PC009

In Vivo Target Engagement and Phagocytosis of Aggregated TTR by a Conformation-Specific TTR Antibody

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SUMMARY

Transthyretin amyloidosis (ATTR) is a rare, progressive, and often fatal disease characterized by the deposition of misfolded transthyretin (mis-TTR) protein primarily in the heart and peripheral nerves, which causes significant morbidity and mortality.[1,2] No therapies have been approved to clear mis-TTR deposits from organs and tissue; hence, novel therapies are needed.[3] We have previously described conformation-specific monoclonal antibodies (mAbs) against mis-TTR that bind to an amyloidogenic epitope uniquely exposed on nonnative conformations of TTR but that are buried and inaccessible in the native TTR tetramer. In vitro, mis-TTR mAbs have the ability to inhibit TTR fibril formation and to induce phagocytic uptake of nonnative forms of TTR. Mis-TTR mAbs also specifically recognize TTR deposits in disease-confirmed heart, nerve, and gastrointestinal tract tissue. Collectively, these findings suggest that mis-TTR mAbs may prove useful in preventing the deposition and/or enhancing the clearance of TTR amyloid in patients with ATTR amyloidosis, regardless of the specific organ or organs involved, while sparing the function of the normal tetrameric form of the protein

The potential mode of action of mis-TTR mAbs to clear TTR deposits in affected organs of patients with ATTR amyloidosis is to specifically bind pathologically relevant mis-TTR for subsequent clearance of amyloid deposits through the recruitment of phagocytes that ingest and clear the mis-TTR deposits. Supporting in vitro evidence for this mechanism of antibody-dependent cellular phagocytosis (ADCP) of mis-TTR Abs has been described with the use of an ADCP assay developed by Prothena.[4] In this assay, aggregated mis-TTR was conjugated to the pH-sensitive fluorescent dye pHrodo. This dye is nonfluorescent in the extracellular environment but turns bright red when phagocytized and localized to low pH endocytic compartments, allowing ingested mis-TTR to be tracked into peripheral phagocytes by flow cytometry

Here we report on in vivo models of target engagement and ADCP by the systemically administered, humanized mis-TTR Ab PRX004 and its murine form, mPRX004. We first show that systemically administered PRX004 binds to mis-TTR aggregates implanted subcutaneously into mice as a demonstration of in vivo target engagement. We also describe an in vivo model of ADCP that demonstrates specific and rapid uptake of pHrodo-tagged aggregated mis-TTR by phagocytic cells in the peritoneal cavity of mice dosed with mPRX004. Collectively, our results demonstrate that in vivo, systemically administered PRX004 can target and predispose mis-TTR aggregates for clearance through an ADCP mechanism

BACKGROUND

- mis-TTR Abs are mAbs that specifically target an epitope—amino acid residues 89-97 (EHAEVVFTA) of TTR—uniquely exposed on monomeric, misfolded, and aggregated forms of TTR but hidden in the native tetramer conformation[4]
- Previous in vitro studies with mis-TTR mAbs showed the ability of these mAbs to
  - Inhibit mis-TTR fibril formation in vitro
  - Stimulate Ab-mediated phagocytic uptake of mis-TTR by human monocytes in a dose-dependent fashion
  - Bind to TTR amyloid deposits in cardiac, peripheral nerve, and gastrointestinal tract tissue derived from patients with ATTR amyloidosis
- These properties suggest that mis-TTR mAbs might have therapeutic potential not only to prevent TTR amyloid deposition but also to enhance the clearance of TTR amyloid through Ab-mediated phagocytic mechanisms.

OBJECTIVE

- To demonstrate the ability of a systemically administered mis-TTR Ab, PRX004, to target aggregated TTR in vivo and to induce clearance of these aggregates through an ADCP mechanism

METHODS

Generation of mis-TTR mAbs

- Conformation-specific TTR Abs targeting amino acid residues 89-97 uniquely exposed on misfolded forms of TTR were generated and characterized as previously described[4]

Preparation and pHrodo-Labeling of Aggregated TTR-V30M

- Recombinant His-tagged TTR-V30M was prepared as described previously.[4] Purified TTR-V30M protein was aggregated in low pH conditions (50 mM sodium phosphate, titrated to pH 2.7 with citric acid) at 37°C, 500 rpm, for 2 weeks, then pH neutralized with NaOH before use. Aggregated TTR-V30M protein was labeled with the pH-sensitive dye pHrodo Red SE (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's standard protocols and was resuspended in 1× phosphate-buffered saline (PBS)

