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

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1Prothena Biosciences, Inc., South San Francisco, CA, USA
### Disclosures

<table>
<thead>
<tr>
<th>Company Name</th>
<th>Honoraria/Expenses</th>
<th>Consulting/Advisory Board</th>
<th>Funded Research</th>
<th>Royalties/Patent</th>
<th>Stock Options</th>
<th>Ownership/Equity Position</th>
<th>Employee</th>
<th>Other (Please Specify)</th>
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<tr>
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All authors are employees of Prothena Biosciences, Inc.
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.

**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.

Aβ, amyloid beta; AD, Alzheimer’s disease.
### Translating Patient Needs Into Antibody Engineering

**Patient-Centric Design Strategy For PRX012**

<table>
<thead>
<tr>
<th>TARGET PROFILE</th>
<th>ANTIBODY DESIGN ATTRIBUTES</th>
<th>POTENTIAL IMPLICATIONS FOR PATIENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effectively clear soluble and insoluble aggregated amyloid</td>
<td>• N-terminal directed</td>
<td>• Associated with efficacy</td>
</tr>
<tr>
<td>Low-volume subcutaneous (SC) delivery</td>
<td>• High binding potency</td>
<td>• Simplicity for patient and caretaker</td>
</tr>
<tr>
<td></td>
<td>• Stability in high concentrations for single syringe use</td>
<td>• More convenient</td>
</tr>
<tr>
<td>Designed for monthly dosing</td>
<td>• Optimize pharmacokinetic profile and immunogenicity</td>
<td>• Increases access</td>
</tr>
<tr>
<td></td>
<td>• Optimal bioavailability</td>
<td>• Minimizes treatment burden</td>
</tr>
<tr>
<td>Treatment outside infusion centers</td>
<td>• Optimal biophysical qualities</td>
<td>• Less time-consuming</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Infrequent dosing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Potential for at-home use</td>
</tr>
</tbody>
</table>

**Maintain From First Generation Antibodies**

**Innovations to Support Patient Needs**
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

- 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.
PRX012 and Surrogate Demonstrate Equivalent Potent Binding Affinity for Aβ

- 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?

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fibril/Plaque</th>
<th>N3pE-Aβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRX012</td>
<td>0.070^a</td>
<td>&gt;67^b</td>
</tr>
<tr>
<td>PRX012s</td>
<td>0.054^a</td>
<td>&gt;67^b</td>
</tr>
</tbody>
</table>

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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**

- **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**

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### Antibody Relative Affinity ($K_D$)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Relative Affinity ($K_D$)</th>
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<tbody>
<tr>
<td>Lecanemab(^1)</td>
<td>1.97 nM</td>
</tr>
<tr>
<td>(Tucker et al., 2015)</td>
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</tr>
<tr>
<td>Lecanemab*</td>
<td>1.91 nM</td>
</tr>
<tr>
<td>PRX012s</td>
<td>0.0975 nM</td>
</tr>
</tbody>
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### SPR Binding Kinetics

<table>
<thead>
<tr>
<th>Antibody</th>
<th>$k_a$ (1/Ms)</th>
<th>$k_d$ (1/s)</th>
<th>$K_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecanemab(^1)</td>
<td>6.60E+05</td>
<td>1.30E-03</td>
<td>1.97E-09</td>
</tr>
<tr>
<td>(Tucker et al., 2015)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lecanemab*</td>
<td>1.80E+05</td>
<td>3.42E-04</td>
<td>1.91E-09</td>
</tr>
<tr>
<td>PRX012s</td>
<td>1.63E+05</td>
<td>1.59E-05</td>
<td>9.75E-11</td>
</tr>
</tbody>
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Sequences for aducanumab, lecanemab, and donanemab were obtained from publicly available sequences.
PRX012s Induced Potent and Robust Clearance of Pyroglutamate-modified Aβ

- 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

**Study Conditions**

<table>
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<tr>
<td><strong>Tissue</strong></td>
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<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td><strong>Microglia</strong></td>
</tr>
<tr>
<td><strong>Culture time</strong></td>
</tr>
</tbody>
</table>

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

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

Microglia (Iba1: green) simultaneously phagocytose Aβ (red) and pyroglutamate-modified Aβ (AβpE3-42: blue) in the presence of PRX012 surrogate, indicating that opsonization of plaques is sufficient to clear both species.

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-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.
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.
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