Rational Design of AML Therapeutic Agents that Specifically Bind and Clear Diverse Amyloid Aggregates

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BACKGROUND

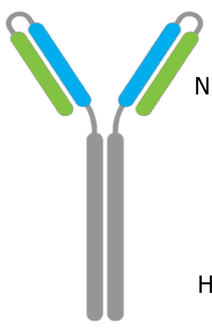
Ig-fusion to the specific g3p N1-N2 domain from M13 bacteriophage has been demonstrated to target and remodel a large variety of amyloid fibers. This binding is mediated by N1N2 hydrophobic and polar residues docking specifically the cross-beta sheet assembly common to amyloid fibers (Krishnan *et al*, 2014). First generation molecules have been developed for treating Alzheimer disease (Levenson *et al*, 2016). Preclinical studies in models for light chain (LC) amyloidosis showed the co-localization of injected radio-iodinated Ig-fusions with subdermal and gut amyloid deposits, demonstrating the expected biodistribution of systemically administrated molecules (Proschitsky *et al*, 2019). Open stabilized variants have been generated exhibiting improved binding potency, inhibition of amyloid assembly and blocking cell to cell propagation of pathologic proteins (Asp *et al, 2019)*. On that basis, new constructs have been made and are evaluated.

OBJECTIVES

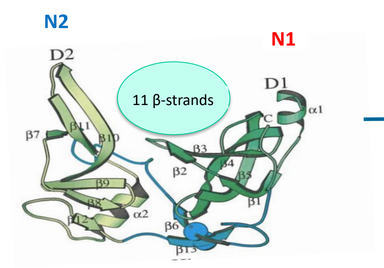
This study compares new AML therapeutic candidates designed for reducing amyloid loads in amyloidosis patients. Focusing firstly to improve LC *in vitro* and *ex vivo* binding, specificity and immunogenicity, we are also generating evidence to confirm the mode of action of our candidates.

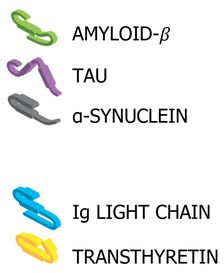
RESULTS

AMYL Tx Candidates : A Conformational Binder

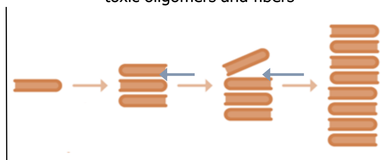


N1-N2 domains can flex to provide a large hydrophobic canonical pocket to bind amyloid folds



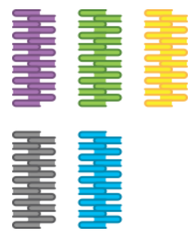


Proteins misfold, aggregate, and assemble into toxic oligomers and fibers



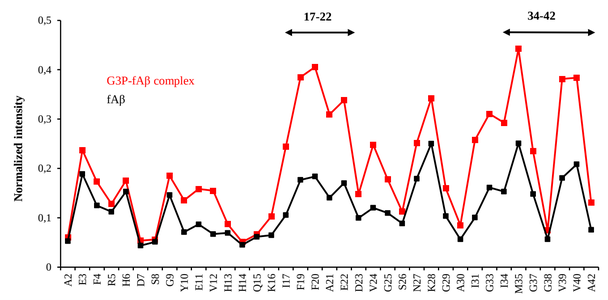
Nucleation and assembly

All aggregates share common amyloid fold

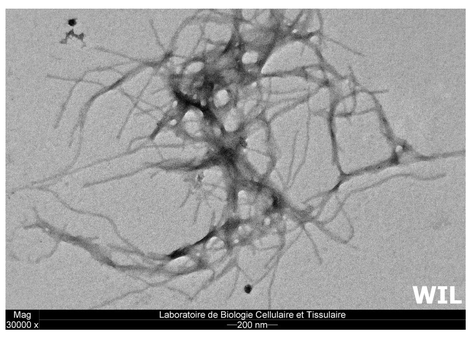


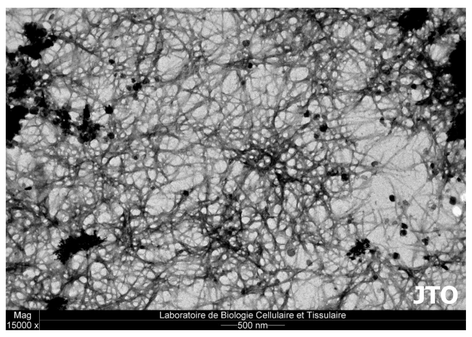
Cross β sheets perpendicular to fiber axis

