PB052

Detection of Misfolded Forms of TTR in Plasma From Patients With Hereditary ATTR Amyloidosis Using Conformation-Specific TTR Antibodies

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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 can inhibit TTR fibril formation and can induce phagocytic uptake of nonnative forms of TTR. Mis-TTR mAbs also specifically recognize TTR deposits in hearts with confirmed disease and in 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.

In addition to their therapeutic potential, mis-TTR mAbs might be used to detect misfolded forms of TTR in ATTR patient blood. This could have applications not only in the diagnosis of ATTR but also in measuring the pharmacodynamic effects of potential therapies targeting misfolded forms of TTR.

The objectives of this study were 2-fold: first, to develop methods using mis-TTR mAbs to detect and measure levels of mis-TTR in plasma derived from patients with ATTR and to correlate these levels with disease; second, to assess target engagement of unbound (free) mis-TTR in ATTR plasma resulting from ex vivo treatment with a conformation-specific mis-TTR mAb (mPRX004).

BACKGROUND

- Misfolded TTR (mis-TTR) antibodies 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 antibody-dependent cellular phagocytic (ADCP) uptake of misTTR 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 ADCP mechanisms. Furthermore, mis-TTR antibodies might be useful in the diagnosis of ATTR amyloidosis and in measuring the pharmacodynamic effects of therapies targeting misfolded forms of TTR

OBJECTIVES

- To compare plasma levels of misfolded TTR in patients with ATTR amyloidosis with levels in normal controls using a mis-TTR–specific bioassay
- To measure pharmacodynamic changes (target engagement) induced by a mis-TTR therapeutic antibody introduced ex vivo to plasma derived from patients with ATTR amyloidosis

METHODS

Generation of mis-TTR mAbs

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

Collection of ATTR and Normal Plasma

- Blood from patients with a positive diagnosis of hereditary ATTR (h-ATTR) or wild-type ATTR (ATTR-WT) amyloidosis was collected in 7-mL Vacutainer (Becton Dickinson, Franklin Lakes, NJ) K2-EDTA tubes and centrifuged to remove cellular debris (**Figure 1**). The plasma fractions were aliquoted into 1.5-mL Eppendorf LoBind (Sigma-Aldrich, St. Louis, MO) tubes and immediately frozen
- Plasma samples from normal controls were collected in the same fashion (**Figure 1**)
- All samples were used immediately after thawing and were not refrozen

Figure 1. Plasma samples for this study were collected from patients with ATTR amyloidosis (left) and normal controls (right).

ATTR

Sample	ID	Age	Sex	TTR Mutation	Clinical Status
1	8120326	37	M	V30M	LT
2	16091078	67	F	V30M	—
4	3098983	44	M	V30M	LT
5	98084122	44	F	Y114C	—
6	1051830	45	F	V30M	LT
7	12004496	44	M	V30M	LT
8	16032804	74	M	V30M	—
10	13097486	39	M	V30M	—
11	11017266	30	F	V30M	LT
12	14094510	72	M	V30M	—
13	14053483	66	M	V30M	—
14	96038112	53	F	V30M	LT
13	11019596	48	M	V30M	—
16	8099635	44	F	V30M	LT
17	8110529	41	F	V30M	—
18	16015447	52	M	G47R	LT
19	95023663	54	F	V30M	LT
20	4080271	38	M	Y114C	—
21	16012192	34	M	S501	LT
22	84041739	54	F	V30M	LT
23	98063581	38	F	S501	AS
24	16123223	24	M	V30M	—
25	15085485	35	F	V30M	—
26	5069417	37	F	V30M	LT
27	8095365	48	F	V30M	LT
28	8099602	70	F	V30M	—
29	16050297	27	F	G47Arg	AS
30	16013388	68	F	V30M	—
31	94045408	63	F	V30M	LT
32	9105775	48	M	V30M	LT
33	8127510	42	F	V30M	AS
34	16129566	62	M	E61L	—
35	9007376	34	F	V30M	AS
36	98029314	41	F	V30M	AS
37	15030924	26	M	V30M	AS
38	15030913	27	F	V30M	AS
39	17032391	39	M	T49S	—
40	12120567	64	M	V30M	—
42	13063964	41	F	Y114C	—
44	17057097	62	M	V30M	—
45	17090197	72	M	V30M	—
46	14044203	27	F	Y114C	AS
47	15048606	38	M	F33V	—
1WT-Kum	15090038	81	M	WT TTR	—
2WT-Kum	85017908	84	F	WT TTR	—
3WT-Kum	16023646	78	M	WT TTR	—
4WT-Kum	14014619	82	M	WT TTR	—
5WT-Kum	16119190	86	M	WT TTR	—
6WT-Kum	16097559	84	F	WT TTR	—
1-Mad	NA	NA	M	A65T	AS
2-Mad	NA	NA	M	E89K	AS
3-Mad	NA	NA	F	E89N	—
4-Mad	NA	NA	F	V122I	—
5-Mad	NA	NA	M	V30M	—
6-Mad	NA	NA	M	WT TTR	—
7-Mad	NA	NA	M	WT TTR	—
8-Mad	NA	NA	M	WT TTR	—

