Liver



Spleen



Heart



# AT-02 Treatment of Progressive AA Amyloidosis in Mice

H2/IL-6 mice – Develop severe systemic AA amyloidosis in response to constitutive expression of IL-6

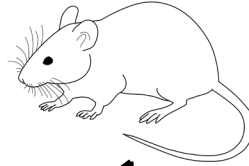


100  $\mu$ g AEF IV (Amyloid induction)

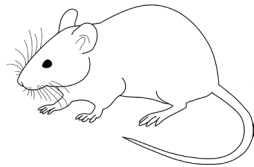
14 d post-AEF (amyloid deposition has started)

AT-02 ( $n=13$ )

PBS ( $n=11$ )

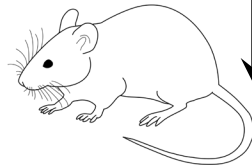


14 d post-AEF: IP 2.5 mg AT-02 or PBS  
21 d post-AEF: IP 2.5 mg AT-02 or PBS  
28 d post-AEF: IP 2.5 mg AT-02 or PBS



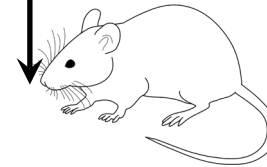
$n=6$

- $^{125}$ I-SAP injection
- SPECT/CT ( $n=2$ )
- Congo red quant



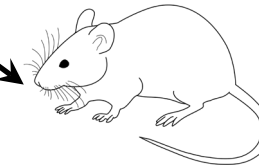
$n=7$

- Bloodwork
- Congo red quant



$n=4$

- $^{125}$ I-SAP injection
- SPECT/CT ( $n=2$ )
- Congo red quant



$n=7$

- Bloodwork
- Congo red quant

Euthanasia 42 d post AEF

# AT-02 Reduced AA Amyloid Deposition and Organ Impairment

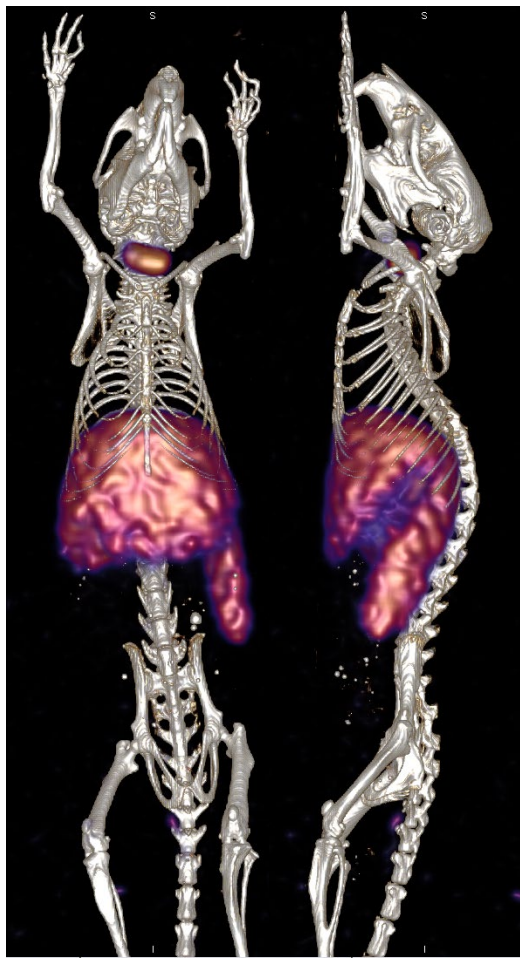
## $^{125}\text{I}$ -SAP SPECT/CT Imaging

