Development and Characterization of Novel Antibodies Targeting Amyloid Beta Oligomers with High Selectivity

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Introduction

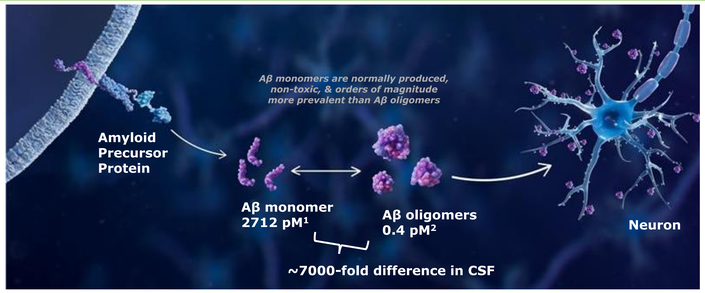

Figure 1. Effective targeting of soluble AβOs requires high selectivity due to significantly greater abundance of Aβ monomers. In the human brain, Aβ monomers are cleaved from the membrane-bound amyloid precursor protein (APP) and then aggregate into soluble Aβ oligomers (AβOs) as well as soluble protofibrils and insoluble fibrils; note the latter two conformers are not pictured here. In Alzheimer's disease (AD), soluble aggregates such as AβOs can bind neuronal synapses and induce synaptic toxicity leading to cognitive decline, which makes them attractive therapeutic targets. Average concentrations of Aβ monomer ($Aβ_{1-40}$ reported here) and AβOs measured in CSF are shown in the schematic.[1,2]

- Soluble amyloid-β oligomers (AβOs) are early, persistent drivers of AD pathogenesis.[3]
- One crucial feature of AβO-targeting therapeutics and immunoreagents is the selectivity for AβOs over Aβ monomers given that monomers are ~7,000-fold more abundant in AD biofluids and tissues.
- We developed three novel antibodies targeting AβOs.
- The purpose of this study was to characterize these novel, highly selective anti-AβO antibodies.