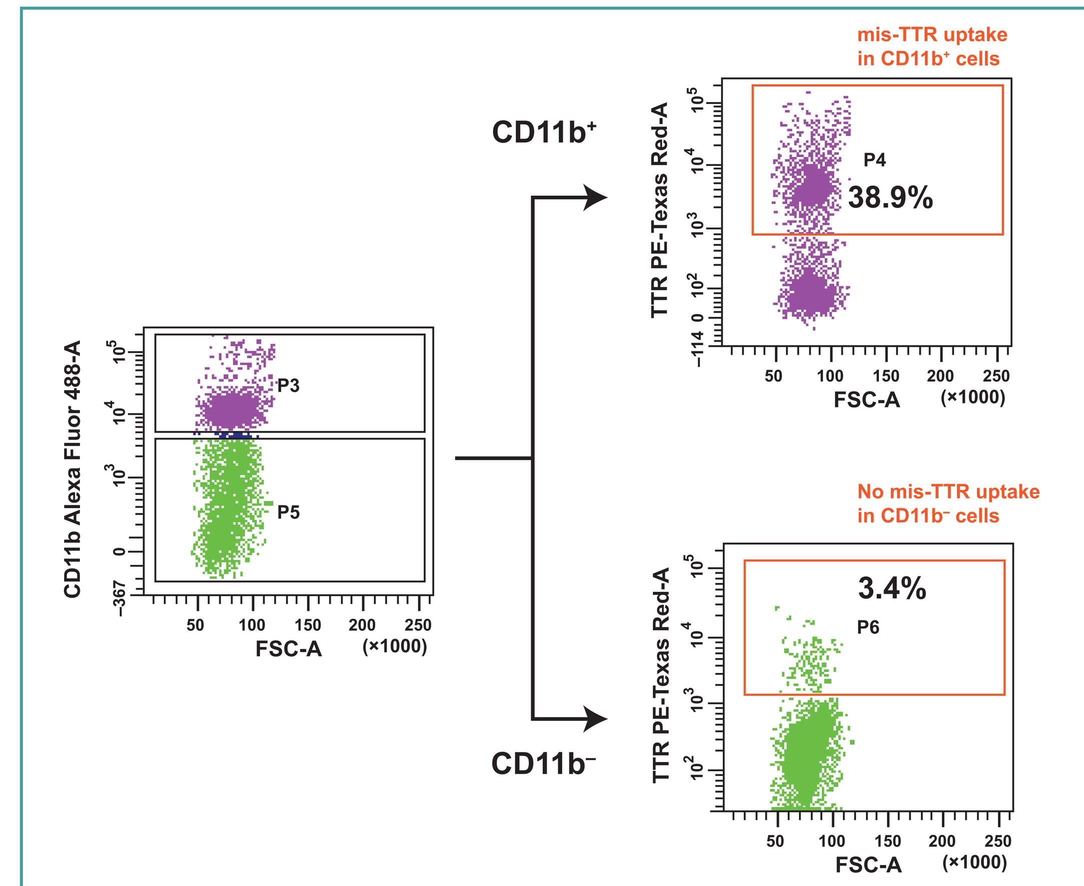
In Vivo Target Engagement Method

- His-tagged aggregated TTR-V30M was suspended 1:1 in basement membrane matrix (Matrigel; Corning, Corning, NY) to a final concentration of 2 mg/mL. Nylon pouches (1.1 cm^2, 18-mm pore) were cut from a nylon mesh membrane filter (Tisch Scientific, North Bend, OH; catalog no. ME17260) and filled with either 100 µL aggregated TTR-V30M/Matrigel mix or 1:1 PBS/Matrigel. The filled pouches were sealed by cauterizing the edges and were implanted subcutaneously and adjacently in the lumbar area of mice 1 week before intraperitoneal administration of test or control Ab
- 1 week after implantation, PRX004 or an h-immunoglobulin G (IgG) isotype control (h-8A9) was injected intraperitoneally (10 mg/kg)
- Implanted pouches were excised 24 hours after dosing and were stained with fluorescence-labeled secondary anti-human IgG (to identify PRX004 and h-8A9) and anti-His Ab (to identify aggregated TTR)