Normal Controls

Sample	Age	Sex
1	38	M
2	22	F
3	24	F
4	42	M
5	29	F
6	34	F
7	51	M
8	30	M
9	28	M

Kum plasma samples generously provided by Y. Ando and M. Ueda, Kumamoto University, Kumamoto, Japan.
Mad plasma samples generously provided by P. Garcia-Pavia, Hospital Universitario Puerta de Hierro Majadahonda, Madrid, Spain.
AS, asymptomatic hereditary ATTR; LT, hereditary ATTR liver transplant patient; WT TTR, wild-type TTR.

Analysis of ATTR Plasma by Western Blot

- Plasma samples were diluted 10-fold into lithium dodecyl sulfate sample buffer (Life Technologies, Waltham, MA), loaded (12 µL) on a 10% NuPAGE bis-tris gel, electrophoresed at 90 V for 105 minutes, transferred to a nitrocellulose membrane, and probed with 1.0 µg/mL mis-TTR mAb1 followed by 1:20,000 dilution of IRDye 800 CW-conjugated goat–anti-mouse secondary antibody (LI-COR, Lincoln, NE)
- The blot was then rinsed and imaged on an Odyssey CLx infrared imager (LI-COR)

mis-TTR Bioassay

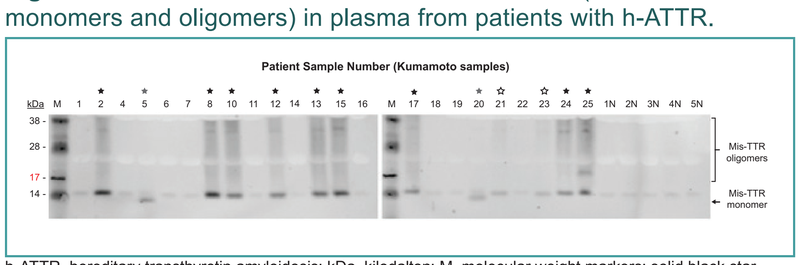
- Total mis-TTR in human plasma was detected using an MSD (Meso Scale Discovery, Gaithersburg, MD) qualitative electrochemiluminescence assay. MSD plates were coated overnight with 4 µg/mL NeutrAvidin (Thermo Fisher Scientific) followed by 1 µg/mL biotinylated mis-TTR mAb1
- Plasma samples were diluted 5-fold in sample diluent (PBS + 0.1% BSA + 0.05% Tween-20) before they were added to the plate. Plates were washed with TBS-T, and then 1 µg/mL anti-TTR polyclonal antibody (Dako, Carpinteria, CA) labeled with MSD Sulfo-tag was added
- Plates were washed with TBS-T and read on a Meso Sector S 600 (Meso Scale Discovery) after the addition of 150 µL/well diluted MSD Read Buffer T. Samples were analyzed using MSD Discovery Workbench 4.0

mis-TTR Pharmacodynamic Assay

- Plasma from patients with ATTR amyloidosis who had elevated levels of mis-TTR were spiked ex vivo with 0, 12, 40, 120, 400, or 1200 µg/mL of an anti–mis-TTR antibody, mPRX004, that competes for binding with mAb1; plasma was then incubated for 30 minutes at room temperature, diluted 1:5, and assayed using the mis-TTR bioassay
- Binding of plasma mis-TTR by mPRX004 (target engagement) prevents mis-TTR from binding to mAb1 coated on the assay plate because mPRX004 and mAb1 compete for the same epitope on mis-TTR. Thus, this assay effectively quantitates only free (unbound) mis-TTR remaining in the plasma after the addition of mPRX004

