Binding Characteristics of Surrogate PRX012 Demonstrate Potent Engagement of Toxic Aβ Protofibrils and Robust Clearance of Pyroglutamate-Modified Aβ

Brian Campbell, PhD[1,*]; Gang Zhang, PhD[1]; Joshua Salmans[1];
Abderrahman Elmaarouf, PhD[1]; Stephen Tam, PhD[1]; Amir Porat, PhD[1];
Michael Skov, DABT[1]; Philip Dolan, PhD[1]; Gene G Kinney, PhD[1]; Wagner Zago, PhD[1]

[1]Prothena Biosciences, Inc., South San Francisco, CA, USA

Disclosures

Compancy Name	Honoraria/ Expenses	Consulting/ Advisory Board	Funded Research	Royalties/ Patent	Stock Options	Ownership/ Equity Position	Employee	Other (Please Specify)
Prothena Biosciences, Inc.					X		X	

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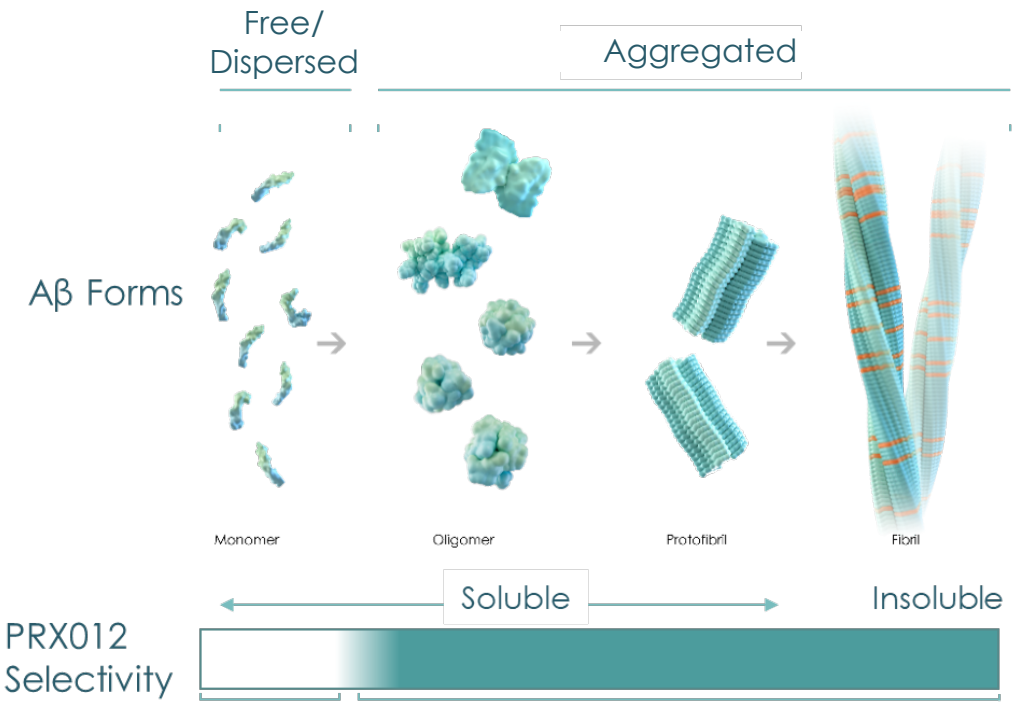
PRX012 Is a High-Affinity N-terminal-Targeted Anti-Aβ Antibody in Clinical Development for the Potential Treatment of Alzheimer's Disease

Objective: To design the best-in-class Aβ-targeted antibody that rapidly and safely depletes Aβ plaques with convenient, infrequent subcutaneous administration in Alzheimer's disease

- Evidence indicates clearance of Aβ plaques is necessary to slow clinical decline in AD
- Approved and investigational late-stage Aβ-targeted antibodies remove plaques in the brains of patients with AD, but target different forms of Aβ aggregates
- Route and frequency of administration of these medications may create barriers for patient access

PRX012 Is Designed to Target and Clear All Toxic Aggregated Forms of Aβ



Aβ, amyloid beta; AD, Alzheimer's disease.

