

Project title: Dissecting the pathogenic role of N-glycosylation in AL amyloidosis: molecular bases, diagnosis, and treatment.

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Background: Light chain (AL) amyloidosis is caused by a typically small, minimally proliferating B cell/plasma-cell clone secreting a patient-unique, unstable, aggregation-prone, toxic light chain (LC). The amyloidogenicity of LCs is encrypted in their sequence, yet molecular determinants of LC pathogenicity remain obscure.

Recent data suggest that N-glycosylation of a monoclonal LC is an independent risk factor for progression to AL amyloidosis. However, clinical and biological correlates of LC N-glycosylation have not been thoroughly assessed and molecular determinants of LC pathogenicity remain obscure.

Our lab has recently established and validated a high throughput, NGS-based technology termed "single-molecule real-time sequencing of the M protein" (SMaRT M-Seq) to identify the entire variable sequence of clonal LCs from a high number of biological samples in parallel¹. By combining bioinformatics with biochemical, proteomics, structural, and genetic analyses, we found an N-glycosylation hot spot associated with κ LCs from AL patients, setting them apart with respect to LCs from other clonal plasma cell disorders².

Besides refining current N-glycosylation-based prognostic assessments for patients with monoclonal gammopathies, our data further support a potential role of N-glycosylation in determining the pathogenic behavior of a subset of amyloidogenic LCs and warrant future investigations.

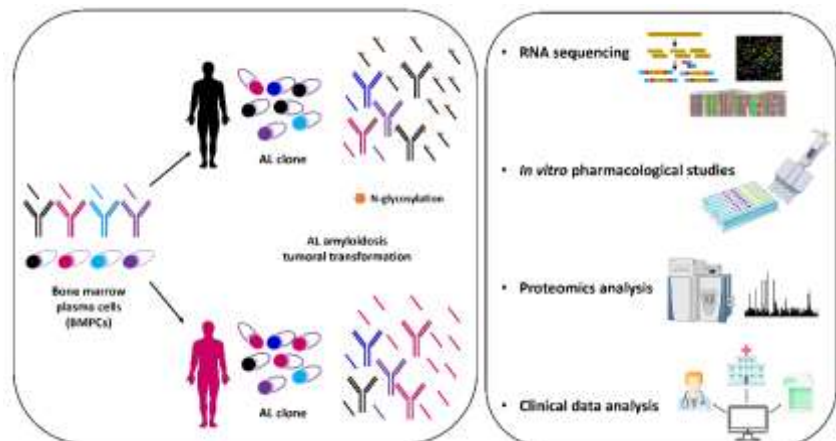
Scientific hypothesis and aims:

We hypothesize that N-glycosylation fundamentally contributes to determining the amyloidogenicity of immunoglobulin LCs in a subset of pts with AL amyloidosis and might influence its clinical phenotype. We also postulate that the synthesis and secretion of unstable LCs that also have to be N-glycosylated might also reverberate on the biology of the plasma cell clone and possibly modulate the sensitivity toward different drugs.

By exploiting a large and well-characterized cohort of samples/patients with AL amyloidosis and other plasma cell disorders, well-established analytical platforms based on cutting-edge technologies and a network of scientific collaborations with research groups with synergistic and complementary expertise, we plan to:

1) Deepen our understanding of the clinical heterogeneity of AL and contribute to refining currently available sequence-based prediction algorithms to identify patients at risk of AL development;

2) Dissect the molecular mechanisms underlying the increased pathogenicity of N-glycosylated LCs;



3) Improve our knowledge on the biology of the amyloidogenic plasma cell clones and explore the potential contribution of LC N-glycosylation towards sensitivity to established and novel pharmacologic interventions.

Techniques: Single molecule real-time DNA sequencing, RNA sequencing, flow cytometry, pharmacologic studies with plasma cell lines and primary plasma cells, biochemical and proteomics analyses.

Bibliography

1. Cascino et al. Single-Molecule Real-Time Sequencing of the M protein: toward personalized medicine in monoclonal gammopathies. *Under revision*
2. Nevone et al. An N-glycosylation hotspot in immunoglobulin κ light chains is associated with AL amyloidosis. *Leukemia* 2022 *In press*