RESULTS

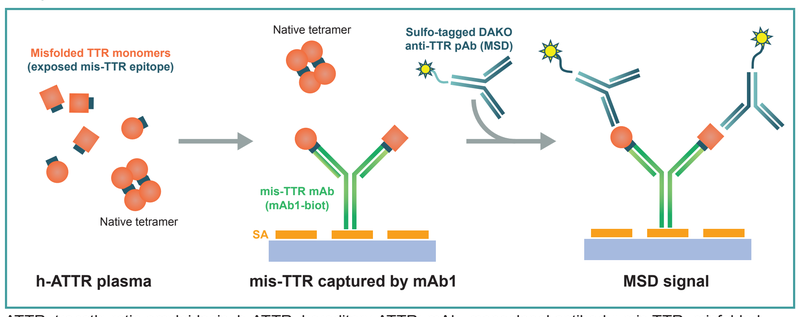
Figure 2. mAb1 detects elevated levels of mis-TTR (misfolded TTR monomers and oligomers) in plasma from patients with h-ATTR.



h-ATTR, hereditary transthyretin amyloidosis; kDa, kilodalton; M, molecular weight markers; solid black star, patients with TTR-V30M who did not undergo liver transplantation; solid gray star, TTR-T114C; open star, TTR-S50I; no star, liver transplantation; 1N-5N, normal.
Primary mAb was 1 µg/mL mAb1.

- The conformation-specific mis-TTR mAb1 detects elevated levels of mis-TTR species (monomers and oligomers) in plasma derived from patients with h-ATTR
- In contrast, plasma from patients with h-ATTR who underwent liver transplantation showed barely detectable levels of mis-TTR, as did plasma from healthy controls

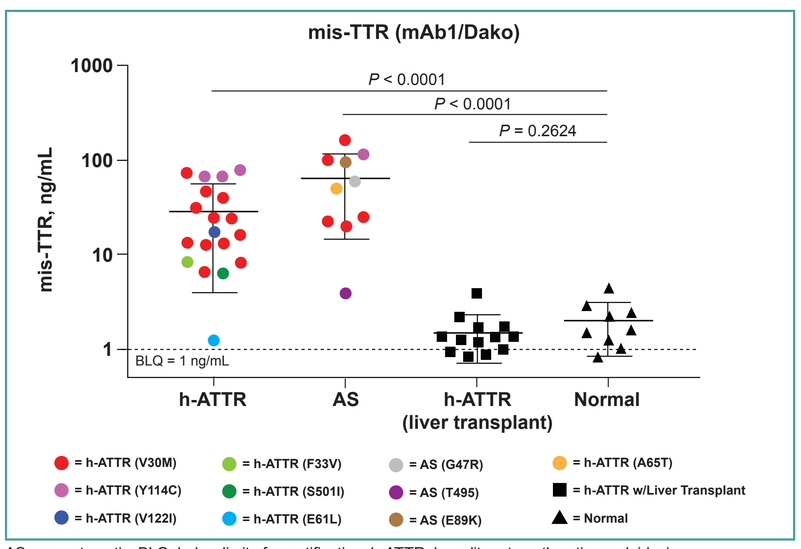
Figure 3. Diagram of an MSD-based bioassay to quantitate levels of free (unbound) mis-TTR in plasma derived from patients with ATTR amyloidosis.



ATTR, transthyretin amyloidosis; h-ATTR, hereditary ATTR; mAb, monoclonal antibody; mis-TTR, misfolded transthyretin; pAb, polyclonal antibody; SA, streptavidin; TTR, transthyretin.

