Small Molecule Kinetic Stabilizers Reduce Amyloidogenicity of Free Light Chain Proteins of Diverse Sequences in λ Light Chain Amyloidosis

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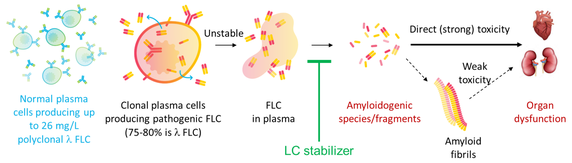
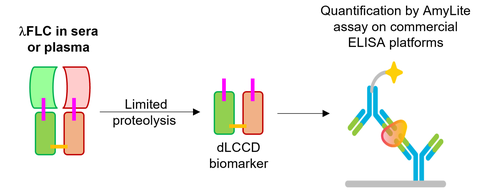
INTRODUCTION

- In light chain (LC) amyloidosis (AL), clonal plasma cells produce unstable free light chains (FLCs) prone to misfolding and aggregation, primarily impacting the heart and kidneys. While current therapies and some development candidates target amyloid fibrils, none address the soluble toxic protein conformations associated with the amyloidosis aspect of the disease.[1]
- We aim to develop the LC kinetic stabilizers, as used in Tafamidis for transthyretin (TTR) amyloidosis[2], to help restore misfolded LCs to a normal, stable state. This approach is expected to lead to faster, deeper organ responses and improved survival outcomes.
- A unique challenge in developing an FLC kinetic stabilizer lies in the fact that FLC proteins exhibit significant sequence diversity, primarily due to somatic recombination in the variable domain (V). Each AL patient has a unique clonal FLC sequence.



NOVEL ASSAY

- The AmyLite™ assay[5] was used to measure the level of unstable FLC in *ex vivo* biofluids through the quantification of a novel biomarker dLCCD (dimeric LC constant domain), of which the baseline levels in λ AL patient plasmas strongly correlate to overall survival.
- **AmyLite assay composes a two-step process:**
  1. Limited proteolysis treatment - generates a conserved fragment (dLCCD) specifically for amyloidogenic λ FLC
  2. Sensitive and specific immunoassay - quantifies unstable FLC directly in *ex vivo* patient plasma samples
- PTG-61 demonstrated *ex vivo* efficacy in randomly selected AL plasma samples *via* the AmyLite assay, protecting endogenous amyloidogenic λ FLC in 71% of tested samples (5 of 7, 18-96% stabilization) and significantly reduced toxicity.

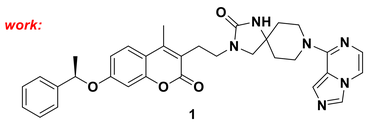


- **AmyLite on commercial MSD ELISA platform:**
  - High sensitivity (> 100x more sensitive than FreeLite[6])
  - Large dynamic range
  - Readout directly indicative of levels of amyloidogenic/ toxic FLC
  - Uses μL of plasma or serum samples
  - Turnaround time within a day

PROOF OF CONCEPT

- Kinetic stabilizer of λ full length LC dimers (FL LC_2), **1**, with a coumarin-based aromatic core, was developed by Scripps Research[3], but it lacks desired plasma selectivity and metabolic stability.
- Using structure-based design, we developed two-component anchor–aromatic core combinations with diverse linker modules and distal substructures, resulting in significantly enhanced *in vitro* potency, binding affinity, and kinetic stabilization of λ FL LC_2 in plasma and increased sequence coverage. The improved novel stabilizer, PTG-61, exhibits high potency and strong plasma stabilization of FL LC_2.[4]

Previous work:



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✓ ***Good Potency*** ✗ ***Poor plasma selectivity*** ✗ ***Poor metabolic stability***

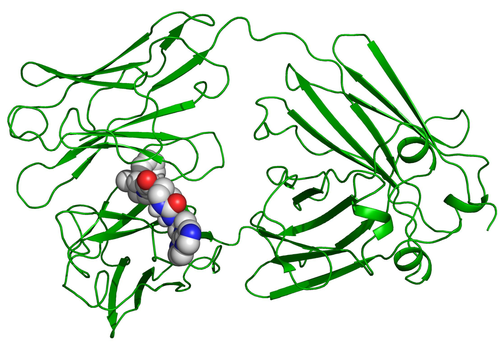
Improved analog:

PTG-61
MW: 527
DSF ΔT_m: 0.3-3.4 °C
FP EC_{50}: 436 nM
SPR K_d: 124 nM
Rat S9 stablity $t_{1/2}$: >30 min

✓ ***Excellent Potency*** ✓ ***Excellent plasma stabilization***

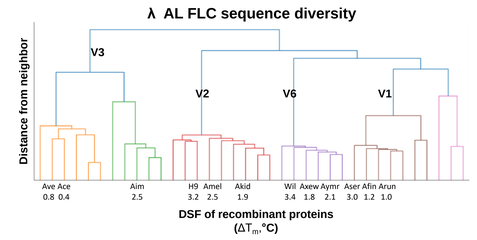
CO-CRYSTAL STRUCTURE

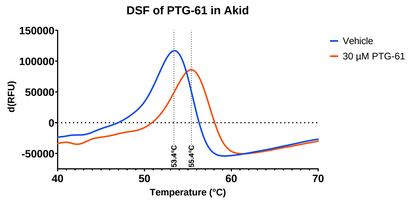
- The human immunoglobulin LC gene comprises three different segments: the variable domain (V), the joining domain (J), and the constant domain (C).
- PTG-61, the most potent kinetic stabilizer of FL LC_2, was crystallized with the amyloidogenic recombinant protein H9 FL LC_2 and resolved at 1.8 Å (PDB: 9AWX).
- PTG-61 binds the core hydrophobic pocket with its (*R*)-enantiomer, occupying the anchor cavity. Notably, PTG-61 is the only known LC stabilizer with this unique linker module–distal substructure binding orientation.



RESULTS UPDATE

- To assess sequence coverage, recombinant amyloidogenic λ FLC protein sequences were selected to represent the diversity of λ AL FLC sequences. These FLC proteins were characterized by pairwise sequence analysis, which closely match the gene family designation assigned to each sequence. The 12 recombinant proteins chosen for further classification are representative of the V1, V2, V3, and V6 families.
- Differential Scanning Fluorimetry (DSF) and limited proteolysis were used to characterize the stability of the recombinant λ FLC proteins with or without λ FLC kinetic stabilizers. PTG-61 successfully stabilized 11 of 12 recombinant λ FLC proteins. ΔT_m values ranged from 0.3°C to 3.4°C, with 10 of 12 proteins achieving $\Delta T_m \geq 1$°C.





CONCLUSIONS

- We have successfully developed novel FL LC_2 kinetic stabilizers as promising candidates for further advancement toward a clinical treatment of the proteinopathy component of AL.

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