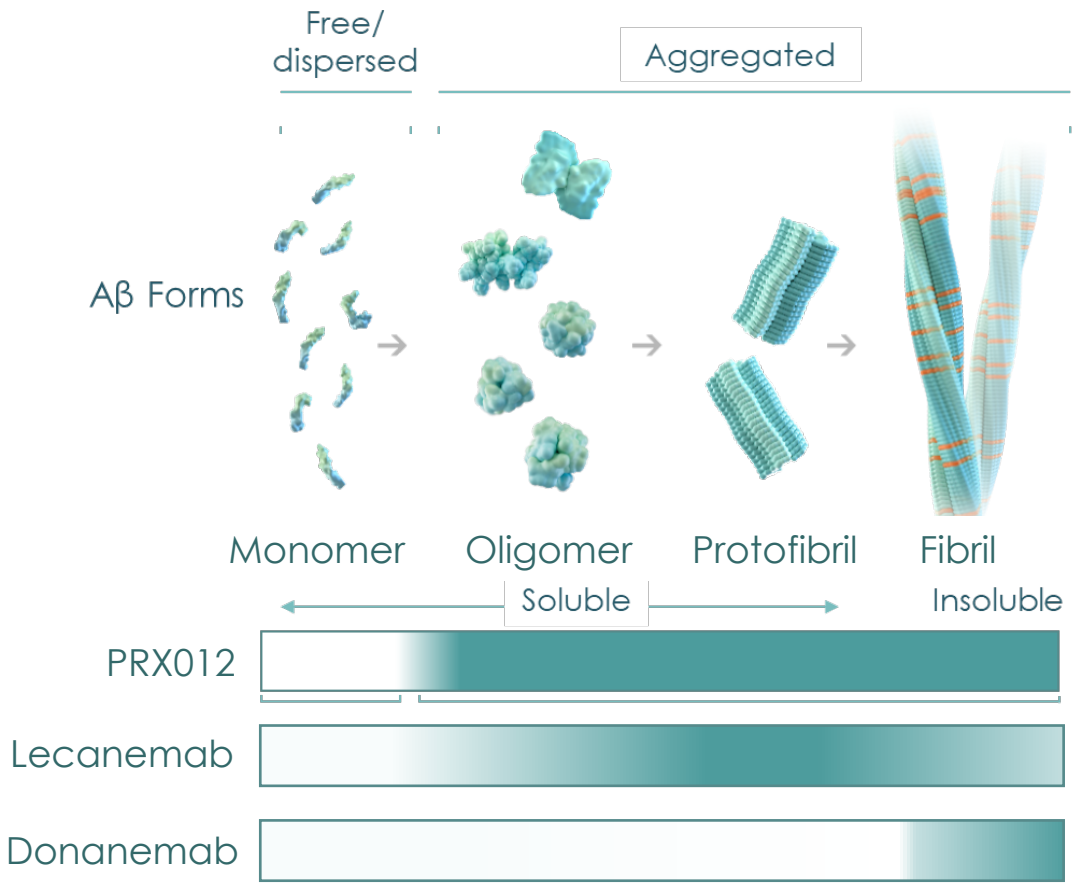
Translating Patient Needs Into Antibody Engineering

Patient-Centric Design Strategy For PRX012

	TARGET PROFILE	ANTIBODY DESIGN ATTRIBUTES	POTENTIAL IMPLICATIONS FOR PATIENTS
Maintain From First Generation Antibodies	**Effectively clear soluble and insoluble aggregated amyloid**	• N-terminal directed	• Associated with efficacy
Innovations to Support Patient Needs	**Low-volume subcutaneous (SC) delivery**	• High binding potency • Stability in high concentrations for single syringe use	• Simplicity for patient and caretaker • More convenient • Increases access • Minimizes treatment burden • Less time-consuming • Infrequent dosing • Potential for at-home use
	Designed for monthly dosing	• Optimize pharmacokinetic profile and immunogenicity • Optimal bioavailability	
	Treatment outside infusion centers	• Optimal biophysical qualities	

How Does the Aβ Binding Profile of PRX012 Compare to Anti-Aβ Antibodies Approved or in Late Development?