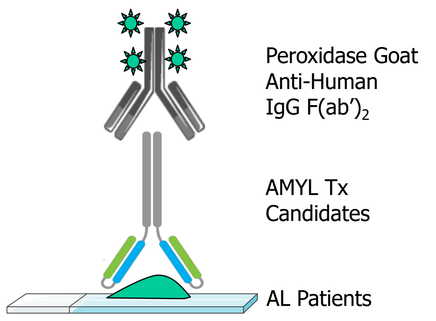
H-D NMR data with A-Beta fibers data confirm that
- **Protected residues on fibers are in core beta-strands**
- **Protected residues in N1-N2 fusions are in core beta-strands**



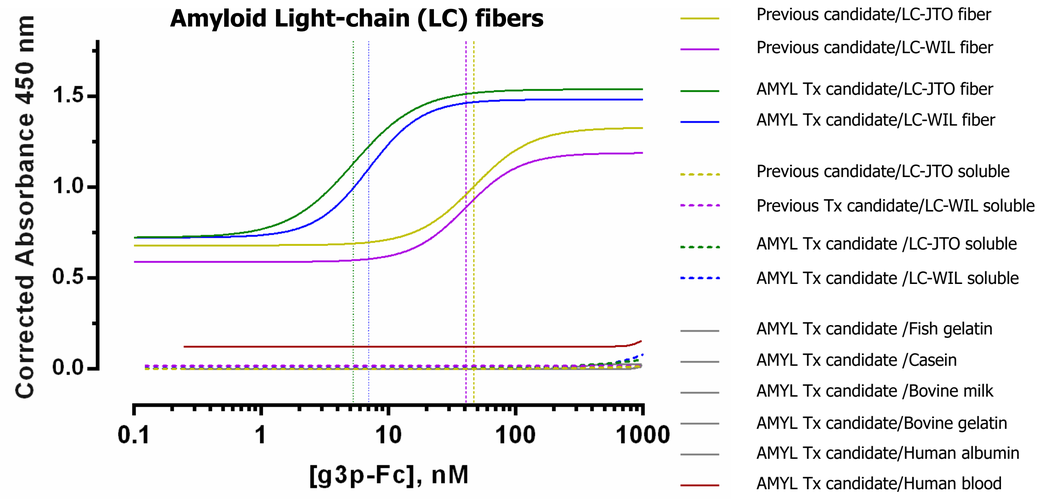
Binding Potency of AMYL Tx Candidates : *in vitro and ex vivo*







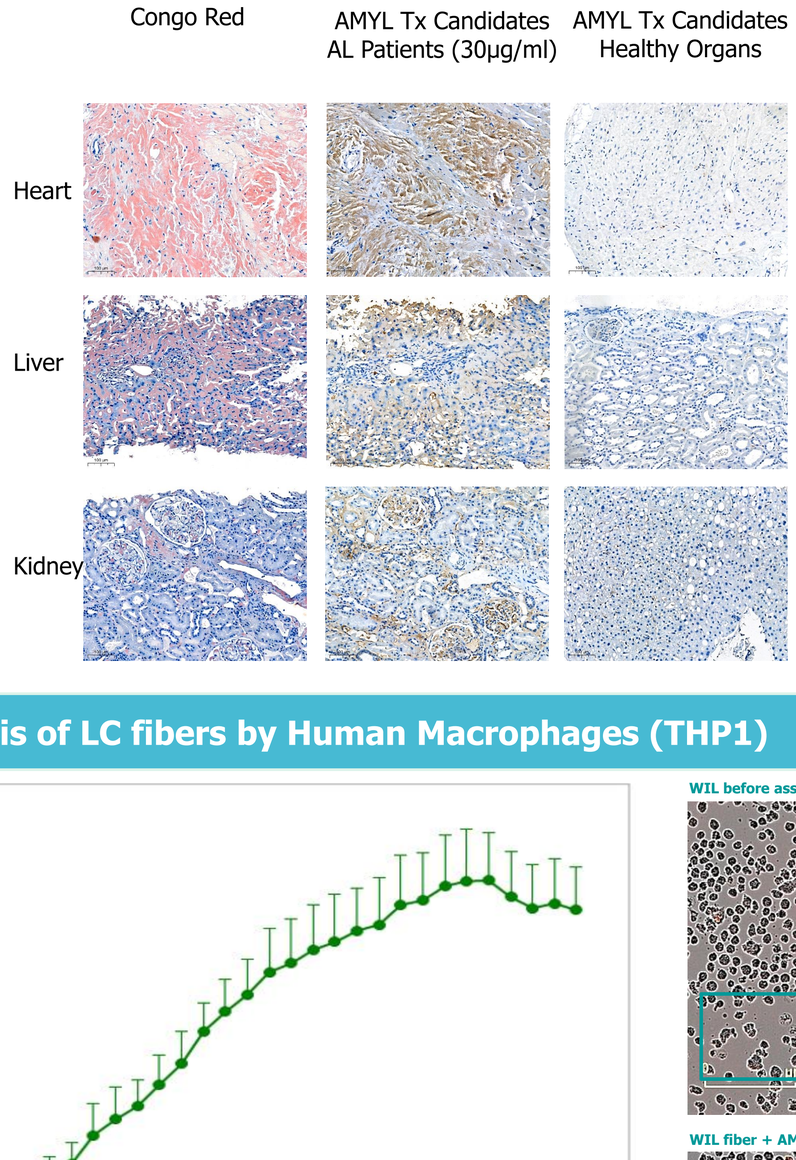
Organ	LC Sub Type	Congo Red	AMYL Tx Candidates (30µg/ml)
Kidney	AL-Lambda	+	+
Kidney	AL-Lambda	+	+
Liver	AL-Kappa	+	+
Heart	Cardiac Amyloidosis	+	+
Kidney	AL	+	+
Kidney	AL-Lambda	+	+
Kidney	AL-Lambda	+	+