PBS - treated

AT-02 - treated

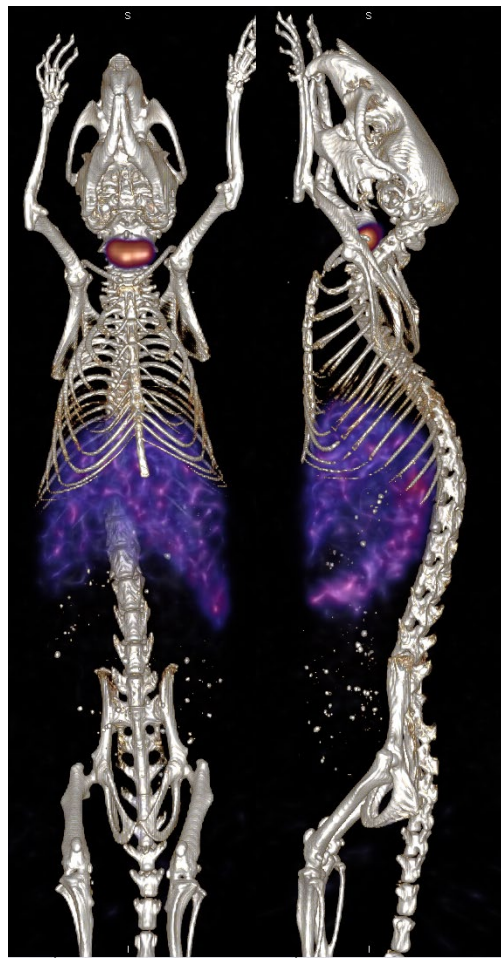
coronal

sagittal



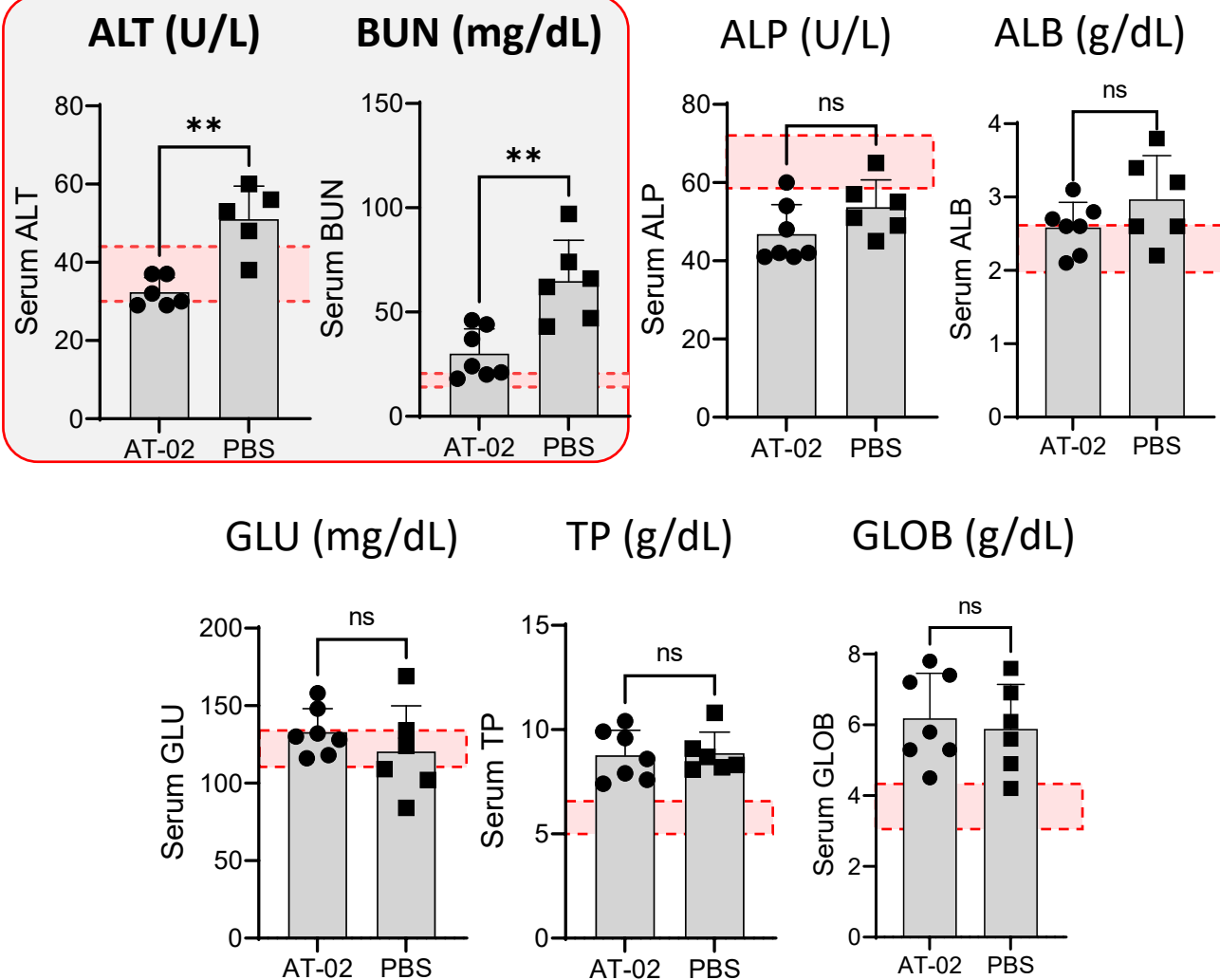
coronal

sagittal

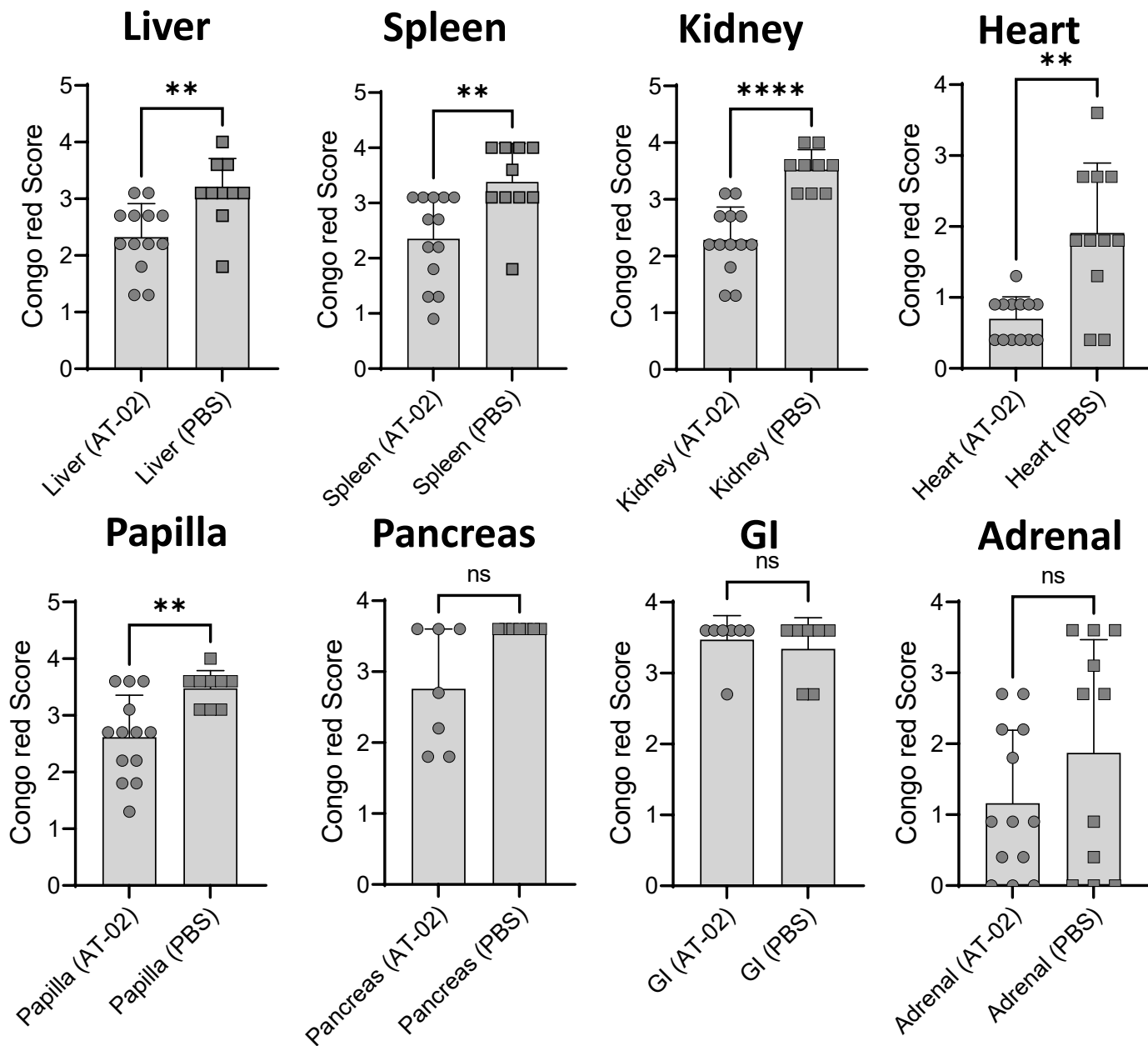


## Bloodwork Analysis

Reduced organ impairment



# AT-02 Reduced AA Amyloid Deposition



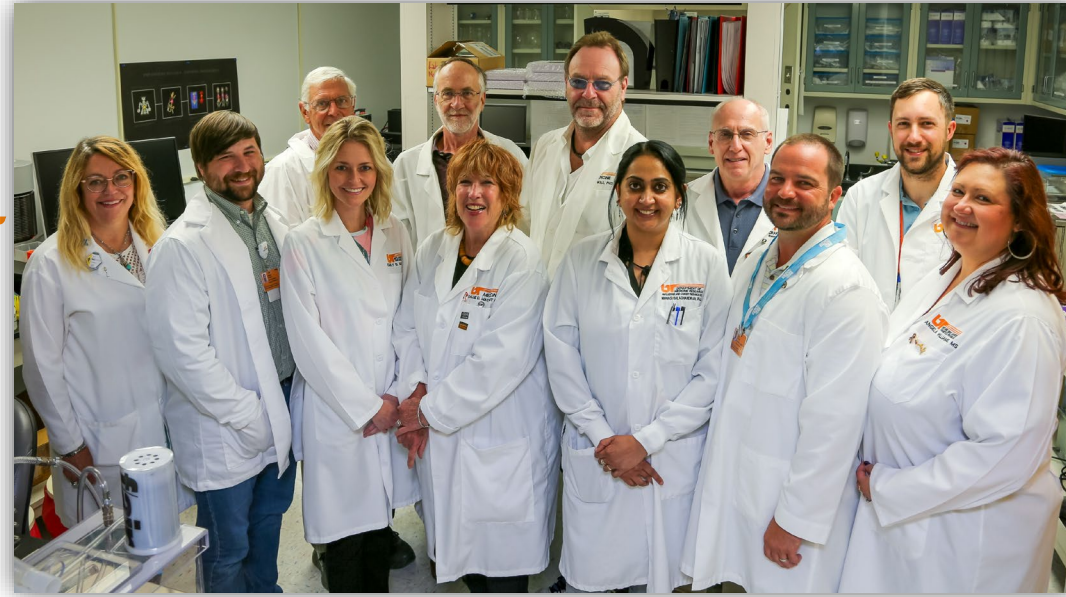
Organ	AT-02 median (n)	PBS median (n)	Mann-Whitney Sig.
<b>Liver</b>	<b>2.2</b> (n=13)	<b>3.1</b> (n=10)	<b>p=0.0029</b>
<b>Spleen</b>	<b>2.7</b> (n=13)	<b>3.35</b> (n=10)	<b>p=0.0023</b>
<b>Kidney</b>	<b>2.2</b> (n=13)	<b>3.6</b> (n=9)	<b>p&lt;0.0001</b>
<b>Heart</b>	<b>0.9</b> (n=13)	<b>1.8</b> (n=11)	<b>p=0.0017</b>
<b>Papilla</b>	<b>2.7</b> (n=13)	<b>3.6</b> (n=9)	<b>p=0.0042</b>