- Diagram of an MSD-based mis-TTR bioassay using biotinylated mis-TTR mAb1 to capture mis-TTR in ATTR plasma samples
- MSD signal intensities were converted to ng/mL equivalent free mis-TTR by extrapolation from a standard curve generated using a guanidine hydrochloride–denatured recombinant wild-type TTR

Figure 4. Elevated levels of mis-TTR in plasma from patients with h-ATTR.



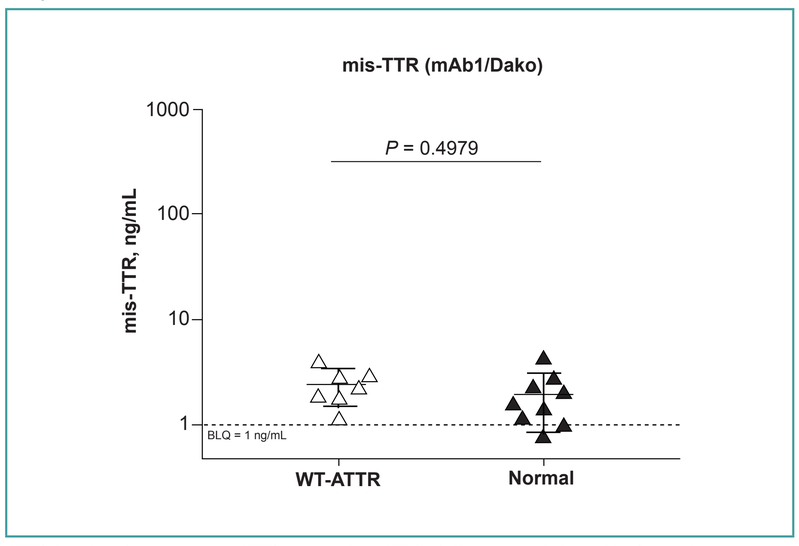
AS, asymptomatic; BLQ, below limit of quantification; h-ATTR, hereditary transthyretin amyloidosis.
Limits of quantification (BLQ) of 1.0 ng/mL. Bars represent group means and SD. P values, Mann-Whitney test.

- mis-TTR levels in plasma from patients with h-ATTR were significantly elevated relative to those from normal controls
- Elevated levels of mis-TTR were also observed in asymptomatic persons with a TTR mutation
- Normal, low levels (<10 ng/mL) of mis-TTR were observed in plasma from patients with h-ATTR who underwent liver transplantation

Disclosures of Interest

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

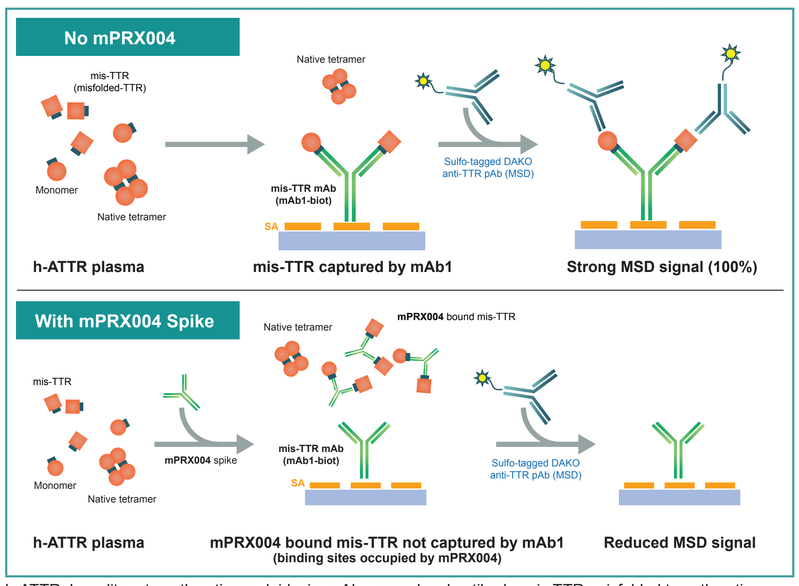
Figure 5. mis-TTR levels in plasma from patients with wild-type ATTR amyloidosis were low.



