Preclinical Assessment of BBB-Crossing Amyloid-β Oligomer-Targeting Peptide Using PET, MRI and CSF Biomarkers

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Translational Challenges

• Animal models – translation across species (mouse, rat, dog)

• **Design of target engagement and efficacy** preclinical study that ‘mirrors’ typical clinical study design

• **Use of imaging (PET, MRI) and CSF biomarkers in preclinical study**

• Translational PK/PD modeling (**small brain to large brain**)

• Analytics that support translation
**Therapeutic Molecule (KAL-ABP-BBB)**

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Molecule Development</th>
<th>Detection (Analytics)</th>
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<tbody>
<tr>
<td><strong>ABP (4 kD)</strong></td>
<td>Amyloid binding regions from human PCM-1; binds <strong>oligomeric</strong> Aβ with nM affinity</td>
<td>C-terminus-specific monoclonal antibody (ELISA, WB); specific peptides for nanoLC-SRM</td>
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<tr>
<td><strong>FC5 (13 kD)</strong></td>
<td><strong>BBB-crossing</strong> V₁H; species cross-reactive; humanized</td>
<td>FC5-specific mouse monoclonal antibody (ELISA, WB); specific peptides for nanoLC-SRM</td>
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<td><strong>FC5-Fc-ABP-M (90 kD)</strong></td>
<td><strong>Surrogate molecule for rodent studies:</strong> mouse Fc; camelid FC5</td>
<td>Anti-mouse Fc antibody (ELISA, WB); specific peptides for nanoLC-SRM</td>
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<tr>
<td><strong>FC5-Fc-ABP-H (90 kD)</strong></td>
<td><strong>Human studies:</strong> humanized FC5, engineered human Fc</td>
<td>Anti-human Fc antibody (ELISA, WB); specific peptides for nanoLC-SRM</td>
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</table>

**BBB ‘Trojan’**

- FC5 (camelid V₃H) – [15 kD]
  - BBB-crossing
- IgG Fc fragment [50 kD]
  - Prolongs serum half-life;
- Peptide [4.5 kD]
  - Amyloid oligomer binding and clearance
**KAL-ABP-BBB crosses in vitro BBB intact**

![Diagram](image)

**KAL-ABP-BBB**

- Anti-ABP
  - Wells: 1, 2, 3
  - Std: 75kD, 100kD

**Rat-BBB**

- Anti-Fc
  - Wells: 1, 2, 3
  - Std: 75kD, 100kD

**Human-BBB**

- Anti-ABP
  - Wells: 1, 2, 3
  - Std: 75kD, 100kD

**Sandwich ELISA**

- Wells: 1, 2, 3
- Std: KAL-ABP-BBB
- Reaction: +
Enhanced Brain Exposure of KAL-ABP-BBB transgenic mice

Time- and dose-dependent appearance of KAL-ABP in the tissue indicates delivery of ABP to target regions of the brain by FC5
Reduction of Amyloid-β levels in KAL-ABP-BBB treated Tg mice

**Brain parenchyma**

- **MRM**
  - Cortex: Aβ (relative MRM values) vs. Control+4h and 24 h, p < 0.007
  - Hippo: Aβ (relative MRM values) vs. Control+4h and 24 h, p < 0.001

- **ELISA**
  - Aβ42 (ng/mL) vs. Control+4h and 24 h, p < 0.02
  - Aβ42 (ng/mL) vs. Control+4h and 24 h, p < 0.03

**CSF**

- KAL-ABP-BBB 24 h post iv injection
- Aβ 24 h post iv injection

**CSF Aβ levels could be used as surrogate for target engagement**
Preclinical Efficacy Study Design: Animal model

McGill-R-Thy1-APP Tg Model (Claudio Cuello)

Leon et al., J Alzheimers Dis 2010
Preclinical Efficacy Study Design: Longitudinal Biomarker Assessment

**PET**

- [18F]NAV4694 (Aβ load)

- MRI
  - rsfMRI
  - Hippocampal volume

**CSF/Plasma**

- Aβ

- FC5
- hFc
- ABP

**Safety:** MR Susceptibility imaging

**iv injection**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Day</th>
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<tr>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
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<td>15</td>
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<td>15</td>
<td>22</td>
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<tr>
<td>15</td>
<td>29</td>
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4-week treatment

Serial collection of serum and CSF at various time points
PK and CSF Biomarker (Aβ) Profile

- KAL-ABP-BBB serum PK similar to that of a mAb
- CSF exposure 25-fold higher than that of a mAb

CSF levels of Aβ inversely correlate with KAL-ABP-BBB levels in transgenic rats
Drug Efficacy:
Aβ load is significantly reduced

$[^{18}F]NAV4694 \text{ BP}_{ND}$ at Baseline and Follow-up
Drug Efficacy:
Hippocampal volume is increased

- Increased hippocampal volume only in Tg-ABP group
Secondary Drug Efficacy: rs-fMRI ACC Connectivity

- Tg-ABP showed greater ACC connectivity compared to Tg-Sal treatment

Tg-ABP > Tg-Sal

Drug Safety: Microhemorrhage

- No evidence of drug-induced microhemorrhage

Before treatment

After treatment
Summary of longitudinal studies in AD rat model

- 27% reduction in global amyloid load
- 7% Increased hippocampal volume
- Restoring rs-fMRI ACC Connectivity
- No evidence of microhaemorrhage
Translation from small to large brain

Serum/CSF PK

Rat

Dog

Aβ in CSF

Rat (Tg)

Dog (aged)
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