PRX012 Is Designed to Target and Clear All Toxic Aggregated Forms of Aβ



PRX012:

- Binds with very high affinity to Aβ fibrils and oligomers[1]
- Potently neutralizes soluble aggregates[2]
- Induces phagocytosis of AD plaques[1]
- Does not bind to pyroglutamate Aβ

- Approved and investigational Aβ antibodies clear plaques and slow cognitive decline through different Aβ engagement mechanisms including binding to protofibrils and pyroglutamate-Aβ

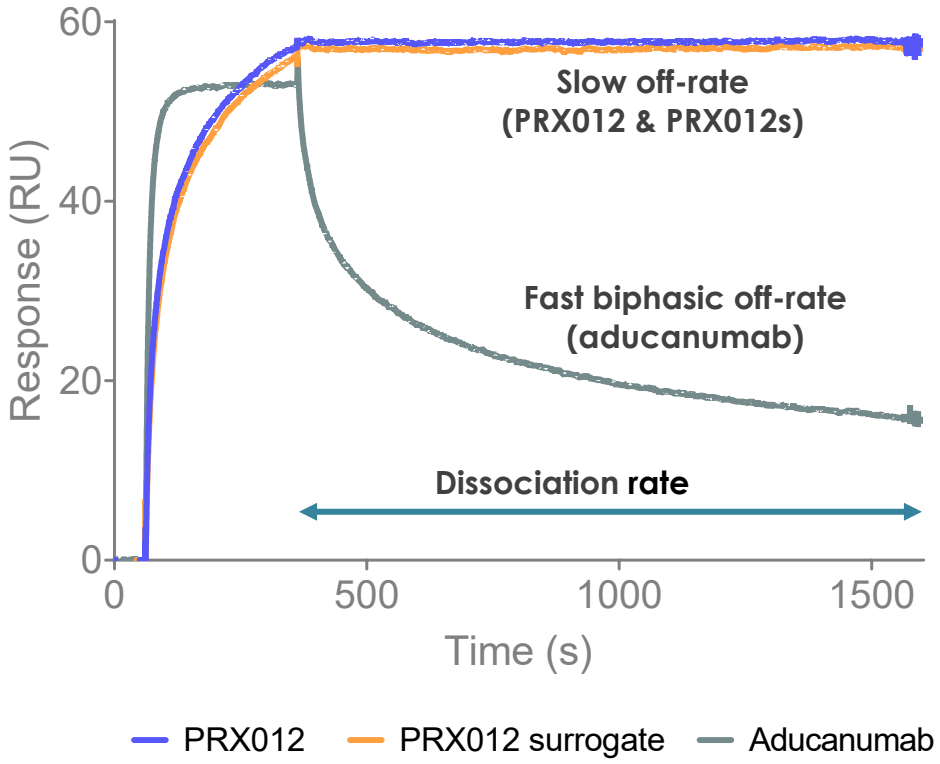
Open questions:

- **Does PRX012 (surrogate) bind to protofibrils with high affinity?**
- **Does PRX012 (surrogate) clear pyroglutamate-Aβ from AD plaques?**

Aβ, amyloid beta; AD, Alzheimer's disease; APP, amyloid precursor protein.
1. Tam SJ, et al. Poster presented at AAIC; July 26–30, 2021; Denver, CO, USA and virtual. 2. Skov M, et al. Poster presented at CTAD; November 4–7, 2020; Boston, MA, USA and virtual.

PRX012 and Surrogate Demonstrate Equivalent Potent Binding Affinity for Aβ

Surface Plasmon Resonance (SPR)
Aβ Fibrils



Affinity for Aβ Species

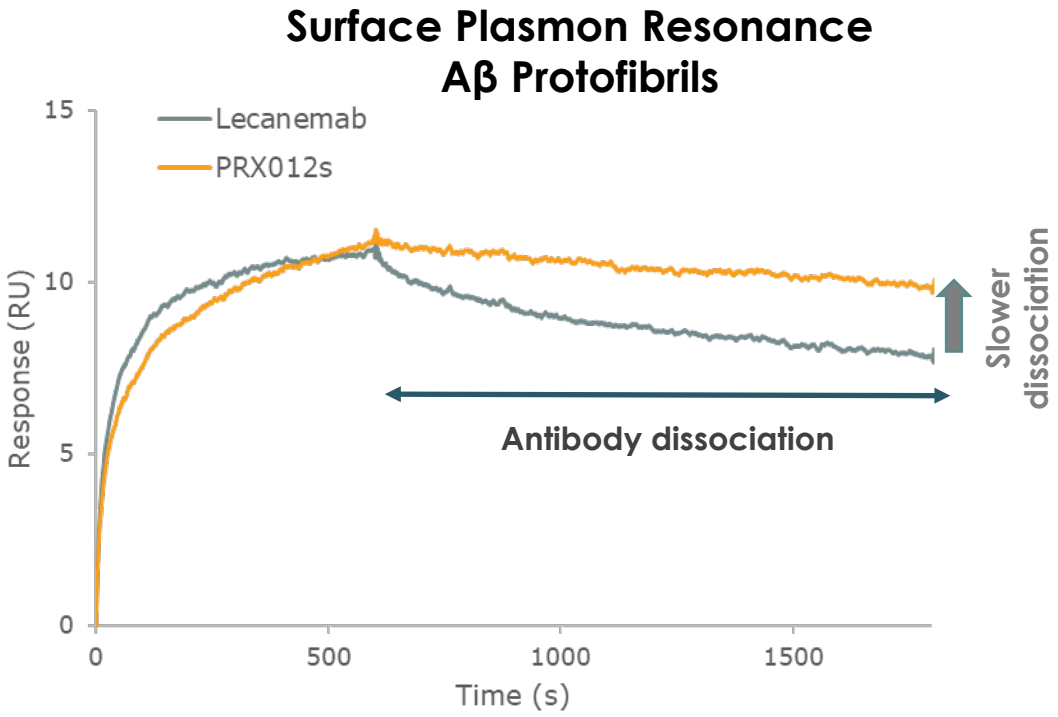
Compound	Fibril/Plaque	N3pE-Aβ
PRX012	0.070[a]	>67[b]
PRX012s	0.054[a]	>67[b]

