Utilizing the Transferrin Receptor-Mediated Transport System for Enhanced Brain Delivery of Anti-Aβ Oligomer Antibodies

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Introduction

Enhanced Brain Delivery™ (EBD™) platform utilizes TfR-mediated transcytosis to increase brain exposure of anti-AβO antibodies

- Soluble amyloid-β oligomers (AβOs) bind neuronal synapses and disrupt synaptic function.
- Anti-amyloid immunotherapies show promise, but limited brain penetration may constrain target engagement.
- To address this challenge, we developed bispecific antibodies targeting both AβOs and transferrin receptors (TfRs) to increase brain penetration of AβO-selective antibodies.

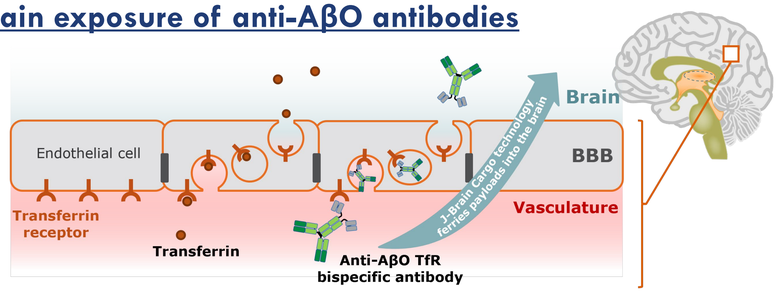


Figure 1. The EBD platform utilizes J-Brain Cargo® technology to exploit TfR-mediated transcytosis to transport antibodies through the blood brain barrier (BBB). J-Brain Cargo technology employs scFv or VHH antibody fragments targeting the transferrin receptor (TfR) conjugated to the C-terminus of the anti-AβO mAb.

- EBD bispecific constructs incorporating two AβO-selective antibodies were evaluated: (1) ACU193 (sabirnetug), currently being studied in early symptomatic Alzheimer's disease (AD) in the phase 2 ALTITUDE-AD trial (NCT06335173), and (2) ACU234, a novel antibody with ~21,000-fold greater selectivity for AβOs than Aβ monomers (Cline et al., AD/PD 2026).

Methods

Bispecific constructs of anti-AβO antibodies and antibody fragments (scFv or VHH) targeting human TfR (hTfR) were developed, characterized, and optimized using a panel of in vitro, ex vivo, and in vivo assays. Construct AβO binding affinity and selectivity were measured by surface plasmon resonance (SPR), and TfR binding affinity by bio-layer interferometry (BLI). Pharmacokinetics (PK) of EBD constructs in plasma and brain were evaluated in heterozygous hTfR transgenic (Tg) mice following intravenous (IV, 2 mg/kg) or subcutaneous (SC, 5 mg/kg) administration. Construct concentrations were quantified using a Meso Scale Discovery (MSD) electrochemiluminescence assay with anti-human antibodies for capture and detection. Immunohistochemical (IHC) studies confirmed construct binding to endogenous AβOs in human AD brain.

Results

In hTfR Tg mice, bispecific antibodies with anti-TfR scFvs had longer half-lives in brain and plasma than constructs with anti-TfR VHHs

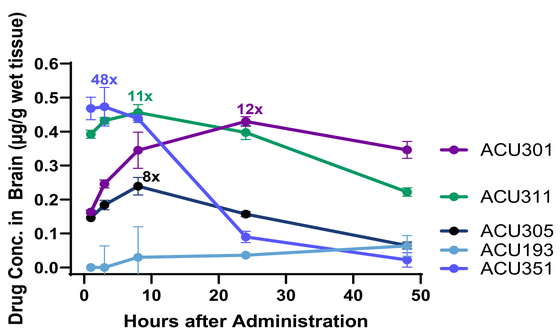
- We screened a library of EBD bispecific antibodies, varying the anti-TfR portion as either bivalent scFvs or mono- or bivalent VHHs. Different linker lengths were also tested. ACU193 (sabirnetug) was the cargo for all constructs in this initial library.
- Below is a representative study of hTfR binding and brain penetration with 4 constructs from this library.

