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# PRX012 Induces Microglia-Mediated Clearance of Pyroglutamate-Modified and -Unmodified Aß in Alzheimer's Disease Brain Tissue

Stephen J Tam, Gang Zhang, Lauri Li, Abderrahman Elmaarouf, Joshua Salmans, Michael Skov, Philip Dolan, Gene G Kinney, Brian Campbell, Wagner Zago

Prothena Biosciences Inc, South San Francisco, CA, USA

## INTRODUCTION

- PRX012 is a high-potency, fully humanized, immunoglobulin G1 monoclonal antibody targeting the N-terminus of amyloid-beta (Aβ) with ~70 picomolar (pM) affinity equilibrium dissociation constant ($K_D$) for Aβ fibrils
- PRX012 has previously been shown to promote phagocytic-mediated clearance of $Aβ_{1-42}$ fibrils
- In addition to unmodified $Aβ_{1-42}$, pyroglutamate-modified Aβ ($Aβ_{pE3-42}$) has also been described as a component of senile plaques and vascular amyloid in Alzheimer's disease (AD)
- We hypothesized that N-terminal antibodies, such as PRX012 and aducanumab (Aduhelm, Biogen Inc., Cambridge, MA), are capable of promoting phagocytic-mediated clearance of $Aβ_{pE3-42}$ co-deposited in plaques

## AIM

- To determine whether PRX012 and aducanumab stimulate microglial-mediated clearance of $Aβ_{pE3-42}$ from plaques of AD brains, at concentrations expected to be achieved in the central nervous system (CNS) at therapeutic doses

## METHODS

### Generation of Antibodies

- Prothena antibody PRX012 and aducanumab were expressed, purified by Protein A affinity chromatography, and characterized by capillary electrophoresis and size-exclusion chromatography

### Aβ Binding Assays

- Binding kinetics to $Aβ_{1-42}$ fibrils were performed with the Biacore T200 system (GE Healthcare, Princeton, NJ)
  - Fibrils were immobilized on sensor chip CM5 (GE Healthcare Life Sciences, Princeton, NJ)
  - Antibodies were injected at 30 µL/min in running buffer (HEPES buffered saline + 0.05% P-20, 1 mg/mL bovine serum albumin [BSA]) for 300 seconds association time and 1200 seconds dissociation time
  - The analysis was performed using a global 1:1 fit with Biacore Insight Evaluation software (v2.0)
- Enzyme-linked immunosorbent assay (ELISA) was performed to compare binding of PRX012 to $Aβ_{1-42}$ and $Aβ_{pE3-42}$
  - Peptides were coated at 1 µg/mL and then blocked with 1% BSA in phosphate-buffered saline (BSA/PBS)
  - Antibody diluted in 0.1% BSA/PBS and 0.1% Tween 20 were incubated on the plate, followed by incubation with goat anti-human IgG horseradish peroxidase (Invitrogen, Carlsbad, CA)
  - Plates were developed with o-phenylenediamine dihydrochloride