In Vivo Phagocytosis Method

- To assess whether mPRX004 induced Ab-dependent phagocytosis of mis-TTR in vivo, wild-type mice were injected intravenously with mPRX004 or mouse isotype control (EG27/1) (0.1-30 mg/kg); 24 hours later, aggregated mis-TTR-V30M conjugated to the pH-sensitive fluorogenic dye pHrodo, which exhibits bright red fluorescence following phagocytosis, was injected intraperitoneally (5 mg/kg)
- Peritoneal cells were then lavaged and harvested within 3 hours and stained with a CD11b phagocyte-specific Ab
- Phagocytic uptake of aggregated mis-TTR was evaluated by flow cytometry (**Figure 1**)
  - Representative flow cytometry plots demonstrated gating of cells for CD11b$^+$ (P3) and CD11b$^-$ (P5) cells, then showed the mis-TTR uptake (P4 and P6) within the CD11b$^+$ and CD11b$^-$ populations, respectively (**Figure 1**)
  - Most mis-TTR was taken up by CD11b$^+$ cells (phagocytes) (**Figure 1**)

Figure 1. Fluorescence-activated cell sorter gating strategy measured the uptake of aggregated pHrodo-TTR-V30M by phagocytic (CD11b$^+$) cells (representative animal).



mis-TTR, misfolded transthyretin; TTR, transthyretin.

RESULTS

- Immunofluorescence staining was used on implanted Matrigel containing His-tagged aggregated-TTR-V30M (**Figure 2**, top rows) or Matrigel alone (**Figure 2**, bottom rows) in mice treated with 10 mg/kg PRX004 or with isotype control h-8A9
- Aggregated-TTR-V30M containing Matrigel pouches from mice dosed with PRX004 showed colocalization of the PRX004 (green) with aggregated-TTR (red), indicating that systemically administered PRX004 can target TTR deposits in vitro (**Figure 2**)
- Animals dosed with isotype control h-8A9 or implanted with Matrigel-only pouches did not show colocalization (negative controls) (**Figure 2**)

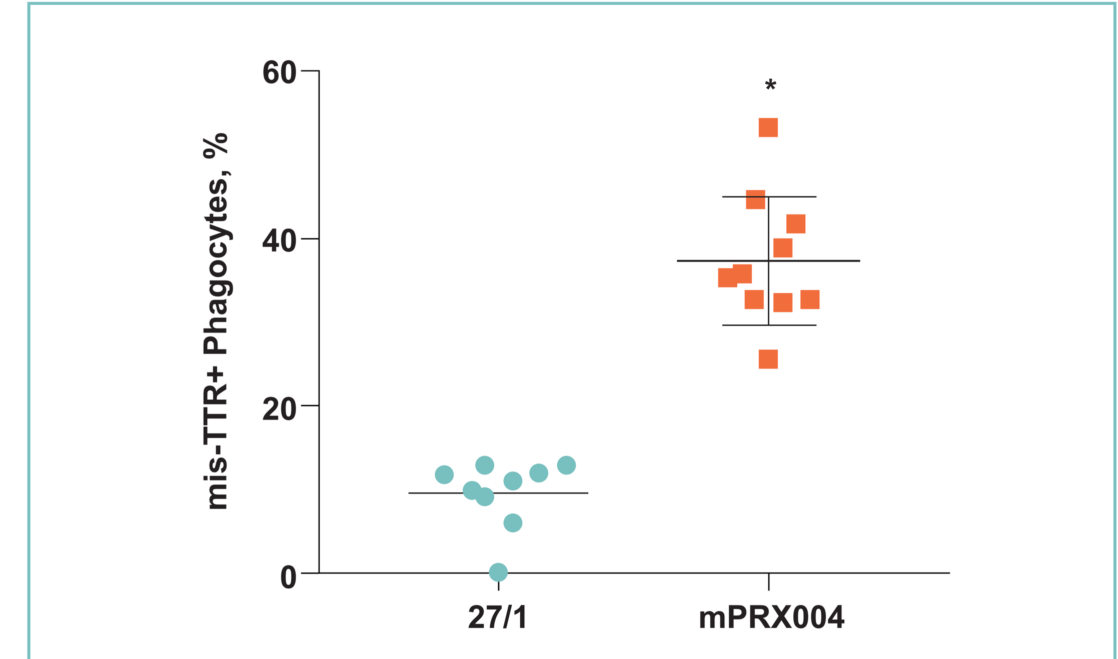
Figure 2. Systemically administered PRX004 targets Matrigel-embedded aggregated TTR-V30M in mice.