Data represent K_D values from SPR[a] (nM) or IC50 from ELISA[b] (nM).

- **Potent binding strength of PRX012 and its surrogate (PRX012s) to fibrillar Aβ are equivalent, both demonstrating a very slow rate of dissociation**
  - **PRX012 and PRX012s share >99.5% sequence homology**
- **How does binding to protofibrils compare?**

Aβ, amyloid beta; N3pE-Aβ, pyroglutamate-modified Aβ; SPR, surface plasmon resonance.
PRX012s: 'Surrogate' is defined as an antibody with the same binding epitope and equivalent binding profile to forms of Aβ where directly compared.
Sequences for aducanumab, lecanemab, and donanemab were obtained from publicly available sequences.

PRX012s Binds Aβ Protofibrils With Very High Affinity



SPR protofibril binding was performed as described in Tucker et al., 2015[1]

Antibody	Relative Affinity (K_{D1})
Lecanemab[1] (Tucker et al., 2015)	1.97 nM
Lecanemab*	1.91 nM
PRX012s	0.0975 nM

SPR Binding Kinetics

	ka1 (1/Ms)	kd1 (1/s)	K_{D1}
Lecanemab[1] (Tucker et al., 2015)	6.60E+05	1.30E-03	1.97E-09
Lecanemab*	1.80E+05	3.42E-04	1.91E-09
PRX012s	1.63E+05	1.59E-05	9.75E-11

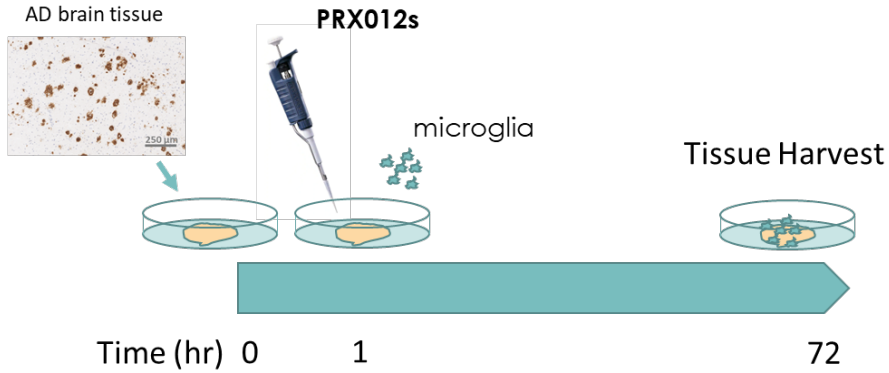
- **PRX012s binds to Aβ protofibrils with approximately 20-fold greater affinity than lecanemab when tested under the same conditions**
- **Greater affinity is driven largely by a slower binding dissociation**

Aβ, amyloid beta; ka, association constant; kd, dissociation constant; K_D, equilibrium constant; SPR, surface plasmon resonance. *Determined by Prothena.