### Immunohistochemistry of AD Tissue

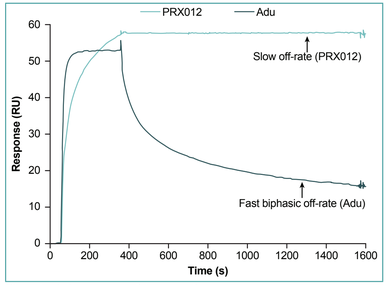
- Cryostat sections were obtained from frozen tissue blocks embedded in optimal cutting temperature compound (Sakura Finetek, Torrance, CA). Human AD tissues were from Banner Sun Health Research Institute (Sun City, AZ)
- *Brightfield Immunohistochemistry (IHC)*: Staining was performed in an automated stainer (Leica Biosystems, Buffalo Grove, IL). Biotinylated human N-terminal anti-Aβ antibody was visualized using the ABC Elite Standard kit (PK-6100; Vector Laboratories, Burlingame, CA) and DAB (Agilent Dako, Santa Clara, CA). The mouse anti-pyroglutamyl antibody (822301; BioLegend, San Diego, CA) was detected using the Bond polymer refine detection kit (Leica Biosystems)
- The slides were digitally imaged (NanoZoomer 2.0-HT, Hamamatsu Corporation, Bridgewater, NJ) and analyzed using HALO software (Indica Labs, Albuquerque, NM) to measure percentage of stained tissue, and results were plotted using Prism software (GraphPad Software, San Diego, CA)
- *Immunofluorescence:* An N-terminal anti-Aβ antibody was conjugated to a Cy3-secondary anti-human antibody (Jackson Laboratories) before application to tissues. $Aβ_{pE3-42}$ was detected using a mouse anti-$Aβ_{pE3-42}$ antibody with a 488-Alexa fluor-conjugated anti-mouse secondary antibody. Slides were imaged using a Metamorph-assisted IX81 Olympus microscope connected to a Hamamatsu camera (C10600-10B)

### *Ex Vivo* Phagocytosis Assays

- Cryostat sections of human AD brain (Braak stage V/VI) were thaw-mounted onto poly-D-lysine coated coverslips, placed in 24-well tissue culture plates, and incubated with test antibodies for 1 hour at 37°C 5% $CO_2$
- Primary mouse microglial cells were then seeded at 800,000 cells/mL, and the cultures were maintained at 37°C 5% $CO_2$ for 72 hours
- Media was carefully aspirated, and sections were washed with PBS
- The sections were resuspended in 8M urea for quantification by ELISA and MSD
- $Aβ_{pE3-42}$ was quantified using an ELISA kit (Immuno-Biological Laboratories, Minneapolis, MN) that specifically detects pE3-42 species, with no detection of full-length Aβ
- $Aβ_{1-40}$ was quantified using an MSD kit (Meso Scale Diagnostics, Rockland, MD)

## RESULTS

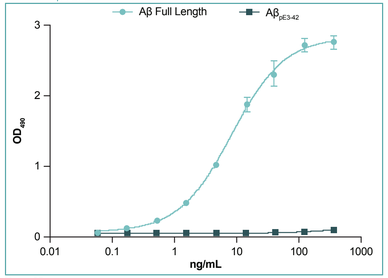
### Figure 1. PRX012 Binds Aβ Fibrils With Greater Avidity Than Aducanumab




Aβ, amyloid-beta; Adu, aducanumab; RU, response units; s, seconds.

- Binding of antibodies to $Aβ_{1-42}$ fibrils was assessed by surface plasmon resonance binding kinetics (**Figure 1**)
- PRX012 has slower dissociation compared to aducanumab, resulting in approximately 10-fold greater avidity

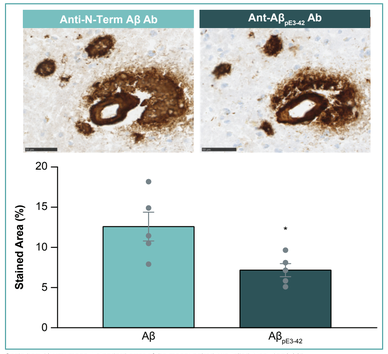
### Figure 2. PRX012 Binds With High Apparent Affinity to the N-Terminus of Full-Length Aβ and Does Not Directly Bind $Aβ_{pE3-42}$




Mean ± SD.
Aβ, amyloid-beta; $Aβ_{pE3-42}$, pyroglutamate-modified Aβ; $OD_{490}$, optical density 490 nanometers; SD, standard deviation.