Agg-TR in Matrigel	Anti-human IgG	Anti-His	Merge
PRX004			
h-8A9			
Matrigel Only	**Anti-human IgG**	**Anti-His**	**Merge**
PRX004			
h-8A9			

Agg, aggregated; h-8A9, h-immunoglobulin G isotype control; IgG, immunoglobulin G; TTR, transthyretin.
Aggregated mis-TTR was detected using an anti-His Ab (red), and PRX004 was detected using an anti-human IgG (green).

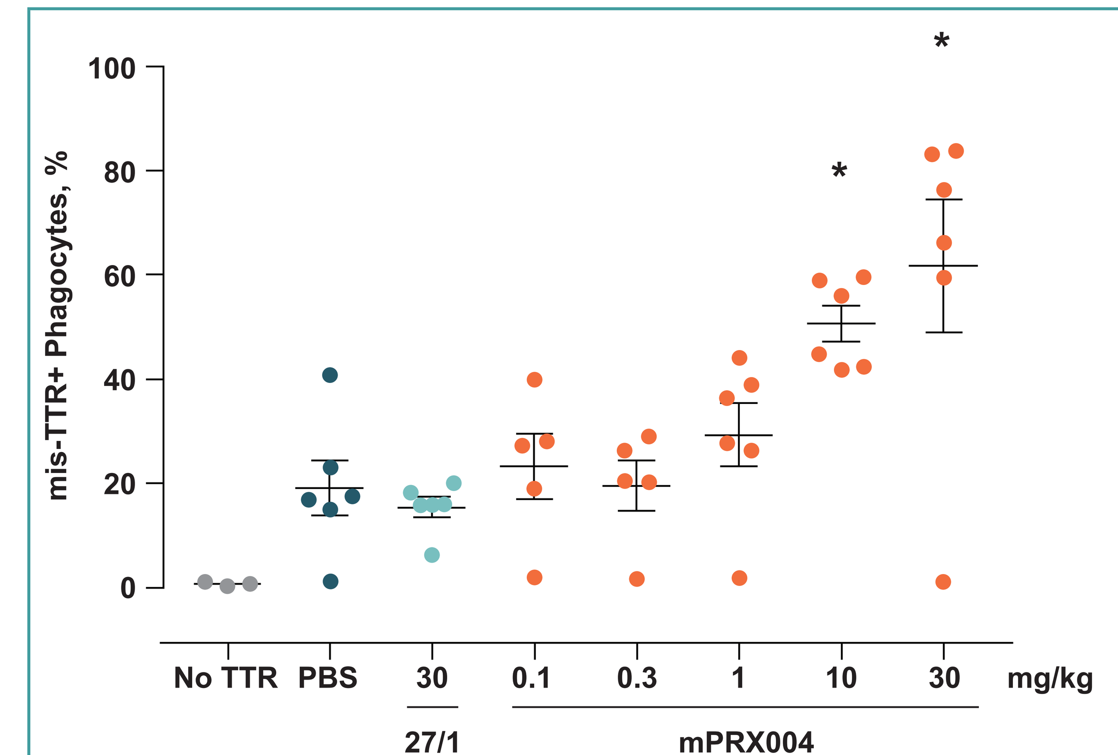
- Mice treated with a single 10-mg/kg dose of mPRX004 showed significant increases ($P < 0.0001$) in the percentage of phagocytes that ingested pHrodo mis-TTR aggregates compared with the isotype control (**Figures 3** and **4**)
- These in vivo results support the concept that mPRX004 administered systemically opsonizes mis-TTR aggregates in vivo and promotes the engagement of phagocytes, resulting in clearance of the target amyloid by ADCP

Figure 3. Systemically administered mPRX004 induces ADCP of aggregated TTR-V30M in mice.



27/1 = isotype control monoclonal antibody; ADCP, antibody-dependent cellular phagocytosis; mis-TTR, misfolded transthyretin; TTR, transthyretin.
Individual data are shown as 1 point for each mouse, representing the percentage of CD11b$^+$ phagocytic cells containing mis-TTR. Bars represent the group mean ± SD (Mann-Whitney test). Measurements were taken 3 hours after injection with mis-TTR aggregates.
*$P < 0.0001$.