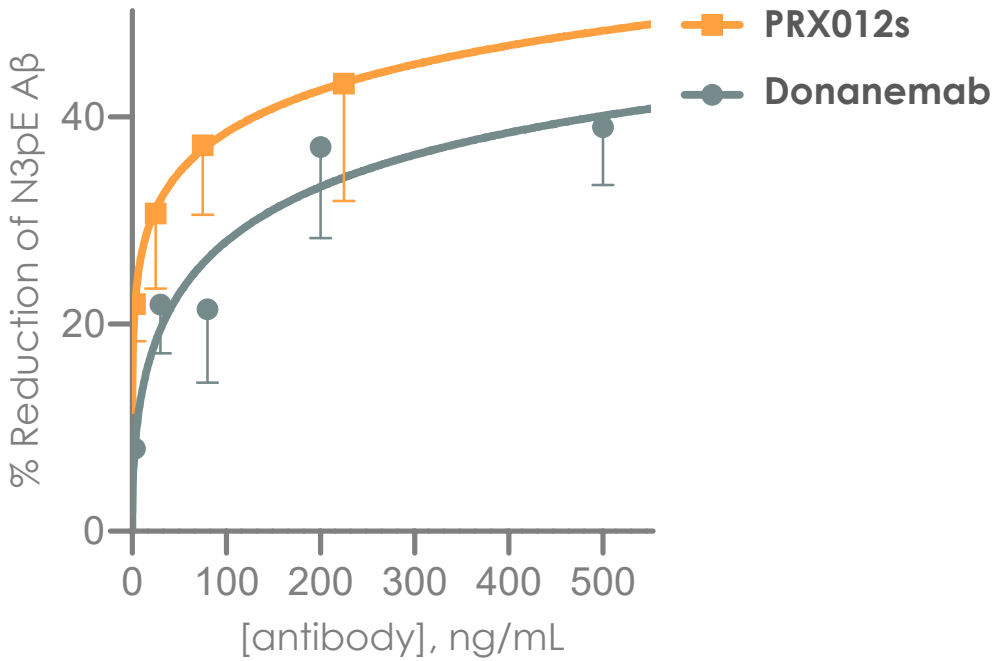
1. Tucker S, et al. *J Alzheimers Dis*. 2015;43:575-588.

Sequences for aducanumab, lecanemab, and donanemab were obtained from publicly available sequences.

PRX012s Induced Potent and Robust Clearance of Pyroglutamate-modified Aβ



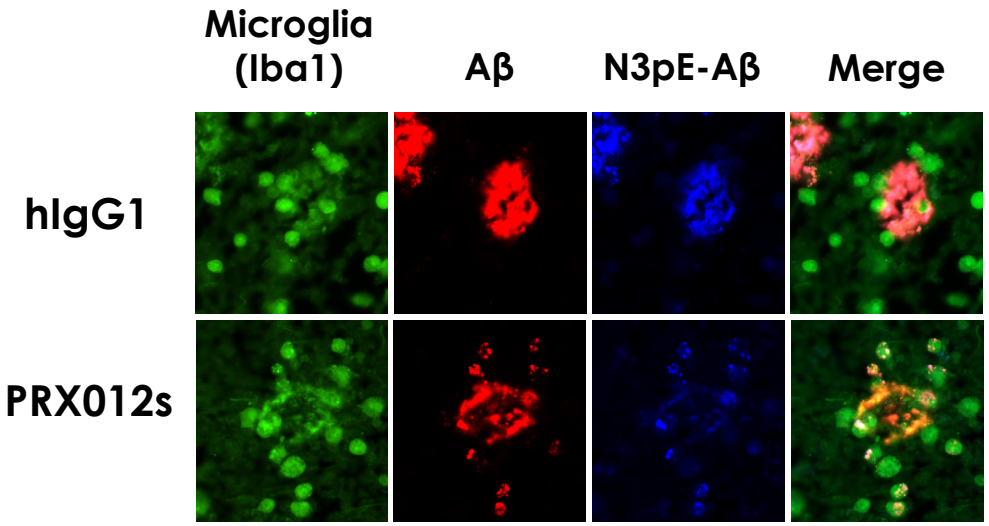
	Study Conditions
Tissue	Post-mortem AD brain tissue (same donor used for all conditions)
Treatment	PRX012s, donanemab, or IgG1 isotype control
Microglia	Primary mouse microglia (800,000 cells/mL)
Culture time	72 hours at 37°C



- **PRX012s facilitates concentration-dependent clearance of pyroglutamate-modified Aβ (N3pE-Aβ) at concentrations that may be relevant for PRX012 clinical exposure**
- **PRX012s clears equivalent or more N3pE-Aβ at ~3–8x lower concentrations than donanemab**