A.

No.	ACU301	ACU311	ACU305	ACU351
Candidate Molecules	ACU193 scFv #1	ACU193 scFv #2	ACU193 Knob into holes, Tag, VHH #1	ACU193 Knob into holes, Tag, VHH #2
hTfR affinity (K_D)	2.81 nM	0.41 nM	24.20 nM	4.03 nM

Figure 2A. Schematic representation of EBD bispecific antibodies. Here, two different anti-TfR scFv sequences and two different anti-TfR VHH sequences are tested.

B.



- Brain PK analysis in heterozygous hTfR-expressing mice showed that hTfR affinity in low nM range (< 15 nM) appeared to maximize brain penetration.
- IHC analysis demonstrated that bispecific antibodies with hTfR affinity in the pM range were retained at the blood vessel wall and did not reach brain parenchyma (Shughrue et al., AD/PD 2026).
- Bispecific antibodies with anti-TfR scFvs had longer half-lives in both brain (Fig. 2B) and plasma (data not shown) than those with anti-TfR VHHs.

Figure 2B. PK profile of EBD bispecific antibodies. Brain PK profile of selected constructs after dosing heterozygous hTfR transgenic mice at 2 mg/kg IV. X-fold increases in brain concentration relative to ACU193 are shown above the plot.

References: Cline E, et al. Development and characterization of novel antibodies targeting amyloid beta oligomers with high selectivity. Poster presented at: International Conference on Alzheimer's and Parkinson's Diseases (AD/PD); March 17–21, 2026; Copenhagen, Denmark. Shughrue P, et al. Enhanced brain delivery of sabirnetug after fusion with an anti-transferrin receptor antibody fragment in a mouse model of Alzheimer's disease. Oral presentation at: International Conference on Alzheimer's and Parkinson's Diseases (AD/PD); March 17–21, 2026; Copenhagen, Denmark.

Results

EBD bispecific antibodies were selected for further evaluation

A.

No.	ACU301	ACU401	ACU351	ACU451
Candidate Molecules	ACU193 scFv	ACU234 scFv	ACU193 Knob into holes, Tag, VHH	ACU234 Knob into holes, Tag, VHH
hTfR affinity (K_D)	3.29 nM	2.35 nM	13.31 nM	4.98 nM
AβO affinity (K_D)	0.315 nM	0.808 nM	0.434 nM	0.389 nM
Aβ affinity (K_D)	2,990 nM	10,818 nM*	2,873 nM	10,044 nM*
AβO selectivity	9,492-fold	13,389-fold	6,620-fold	25,820-fold

Figure 3A. In vitro characterization of selected bispecific antibodies. Schematic representation of the constructs and their binding affinities to hTfR and Aβ conformers. *Extrapolated K_D (> highest tested concentration).

B.

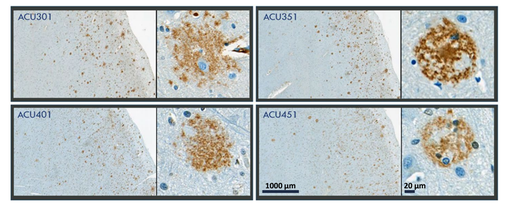


Figure 3B. In vitro characterization of selected bispecific antibodies. IHC analysis of Aβ species in human AD cerebral cortex. Bispecific antibodies were applied at 0.5 µg/mL for images at lower magnification or 0.25 µg/mL for images at higher magnification.

- From the initial panel, ACU301 and ACU351 were selected for further studies as they maximized brain penetration and had contrasting PK profiles. The ACU234 cargo was utilized to create the additional bispecific antibodies ACU401 and ACU451.
- These 4 bispecific antibodies have similar binding profiles to synthetic (Fig 3A) and endogenous (Fig 3B) Aβ species of interest with > 6,000-fold higher selectivity for AβOs over Aβ monomers. Binding in both model systems was comparable to the monoclonal antibodies ACU193 and ACU234 (data not shown).