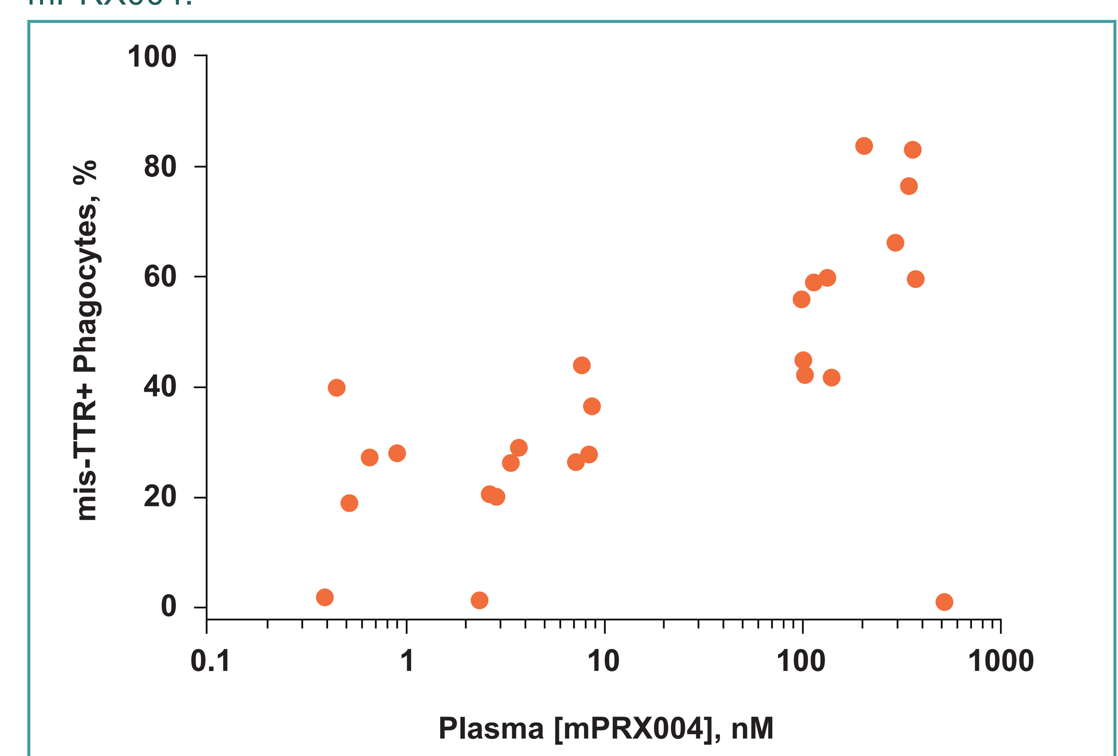
Figure 4. mPRX004-induced ADCP of aggregated TTR-V30M is dose dependent.



27/1, isotype control mAb; ADCP, antibody-dependent cellular phagocytosis; PBS, phosphate-buffered saline (vehicle control); mis-TTR, misfolded transthyretin; No TTR, untreated control; SD, standard deviation; TTR, transthyretin.
Individual data are shown as 1 point for each mouse, representing the percentage of CD11b$^+$ phagocytic cells containing mis-TTR in peritoneal lavage. Bars represent the group median and interquartile range.
*$P < 0.05$ vs isotype control (nonparametric Kruskal-Wallis test with Dunn's multiplicity adjustment).

- All animals dosed with mPRX004 had measurable levels of Ab in terminal plasma 25 to 26 hours after dose (time of lavage). Plasma concentrations increased in a roughly dose-proportional manner
- At time of lavage, plasma mPRX004 concentrations in the range of 10 to 30 µg/mL (67-200 nM) appeared to correspond with minimally effective exposures with respect to increased phagocytic uptake of mis-TTR (**Figure 5**)

Figure 5. Plasma antibody concentrations in animals dosed with mPRX004.



mis-TTR, misfolded transthyretin.
Each data point represents an individual animal treated with 0.1-30 mg/kg mPRX004.

CONCLUSIONS

- The mis-TTR Ab PRX004 specifically binds to nonnative, aggregated TTR protein when administered peripherally
- Peripherally administered mPRX004 opsonizes nonnative, aggregated TTR protein in vivo and induces its phagocytic uptake through an ADCP mechanism
- Collectively, these results provide in vivo evidence for a possible therapeutic mechanism of clearance of TTR deposits in affected tissue in patients with ATTR amyloidosis

ACKNOWLEDGMENTS

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Disclosures of Interest

JNH, CT, KF, ST, JL, JS, RB, TN, and WZ are employees of Prothena Biosciences Inc. This study was sponsored by Prothena Biosciences Inc.

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