<b>Pancreas</b>	2.7 (n=7)	3.6 (n=7)	p=0.0699
<b>GI</b>	3.6 (n=7)	3.6 (n=7)	p>0.9999
<b>Adrenal</b>	0.9 (n=13)	2.7 (n=11)	p=0.2651

# Summary

- AT-02 is a novel antibody-peptide fusion protein designed to serve as a pan-amyloid clearing therapeutic
- AT-02 bound diverse types of amyloid and amyloid-like fibrils with high potency ( $EC_{50} < 500$  pM)
- When injected IV, AT-02 colocalized with AL amyloid in the heart of a murine model of AL $\lambda$ 6 amyloidosis
- AT-02 enhanced macrophage-mediated phagocytosis of AL and ATTR amyloid extracts *in vitro*, and *in vivo* using a murine model of human AL amyloidoma
- Opsonization of human AL amyloid with AT-02 expedited clearance of the mass *in vivo*
- AT-02 treatment caused significant reduction of cardiac, renal and hepatic amyloid in a transgenic mouse model of aggressive AA amyloidosis
- AT-02 is progressing to phase 1 human trials and could represent a novel therapeutic agent for the removal of systemic amyloid deposits of diverse types

# Acknowledge the Support and Research Activities

THE UNIVERSITY of  
**TENNESSEE**   
GRADUATE SCHOOL  
OF MEDICINE



## UT Graduate School of Medicine

Steve Kennel  
Emily Martin  
Manasi Balachandran  
Joseph Jackson  
Steve Foster  
Angela Williams  
Alan Stuckey  
Tina Richey  
Sallie Macy  
Craig Wooliver  
Dr. Eric Heidel



## University of Limoges

Christophe Sirac  
Roussine Codo



## Attralus Inc.

Spencer Guthrie  
Michael Klein  
Gregory Bell  
Suganya Selvarajah