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INTRODUCTION

• Monoclonal antibodies (mAbs) targeting the Aβ-merit of amyloid beta (Aβ) have been demonstrated clinically to reduce amyloid plaque burden.

• One such antibody currently under FDA priority review, aducanumab, showed significant reduction in plaque burden associated with reduction in clinical decline in Alzheimer’s disease (AD).

• Preclinical studies have also indicated that N-terminal mAbs elicit antibody-dependent microglia-mediated Aβ plaque clearance and neutralization of soluble Aβ aggregates in vitro and in vivo. — It is hypothesized that administration of N-terminal Aβ targeting mAbs slows disease progression via clearance of Aβ plaques and neutralization of soluble Aβ aggregates in patients with AD.

AIM

• To develop and characterize novel humanized N-terminal Aβ-targeting antibodies with greater binding strength (affinity and avidity) for pathologic fibrillar Aβ than has been reported with current experimental therapeutics and with high affinity for soluble toxic forms.

• Antibodies with such characteristics may be efficacious therapies for AD and could enable more convenient dosing strategies, which will enhance patient access.

METHODS

GENERATION OF ANTIBODIES

• The full amino acid sequence of saccharomyces was selected based on publicly available sequence information, and was used to generate the humanized antibody.

• Aducanumab and Prothena (PRO) mAbs (H272, H2731, H2831, H2931) were expressed in Chinese hamster ovary (CHO) cells, purified by Protein-A affinity chromatography, and characterized by capillary electrophoresis and size exclusion chromatography.

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Surface Plasmon Resonance (SPR) Measurements

• Binding kinetics were performed with a Biacore T200 (GE Healthcare, Princeton, NJ).

• h2731 was pre-incubated with antibodies or isotype control for 30 minutes at 37°C in Neurobasal-A medium (GIBCO, Gaithersburg, MD).

• The antibody/antigen complexes were added to the chip and incubated for 30 minutes at 25°C.

• The chip was washed with PBS and air-dried.

• Binding of the biotinylated antibodies was visualized using the avidin-biotin amplification system (ABC Elite Standard, PK-6100; Vector Laboratories, Burlingame, CA).

• The slides were digitally imaged (NanoZoomer 2.0-HT, Hamamatsu Corporation, Japan). The direct binding of PRO mAbs and aducanumab to Aβ fibrils was assessed by ELISA.

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RESULTS

The enhanced relative avidity of PRO mAbs for fibrillar Aβ observed by ELISA was confirmed by SPR equilibrium binding kinetics (Table 1), which indicated a 5- to 11-fold greater avidity (apparent KD*) than aducanumab.

Figure 1. Aβ Phagocytosis Assay

Crystal sections of APPPS1 mouse (w5FNDN) on coverslips were incubated with antibodies for 1 hour.

• Antibodies were then washed, and the cultures were maintained at 37°C for 72 hours.

• Media was carefully aspirated and sections washed with PBS.

• The slides were digitally imaged (NanoZoomer 2.0-HT, Hamamatsu Corporation, Japan).

• The enhanced relative avidity of PRO mAbs for fibrillar Aβ observed by ELISA was confirmed by SPR equilibrium binding kinetics (Table 1), which indicated a 5- to 11-fold greater avidity (apparent KD*) than aducanumab.

• This is explained by the different kinetic binding profiles observed in the SPR experiment (Figure 2).

• Although aducanumab binds fibrillar Aβ at a faster association rate (ka), the much greater binding strength of PRO mAbs indicates that these antibodies are likely to have a stronger and more durable effect in vivo.

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• Although aducanumab binds fibrillar Aβ at a faster association rate (ka), the much greater binding strength of PRO mAbs indicates that these antibodies are likely to have a stronger and more durable effect in vivo.

• The direct binding of PRO mAbs and aducanumab to Aβ fibrils was assessed by ELISA (Table 1) showed greater plaque area binding (percentage increase over negative control) of PRO mAbs to fibrillar Aβ relative to aducanumab.

Table 1. Kinetic Binding Parameters of Antibodies to Fibrillar Aβ Determined by SPR

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<tr>
<th>Antibody</th>
<th>KD (nM)</th>
<th>kA</th>
<th>kD</th>
<th>R</th>
<th>% of Aβ bound</th>
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<td>5.7 x 10^6</td>
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<tr>
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<td>2.5 x 10^5</td>
<td>6.1 x 10^6</td>
<td>61</td>
<td>56.0</td>
</tr>
</tbody>
</table>

Figure 2. SPR Sensogram Kinetic Binding Profiles of Antibodies to Fibrillar Aβ at 100 nM Concentration

<table>
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CONCLUSIONS

The enhanced AD binding properties of PRO mAbs demonstrate their potential as therapeutic candidates that warrant further development. Additionally, it is anticipated that the enhanced binding of these antibodies will enable evaluation of more convenient dosing strategies in the clinic.

• These characteristics could lead to improved patient access and potential therapeutic benefits.

REFERENCES


AUTHOR DISCLOSURES

All authors are employees of Prothena Inc.

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