Methods

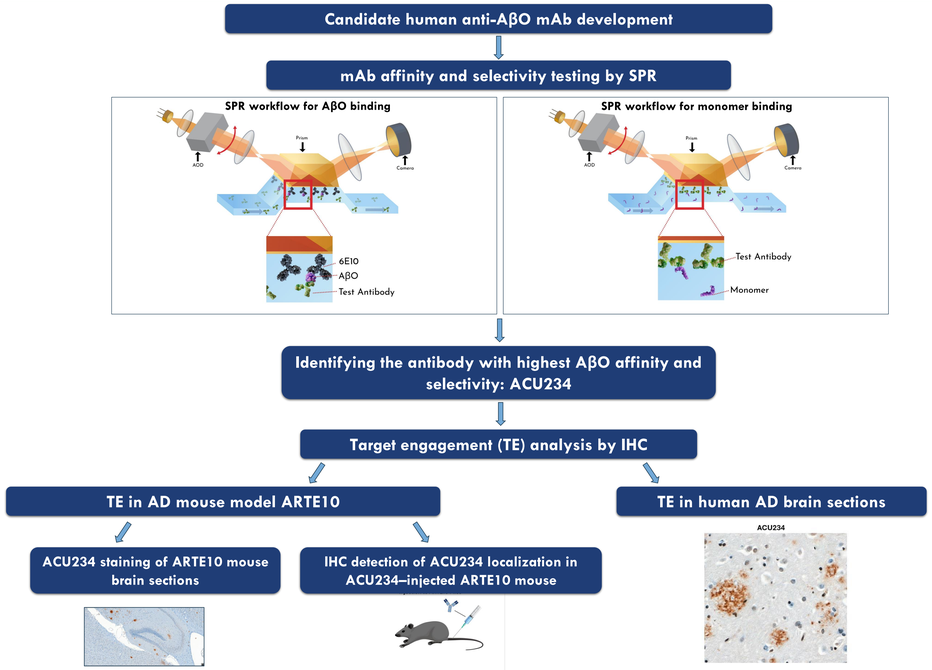

Figure 2. Experiment Flowchart. A panel of novel human anti-AβO mAbs were developed and characterized. The affinity and selectivity of these antibodies for AβOs over Aβ monomers ($Aβ_{1-28}$ and $Aβ_{1-40}$) were measured using surface plasmon resonance (SPR). To measure AβO binding, the N-terminal-Aβ antibody 6E10 was used to capture a fixed concentration of AβOs, and the test antibody was titrated. To measure monomer binding, the test antibody was used as capture and the monomers were titrated. Subsequently, the most selective monoclonal antibody (mAb), ACU234, was evaluated using *in vitro* and *in vivo* models of target engagement (TE). Human brain samples from AD and controls were stained with ACU234 and compared with known Aβ mAbs using immunohistochemistry (IHC). ACU234 was also injected into ARTE10 transgenic mice and brain localization was assessed using IHC.

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Results

Anti-AβO antibodies have nM affinities for AβOs & µM affinities for Aβ monomers

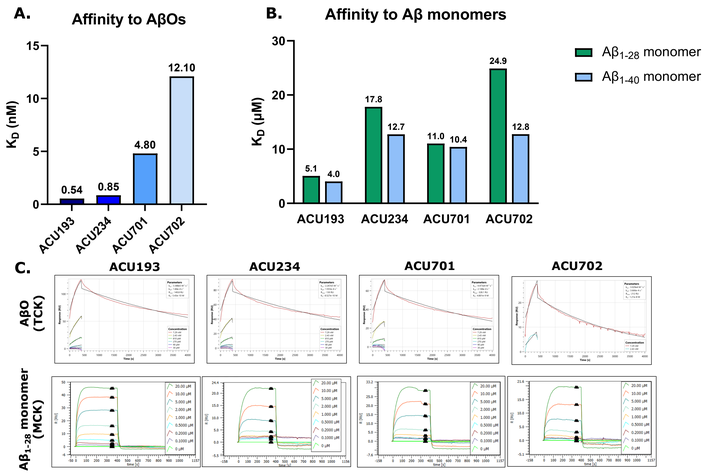
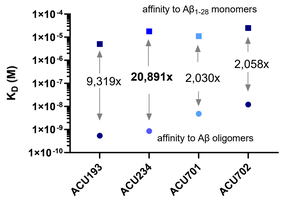


Figure 3. SPR analysis of the interactions between anti-AβO antibodies with synthetic AβOs and Aβ monomers. A. Dissociation constants (K_D) for binding of the test antibodies to AβOs were obtained by a Titration-Cycle Kinetic (TCK) assay and analyzed using a TCK model. B. Dissociation constants were obtained for $Aβ_{1-28}$ and $Aβ_{1-40}$ monomer binding to test antibodies. Note µM units. A Multi-Cycle Kinetic (MCK) assay and the Langmuir Steady-State model were used. C. Representative sensorgrams showing binding to AβOs (upper panel) and monomers (lower panel). Note different y-axis ranges. ACU193 (sabirnetug), a sensitive and selective anti-AβO antibody that is currently being evaluated in a phase 2 clinical trial (NCT06335173), was used as a positive control.

- ACU234 had the highest binding affinity to Aβ oligomers (AβOs) among the tested candidate anti-AβO antibodies, which was driven by fast association rate.
- ACU701 & ACU702 had ~6x & 14x weaker binding to AβOs, respectively.
- All antibodies showed comparable low affinity to both monomeric Aβ proteoforms.

ACU234 demonstrates the highest selectivity for AβOs among the anti-AβO antibodies

A. Selectivity for AβOs over $Aβ_{1-28}$ monomers



B. Candidate mAb fold selectivity for AβOs over $Aβ_{1-28}$ and $Aβ_{1-40}$ monomers

mAb	fold selectivity for AβOs over **$Aβ_{1-28}$** monomer (K_D monomer/K_D AβOs)	fold selectivity for AβOs over **$Aβ_{1-40}$** monomer (K_D monomer/K_D AβOs)
ACU234	**20,891**	**14,921**
ACU701	2,300	2,170
ACU702	2,058	1,054

Figure 4. Selectivity analysis for synthetic AβOs over Aβ monomers. A. Graphic representation of binding affinity data (K_D) to AβOs and $Aβ_{1-28}$, with fold selectivity for AβOs over monomer indicated. Fold-selectivity for AβOs over monomer was calculated as K_D for monomer binding divided by K_D for oligomer binding. B. Summary of selectivity for AβOs over $Aβ_{1-28}$ and $Aβ_{1-40}$ monomers.