ACU401 demonstrated robust brain uptake and prolonged exposure after SC or IV dosing in hTfR mice

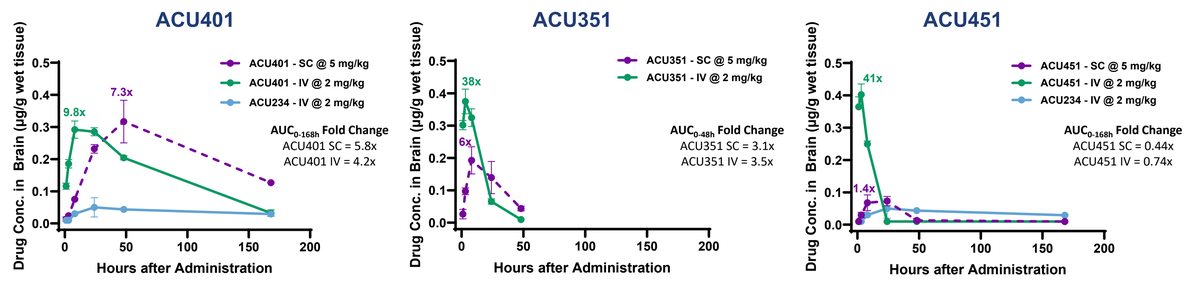


Figure 4. Comparative brain PK studies of ACU401, ACU351, and ACU451 following single IV and SC administration in mice. ACU401 and ACU451 were compared in a single study with duration of 168 h. ACU351 was evaluated in a separate study with a duration of 48 h. Fold change for ACU351 was calculated using ACU234 data from a previous study.

- All 3 bispecific antibody candidates showed greater brain penetration compared to the monoclonal antibody ACU234 with the scFv constructs maximizing brain AUC and the VHH constructs maximizing brain C_{MAX}.
- ACU401 showed comparable brain penetration (C_{MAX} and AUC) whether dosed 2 mg/kg IV or 5 mg/kg SC; the major difference in these dosing regimens was in the timing of uptake and clearance.
- The C_{MAX} of the VHH constructs ACU351 and ACU451 was lower for SC vs. IV, especially for ACU451.

ACU301 and ACU401 have comparable brain uptake after SC dosing in hTfR mice

- Given the robust brain PK profile following SC dosing of ACU401, a comparison with ACU301 was performed.
- ACU301 and ACU401 both displayed a similar, robust uptake into the brain of heterozygous hTfR-expressing mice over 48 h.
- In homozygous hTfR-expressing model systems, including humans, ACU301 and ACU401 may achieve greater brain penetration relative to monospecific mAbs.

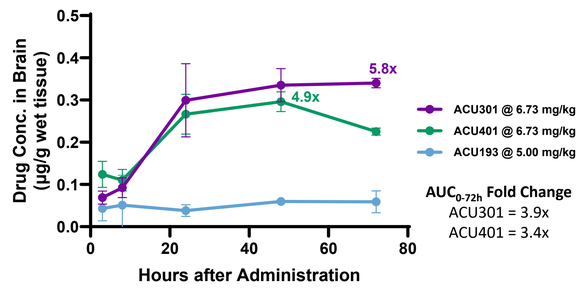


Figure 5. A comparative PK analysis of ACU301 and ACU401 following SC administration in mice. Dosing was normalized by molecular weight (0.03 mmol/kg).

RESEARCH HIGHLIGHTS

- EBD bispecific antibodies targeting both AβOs and hTfR showed improved brain penetration compared to monoclonal anti-AβO antibodies, potentially enabling efficacy at lower doses, improving safety margins, and supporting SC administration.
- Low-nM hTfR affinity was optimal for brain delivery, and VHHs- and scFvs-based hTfR binders exhibited distinct PK profiles.
- Based on favorable PK profiles in mice and nonhuman primates (see Paul Shughrue's Developing Topics oral presentation, 15 July @ 8 am), ACU301 and ACU401 were selected as lead development candidates.