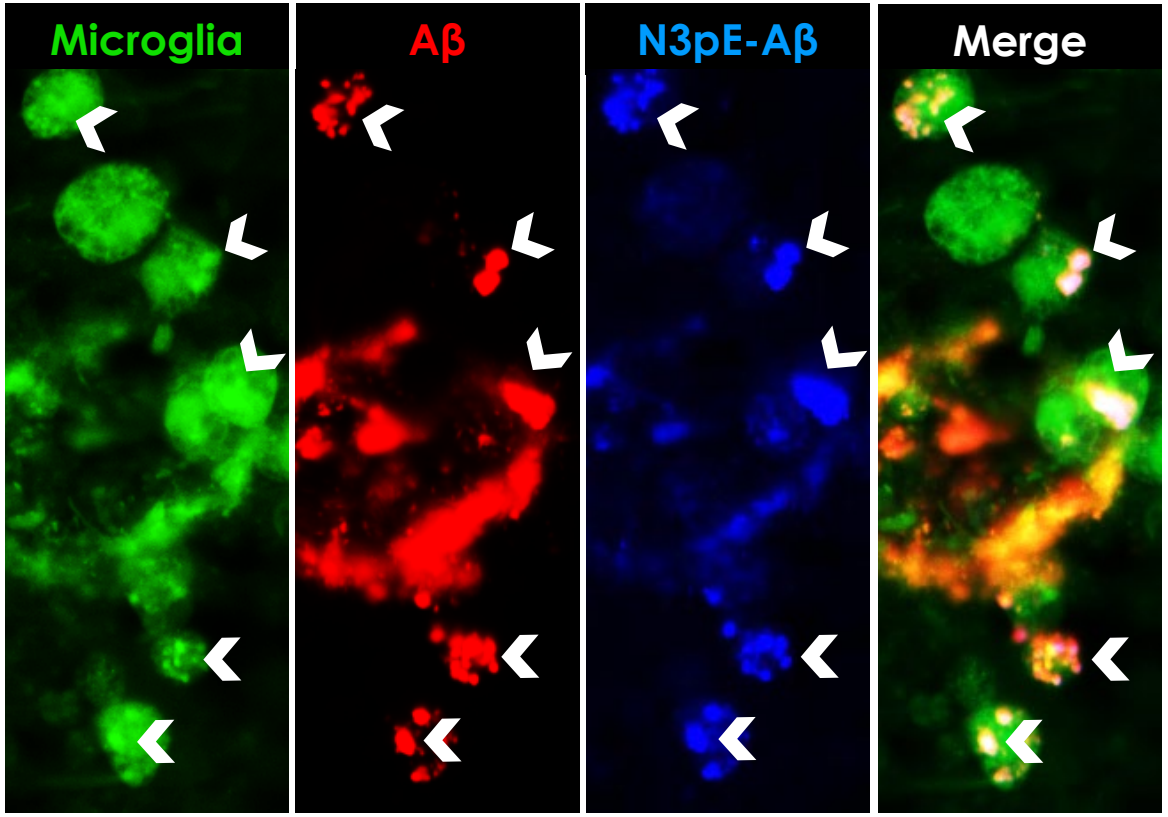
Aβ, amyloid beta; AD, Alzheimer's disease; IgG, immunoglobulin; N3pE-Aβ, pyroglutamate-modified Aβ.
Sequences for aducanumab, lecanemab, and donanemab were obtained from publicly available sequences.

PRX012s Promotes Simultaneous Microglia-Mediated Phagocytosis of Aβ and N3pE-Aβ in Post-mortem Brain Tissue From AD Subjects



Microglia (Iba1: green) simultaneously phagocytose Aβ (red) and pyroglutamate-modified Aβ ($Aβ_{pE3-42}$: blue) in the presence of PRX012 surrogate, indicating that opsonization of plaques is sufficient to clear both species.

