Here, we present evidence that GAIM-Ig fusions: (1) specifically bind recombinant and patient-derived TTR and LC amyloid fibers; (2) co-localize with amyloid deposits in 2 mouse models for light chain amyloidosis; and (3) mediate disruption of amyloidoma tissue consisting of human AL patient-derived LC fibers. These data support the potential of GAIM fusions for treating peripheral amyloidosis.

**METHODS**

GAIM fusion production. All GAIM-IgG1-Fc fusions were expressed by HEK cell transient transfection, followed by protein A and ion exchange chromatography (2). GAIM fusions included NPT088 (wild type), NPT189 (de-immunized), NPT288 (GAIM tetramer), and NPT289 (GAIM tetramer). Synthetic amyloid fiber binding. TTR fibers created by aggregation of rTTR followed by application to dot blot. 200 μL of 0.2 mg/mL TTR solution (100 mM NaAc, 100 mM KCl, 1 mM EDTA, pH 4.3) incubated with or without agitation (350 rpm) 3-5 d. at 37°C. Serial dilutions of TTR tetramers or aggregates were spotted onto nitrocellulose membrane. After washing and blocking, NPT088 (100 mM) was allowed to bind for 1 hr (rt). Goat anti-hu Ab (1:10,000 in TBS-T) used for detection by ECL. AL Patient fiber ex vivo binding. Patient ex vivo AL fibrils and rLC were spotted onto nitrocellulose membrane filters as serial dilutions (10, 5, 2.5, 1.3, 0.6, 0.3 μg/spot). Controls included recombinant AL monomers and dimers. Binding determined after GAIM fusion incubation and detection with anti-GAIM rabbit Ab and donkey anti-rabbit IgG-HRP.

**RESULTS**

**1. GAIM Fusions Recognize TTR Amyloid Variants**

- ELISA: GAIM fusions bind wild-type TTR with low nanomolar affinity (~15 nM). Binding strongly depends on the TTR aggregation conditions (pH).

**2. GAIM Fusions Recognize Patient AL Fibers**

- Immunoblot: GAIM binds fiber aggregates of TTR variants.
  - V3OM is the most prevalent worldwide.
  - T60A most common US variant.
  - V122I most common UK variant.
  - Non-fibrillar tetramer and urea denatured monomer show no binding.

**3. GAIM Fusions Broadly Recognize AL Fibers**

**4. GAIM Fusions Target AL Amyloidomas in vivo**

- Systemic dosing with GAIM fusion NPT288 for 2 weeks results in AL Light chain immunoblot.

**5. NPT189 Co-localizes to AL Deposits in 1.6 Tg Mice**

- NPT189 was radioiodinated with 125Iodine, purified and administered to SCID mouse amyloidoma model. Radioisotope studies, SCID mice bearing amyloidomas received IV 6 μg 125I-NPT189 or 125I-NPT288. SPECT imaging performed 48h after injection.

**6. GAIM-Ig Fusions Co-localize to 1.6 AL Deposits in Patient Kidney Biopsies**

**CONCLUSIONS**

- GAIM fusions specifically recognizes LC and TTR amyloid fibers
- Recombinant LC and TTR
- Extracted AL patient LC
- Transgenic mouse stomach and kidney
- AL patient kidney tissue
- 125I-NPT189 IV distributes preferentially to AL amyloidoma tissue
- NPT189 represents a candidate therapeutic for treating peripheral amyloidosis

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