- ACU234 showed the highest selectivity for AβOs among the tested candidate anti-AβO antibodies.
- The high selectivity of ACU234 resulted from a combination of high affinity for AβOs and low affinity for monomers.

ACU234 detection of AβOs in ARTE10 AD mouse model brain with IHC

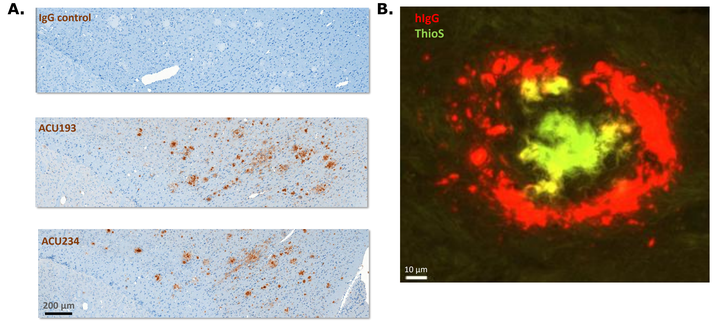


Figure 5. IHC analysis of ACU234 localization in ARTE10 mouse brain cortex. A. ARTE10 mouse brain sections were stained with human IgG2 (negative control), ACU193 (positive control), and ACU234. Note the brown reaction product indicating staining of Aβ species. B. Brain sections of ARTE10 mouse injected with ACU234 and stained with anti-human IgG (red) and Thioflavin S (ThioS, green). Immunodecoration of injected antibody is seen on the periphery, or "halo," of amyloid plaques.[4-6]

ACU234 engages AβOs in human AD brain sections (IHC)

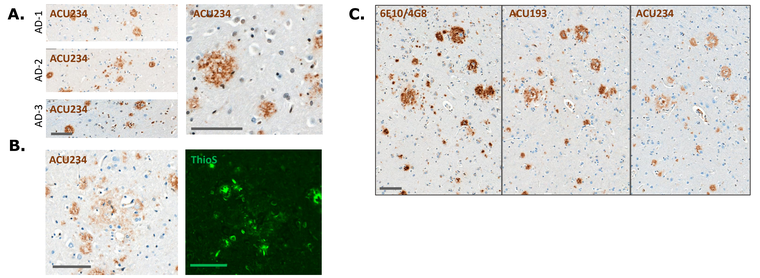


Figure 6. IHC analysis of ACU234 localization in human AD brain sections. Five-micron sections from paraffin-embedded AD brains were stained with ACU234 at 0.25 µg/ml or ThioS for plaque visualization. Scale bar:100 µm for all images. A. Left panel: ACU234 staining of Aβ species in three AD patient brain samples (brown). Right panel: ACU234 staining of Aβ species in AD brain. B. IHC images comparing ACU234 staining of Aβ species in AD brain (left panel, brown) with the staining of plaques by ThioS (right panel, green). C. Pan-Aβ antibody cocktail 6E10/4G8, ACU193, and ACU234 staining in AD brain.

- Staining with ACU234 showed immunoreactivity in mouse and AD patient brain samples similar to that of the AβO-targeting antibody ACU193. Both antibodies showed partial overlap with the pan-Aβ mAbs.
- When applied onto fixed brain slices, ACU234 bound plaques. However, in an *in vivo* setting (injection into AD mouse model), when a therapeutically relevant concentration of ACU234 is exposed to AβOs and plaques unaltered by fixation, ACU234 preferentially binds AβOs, such as those present in a halo surrounding ThioS-positive plaques.[4-6]

RESEARCH HIGHLIGHTS

- The present studies have identified three novel AβO-targeting mAbs, with ACU234 being ~21,000-fold selective for oligomeric versus monomeric Aβ.
- ACU234 was able to engage endogenous AβOs in both AD mouse model and human AD brains.
- Further work is needed to characterize ACU234 and explore its potential as a novel AβO-targeting mAb.