- **PRX012s promoted microglia-mediated phagocytosis of Aβ and pyroglutamate-modified Aβ (N3pE-Aβ) simultaneously**



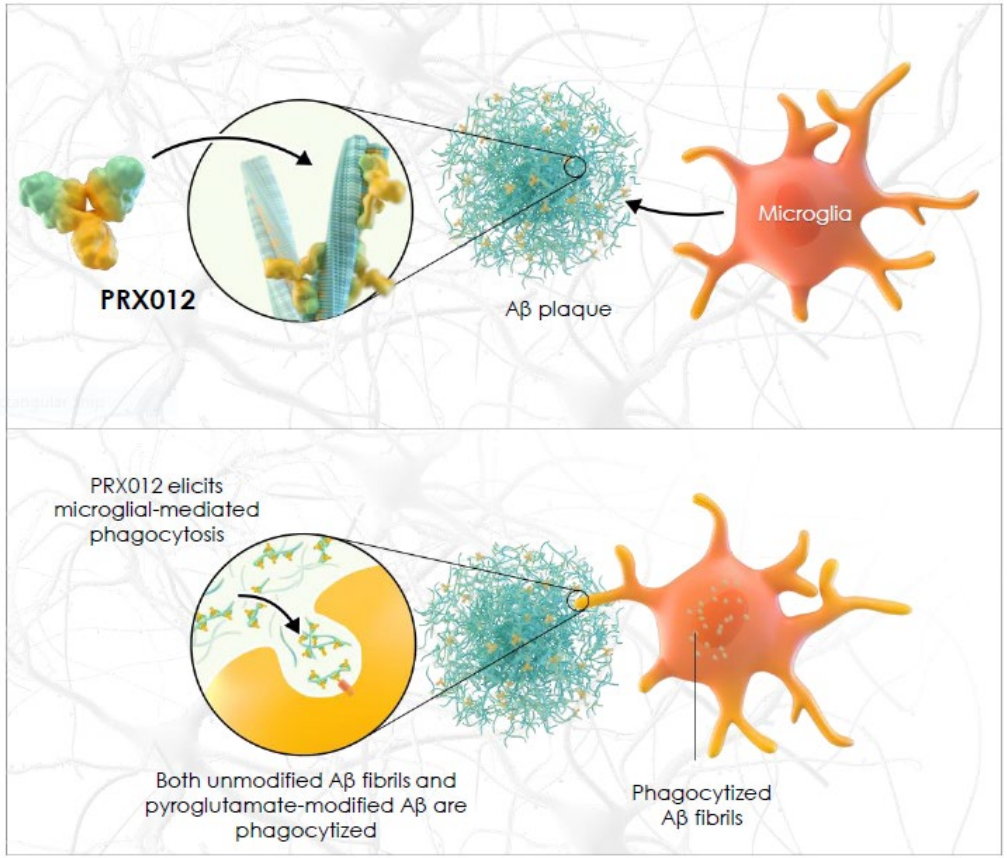
Arrows indicate examples of phagocytosed Aβ and N3pE-Aβ that co-localize inside microglia cells (immunostained with anti-Iba1 antibody).

Aβ, amyloid beta; AD, Alzheimer's disease; IgG, immunoglobulin; N3pE-Aβ, pyroglutamate-modified Aβ.

Key Takeaways

- PRX012 is a high-affinity monoclonal antibody that binds to aggregated forms of Aβ
  - PRX012s bound to protofibrils with low picomolar affinity
  - Binding affinity to protofibrils was approximately 20-fold more potent than lecanemab under the same testing conditions
- Binding to aggregated Aβ by PRX012[1] and PRX012s promotes clearance of Aβ and N3pE-Aβ in AD brain tissue
  - PRX012s eliminated N3pE-Aβ in AD brain tissue with greater potency than donanemab, consistent with very high affinity toward Aβ plaques
- These data suggest that high-binding potency N-terminal-targeted antibodies like PRX012 may produce rapid clearance of toxic Aβ species in patients with Alzheimer's disease
  - Experiments confirmed that PRX012s binds Aβ protofibrils and removes N3pE-Aβ, two mechanisms associated with Aβ plaque clearance and slowing of cognitive decline in Alzheimer's disease

Microglia Recognize and Engulf PRX012-Opsonized Aβ Fibrils



Aβ, amyloid beta; AD, Alzheimer's disease; N3pE-Aβ, pyroglutamate-modified Aβ.

1. Tam SJ, et al. Poster presented at AAIC; July 26–30, 2021; Denver, CO, USA and virtual.

Key Takeaways

- These data add to the body of evidence supporting the profile design of PRX012, which is designed to target all aggregated forms of Aβ with high binding potency and further support the ongoing clinical development of PRX012 as a potential best-in-class treatment for Alzheimer's disease that could enable greater accessibility and more convenient administration for patients and caregivers

Aβ, amyloid beta

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