- Binding of PRX012 to Aβ was assessed by ELISA (**Figure 2**)
  - PRX012 binds with a half-maximal effective concentration of 54 pM (8.1 ng/mL) to fibrillar Aβ species with an unmodified N-terminus
  - PRX012 has no detectable binding to $Aβ_{pE3-42}$

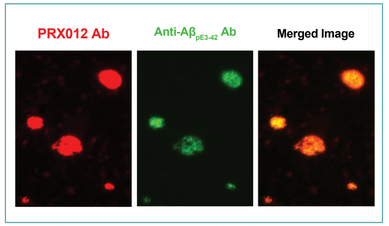
### Figure 3. Pyroglutamate-Modified Aβ Represents a Subset of Total Aβ Distributed in Plaques and Vessels in Human AD Brain Sections




Scale bar: 50 µm; mean ± standard error of the mean; paired two-tailed *t*-test, *p=0.007.
Aβ, amyloid-beta; $Aβ_{pE3-42}$, pyroglutamate-modified Aβ; Ab, antibody; AD, Alzheimer's disease; N-term, N-terminal.

- The distribution of $Aβ_{1-XX}$ (detected with an N-terminal Aβ antibody) and $Aβ_{pE3-42}$ immunostaining was assessed by IHC (**Figure 3**)
  - Anti-$Aβ_{pE3-42}$ antibody was confirmed to not cross-react with $Aβ_{1-42}$ (data not shown)
- Evaluation of $Aβ_{1-XX}$ and $Aβ_{pE3-42}$ confirmed widespread distribution of both species in tissue samples from subjects with AD. The distribution pattern and quantification of the percent area covered by $Aβ_{1-XX}$ compared to $Aβ_{pE3-42}$ was consistent with prior studies, suggesting that $Aβ_{pE3-42}$ represents a relatively smaller pool of modified Aβ intermingled with the unmodified Aβ targeted by N-terminal Aβ antibodies
  - The images show Aβ plaques with modified $Aβ_{pE3-42}$ near and around a blood vessel

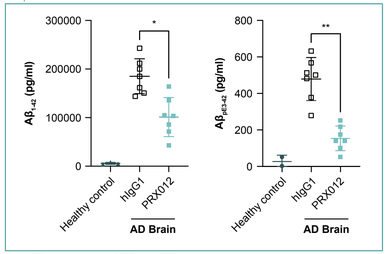
### Figure 4. PRX012 Binding Colocalizes With $Aβ_{pE3-42}$ in an AD Brain




Aβ, amyloid-beta; $Aβ_{pE3-42}$, pyroglutamate-modified Aβ; Ab, antibody; AD, Alzheimer's disease.

- Colocalization of PRX012 immunostaining and $Aβ_{pE3-42}$ was assessed by immunofluorescent microscopy (**Figure 4**)
- PRX012 and anti-$Aβ_{pE3-42}$ immunostaining colocalizes in Aβ plaques, and the overlapping signal appears more prominent in dense core regions

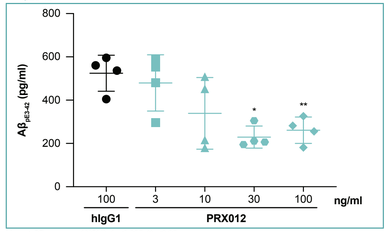
### Figure 5. PRX012 Promotes Clearance of $Aβ_{1-42}$ and $Aβ_{pE3-42}$ From AD Brain Tissue *Ex Vivo*




Mean ± SD; unpaired *t*-test; *p<0.005, **p<0.0001.
Aβ, amyloid-beta; $Aβ_{pE3-42}$, pyroglutamate-modified Aβ; AD, Alzheimer's disease; hIgG1, human immunoglobulin G1.