ATTR, transthyretin amyloidosis; ATTR-WT, wild-type ATTR; BLQ, below limit of quantification; mAb, monoclonal antibody; mis-TTR, misfolded transthyretin.
Limit of quantification was 1.0 ng/mL; bars represent group means and SD; *P* values, Mann-Whitney test.

- Even though mis-TTR mAb1 reacts with the same affinity to variants of both misfolded WT-TTR and hereditary TTR, no differences in mis-TTR levels were seen between WT-ATTR and normal control plasma
- This result suggests that the stability of wild-type TTR does not lead to dissociation and misfolding of TTR in the plasma before deposition in affected tissue

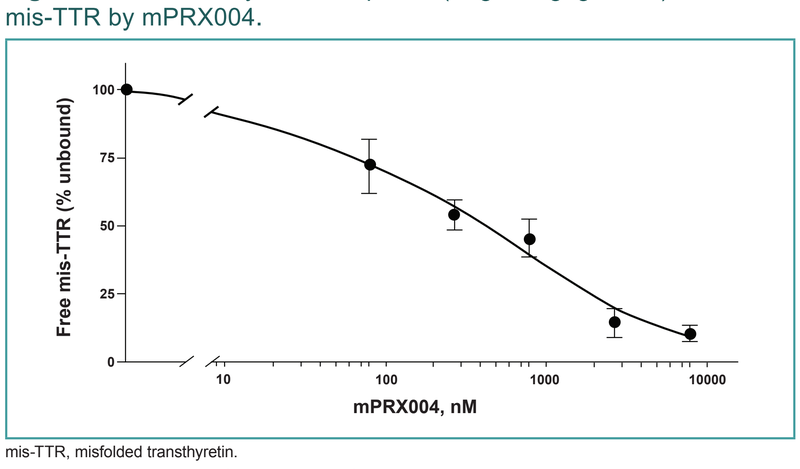
Figure 6. Diagram of a pharmacodynamic assay to measure binding of a mis-TTR mAb (mPRX004) to plasma mis-TTR (target engagement).



h-ATTR, hereditary transthyretin amyloidosis; mAb, monoclonal antibody; mis-TTR, misfolded transthyretin; pAb, polyclonal antibody; SA, streptavidin.

- To measure the ability of a potential mis-TTR therapeutic antibody (mPRX004) to bind to plasma mis-TTR (target engagement), ATTR plasma (V30M) with high levels of mis-TTR were treated ex vivo with increasing amounts of mPRX004
- Binding of mPRX004 to mis-TTR (target) in plasma should decrease the amount of remaining unbound (free) mis-TTR because binding sites become occupied with mPRX004, resulting in a concentration-dependent decrease in the MSD signal and evidence of target engagement by this mis-TTR mAb

Figure 7. Pharmacodynamic response (target engagement) of free mis-TTR by mPRX004.



mis-TTR, misfolded transthyretin.
Mean (point) ± standard error of the mean (bar) of 12 h-ATTR plasma samples (each spiked sample value was normalized to its pre-spike electrochemiluminescence signal at 0 nM mPRX004 in plasma (ie, 100% unbound mis-TTR). Line represents asymmetric sigmoidal nonlinear curve fit of data.

- Treatment of plasma from a patient with h-ATTR (V30M) with increasing amounts of mPRX004 resulted in a concentration-dependent decrease in the free mis-TTR MSD signal as free mis-TTR binding sites became occupied
- The IC_{50} (inhibitory concentration of mPRX004 giving half-maximal response) was determined to be 343 nM (51.4 µg/mL)

CONCLUSIONS

- We have developed a potential diagnostic bioassay using a conformation-specific TTR antibody (mAb1) to detect misfolded TTR species in the plasma of patients with h-ATTR
- Using this assay, we have shown that
  - Elevated levels of mis-TTR in the plasma of patients with h-ATTR were observed in both symptomatic and asymptomatic patients
  - Patients with h-ATTR who underwent liver transplantation had low levels of mis-TTR in their plasma
  - Low levels of mis-TTR were also seen in patients with ATTR-WT
- This bioassay might also be used to measure the pharmacodynamic effects (target engagement) of a mis-TTR mAb (PRX004) when introduced into the plasma of patients with h-ATTR

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