Specific binding to lambda JTO and WIL fibers

- **Absence of binding to soluble forms**
- **Absence of binding to other substrates**
- **Increased binding potency as compared to historical candidates**

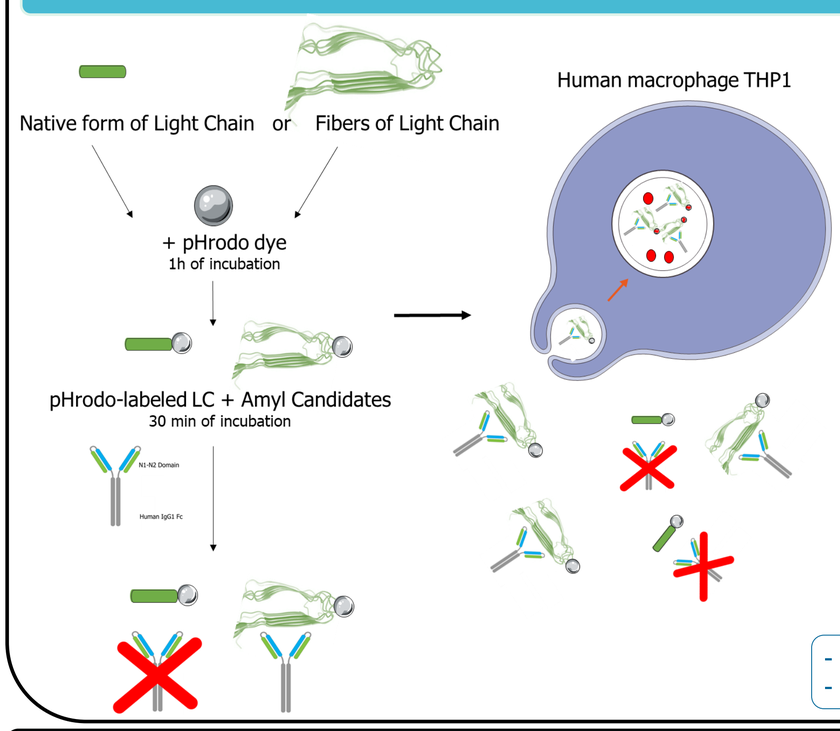
	EC_{50} Fiber LC JTO (nM)	EC_{50} Fiber LC WIL (nM)
AMYL Tx Candidates	~6,7-13	~5,4-10
Previous Candidate	~58-75	~49-70