- $Aβ_{pE3-42}$ ELISA assay is highly specific to $Aβ_{pE3-42}$ when compared to unmodified $Aβ_{1-42}$ (data not shown)
- PRX012 promotes clearance of both $Aβ_{1-42}$ **and** $Aβ_{pE3-42}$ when incubated on AD brain tissue sections with primary mouse microglia for 72 hours (**Figure 5**)
- Results confirm the hypothesis that PRX012 clears both $Aβ_{1-42}$ **and** $Aβ_{pE3-42}$ in the human pathology setting

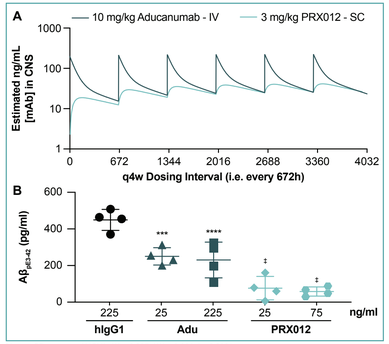
### Figure 6. PRX012 Promotes *Ex Vivo* Clearance of $Aβ_{pE3-42}$ in a Dose-Dependent Manner




Mean ± SD; one-way ANOVA; *p<0.005; **p<0.05.
Aβ, amyloid-beta; $Aβ_{pE3-42}$, pyroglutamate-modified Aβ; AD, Alzheimer's disease; ANOVA, analysis of variance; hIgG1, human immunoglobulin G1.

- PRX012 robustly promotes clearance of $Aβ_{pE3-42}$ from AD brain tissue sections by microglial phagocytosis in a concentration-dependent manner and during a relatively short incubation period (72 hours) (**Figure 6**)

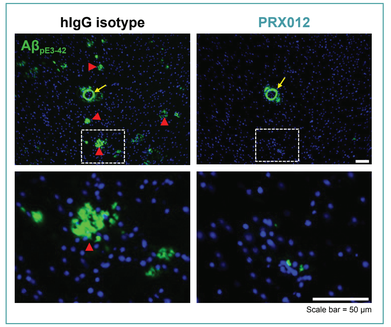
### Figure 7. PRX012 Promotes *Ex Vivo* Clearance of $Aβ_{pE3-42}$ From an AD Brain at a Concentration Range Expected to Be Reached With Subcutaneous Administration and With Greater Biological Activity Than Aducanumab




Mean ± SD; one-way ANOVA; ***p<0.005; ****p<0.001; ‡p<0.0001.
Aβ, amyloid-beta; $Aβ_{pE3-42}$, pyroglutamate-modified Aβ; AD, Alzheimer's disease; Adu, aducanumab; ANOVA, analysis of variance; CNS, central nervous system; hIgG1, human immunoglobulin G1; IV, intravenous; q4w, every 4 weeks; SC, subcutaneous.

- The tested antibody concentrations were based on CNS ranges estimated at 0.1% of steady-state plasma minimum and maximum concentrations from modeled pharmacokinetics following monthly administration of 3 mg/kg subcutaneous PRX012 (25-75 ng/mL) or 10 mg/kg of intravenous aducanumab (25-225 ng/mL) in humans (**Figure 7A**)
- PRX012 exhibited superior $Aβ_{pE3-42}$ clearance when compared to aducanumab, even at 9-fold lower concentrations (**Figure 7B**)

### Figure 8. PRX012 *Ex Vivo* Treatment Reduces $Aβ_{pE3-42}$ Staining in AD Brain




- $Aβ_{pE3-42}$ staining was observed in plaques (red arrowheads) and associated with blood vessels (yellow arrows) (**Figure 8**)
- PRX012 efficiently promotes microglia-mediated clearance of $Aβ_{pE3-42}$

## CONCLUSIONS

- **PRX012 promotes microglia-mediated clearance of $Aβ_{1-42}$ in brain tissue from subjects with AD**
- **Though PRX012 does not target the pyroglutamate modification directly, it effectively clears $Aβ_{pE3-42}$ at concentrations predicted to be clinically relevant and with higher potency and greater biologic activity than aducanumab**
- **Clearance of pyroglutamate species by PRX012 may be due to the ability of microglia to recognize opsonized plaques and engulf large particles with diverse content. PRX012 may therefore clear other neurotoxic elements co-deposited in plaques by this same mechanism**
- **These results support the clinical development of PRX012 in AD, which is expected to begin in 2022**

### AUTHOR DISCLOSURES

All authors are employees of Prothena Inc.

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