

SPECT IMAGING OF AL AMYLOID IN MICE USING AN ANTIBODY TO A
FIBRIL-SPECIFIC, CRYPTIC EPITOPE

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Amyloidosis is a protein misfolding disorder, associated with a diverse group of often fatal hereditary and sporadic diseases characterized by the deposition of protein fibrils in vital organs and tissues. The incidence of peripheral amyloidosis in the USA, is estimated to be ~ 5300 per yr. Treatment options for peripheral amyloidosis currently are limited and focus on reducing synthesis of the amyloidogenic protein e.g., with high dose chemotherapy but there are currently 31 new trials for treating light chain (AL), reactive (AA), and senile cardiac (ATTR) amyloidosis underway. Unfortunately, there are no methods available in the USA to monitor changes in amyloid burden during disease progression or response to therapy either clinically or during clinical trials.

In a routine screen of monoclonal antibodies (mAbs) that bind AA amyloid deposits we identified a reagent, designated 7D8 that exhibited cross-reactivity with AL amyloid, composed of immunoglobulin light chains. The mAb 7D8 bound relatively specifically to V_λ6 light chain fibrils with an apparent midpoint concentration (EC₅₀) of ~ 0.1 μM; however the interaction with monomeric V_λ6 was weaker (EC₅₀ > 0.5 μM). Furthermore, the presence of 160-fold excess of monomeric V_λ6 did not inhibit the binding of mAb 7D8 to fibrils. Immunostaining of AL amyloid in tissue samples was also achieved using mAb 7D8 at 3 μg/mL. These data support the cross-reactivity of this mAb to an as yet unidentified cryptic epitope present on AL amyloid fibrils but not the monomeric precursor proteins.

To evaluate amyloid-specific binding of this reagent *in vivo* we injected ~200 μCi of ¹²⁵I-labeled mAb 7D8 (~ 20 μg) into mice bearing sub cutaneous human ALκ or ALλ amyloidomas. After 48 h the distribution of the ¹²⁵I-7D8 was visualized by microSPECT imaging. The mean % injected dose per gram in the amyloid mass was 9.9% and 17.4% for ALλ and κ amyloid, respectively, which was significantly greater than the 1.4% and 2.4% injected dose observed using an isotype-matched control mAb. The amyloid-to-liver ratio was 2.5:1 and 3.6:1 rendering the amyloid masses readily visible above background in the microSPECT images.

These data indicate that mAb 7D8 selectively binds amyloid fibrils, even in the presence of significant concentrations of monomeric precursor protein and that when radiolabeled this reagent can be used to image AL amyloid deposits *in vivo*.