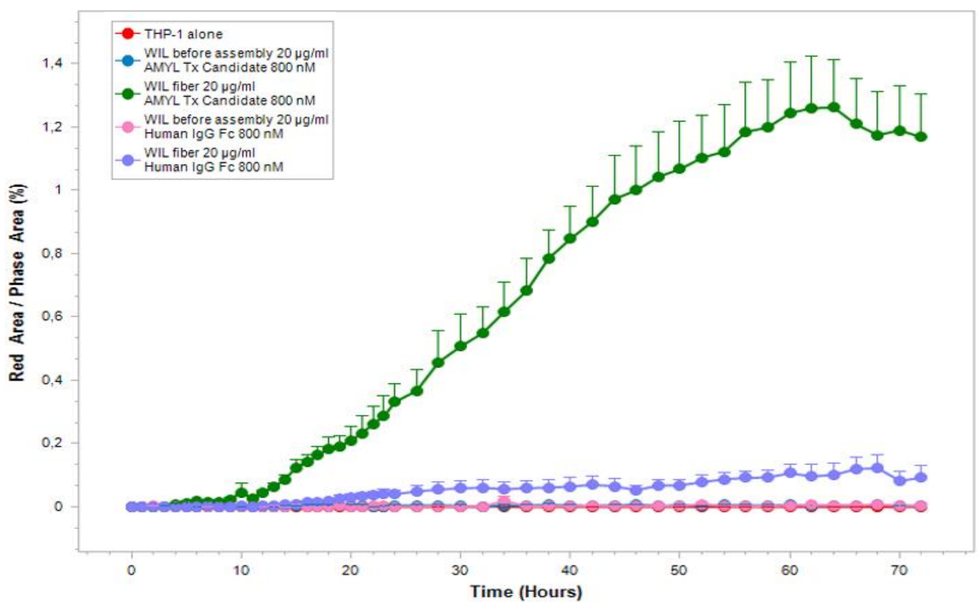


***Ex Vivo* , specific binding to amyloid deposits in AL amyloidosis patients tissues:**

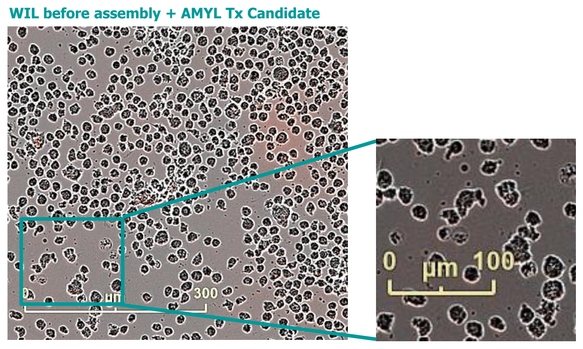
- **LC in heart, kidney and liver tissues**
- **Both Kappa and Lambda LC**
- **No binding to healthy tissues**
- **No binding by the anti-Human IgG F $(ab')_2$**
- **No binding by the control Isotype (Human IgG Fc)**

AMYL Tx Candidates Induce Phagocytosis of LC fibers by Human Macrophages (THP1)

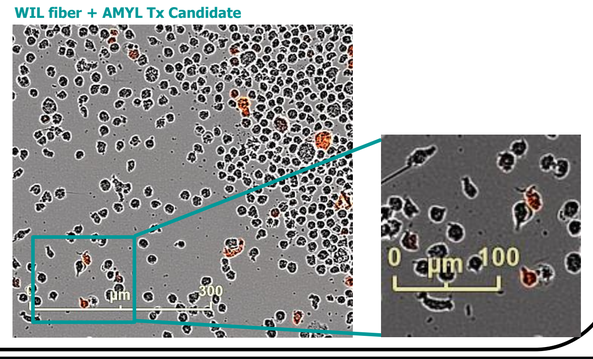




WIL before assembly + AMYL Tx Candidate



WIL fiber + AMYL Tx Candidate



- **LC fibers bound to Amyl Tx candidates are phagocytosed by macrophages**
- **Soluble LC or LC fibers without Amyl candidates are not taken up by macrophages**

CONCLUSIONS

New AMYL Tx Candidates were designed to improve binding potency and specificity to LC amyloid fibers. The data presented here confirms:
- Specific recognition of lambda and kappa light chain aggregates representing different allotypes
- Ability to induce phagocytosis by macrophages (critical step for amyloid elimination)
- In addition, our new candidates display a significantly improved immunogenicity profile (data not shown)

N1-N2 fusion proteins present advantages:
- To target conformational and sequence-independent binding sites
- To lead to loss of amyloid conformation (published remodeling data-see reference)

Based on our global data set, Amyl Tx moves with one candidate to clinical development, generating in parallel POC data in other indication

RESTORING CLARITY

www.amyltx.com

Krishnan, Rajaraman *et al.,* A Bacteriophage Capsid Protein Provides a General Amyloid Interaction Motif (GAIM) That Binds and Remodels Misfolded Protein Assemblies. Journal of Molecular Biology. 2014;426:2500-2519

Proschitsky Ming *et al.,* GAIM fusions are therapeutic candidates for peripheral amyloidosis. Amyloid. 2019 ;26(sup1)/85-86

Levenson Jonathan M *et al.,* NPT088 reduces both amyloid-β and tau pathologies in transgenic mice. Alzheimer's & Dementia. 2016;2(3):141-155

Asp Eva *et al.,* Stability and Inter-domain Interactions Modulate Amyloid Binding Activity of a General Amyloid Interaction Motif. Journal of Molecular Biology. 2019 ;431(10):1920-1939