Fusing Transferrin Receptor Binders to the AβO-targeting Antibody Sabirnetug (ACU193) Achieves Increased Brain Penetration in Mice While Preserving Target Binding

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Introduction

TfR-mediated uptake: A strategy for Enhanced Brain Delivery™ (EBD) of drugs

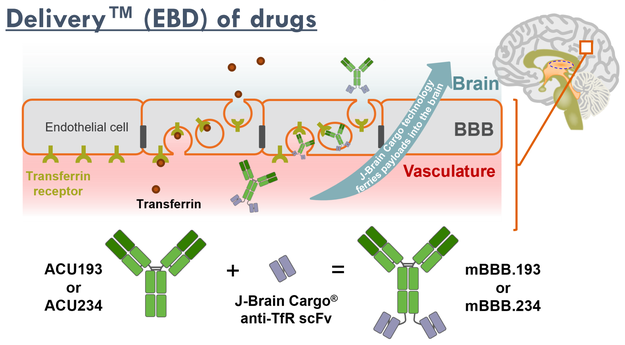


Figure 1. J-Brain Cargo® technology utilizes TfR-mediated transcytosis to enhance brain delivery of drugs. Schematic representation of antibody brain delivery using TfR-targeting fusion proteins.

EBD fusion proteins tested

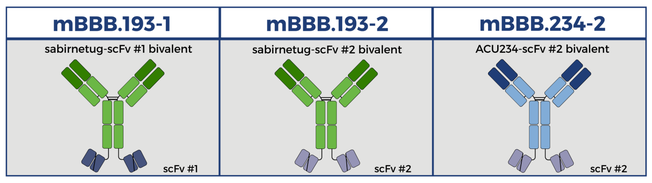


Figure 2. mTfR–targeting EBD fusion proteins. Schematic representation of the drug-cargo fusion proteins tested in this study. The drug is either ACU193 (green) or ACU234 (blue). The cargo is a single-chain variable fragment (scFv) targeting the murine TfR (mTfR). Two different scFv sequences were tested: scFv #1 and scFv #2.

- Immunoglobulin G (IgG) antibodies delivered intravenously (IV) have poor brain penetration (~0.1% of antibody administered) and elevated risk for ARIA in Alzheimer's disease (AD) patients.
- Receptor mediated transcytosis (RMT) has been used to increase brain availability of therapeutic antibodies.
- We used the J-Brain Cargo® technology targeting the transferrin receptor (TfR)[1] for enhanced brain delivery of the candidate AD immunotherapeutic sabirnetug (ACU193) or the non-clinical antibody ACU234.
  - Both antibodies are highly selective for soluble amyloid β oligomers (AβOs), implicated in the synaptic toxicity at early stages of AD.
  - Accessing the brain through the TfR is predicted to result in low amyloid-related imaging abnormalities (ARIA) rates due to the high expression of TfR on capillaries and low expression on arteries, where vascular amyloid is located primarily.[2,3]
  - The potentially enhanced safety profile of this brain delivery strategy is expected to enable dose selection to be focused on maximizing efficacy.
- Here, we evaluated fusion proteins of sabirnetug or ACU234 with single-chain variable fragments (scFv) targeting the murine TfR (mTfR) for evaluation of pharmacokinetic and AβO binding properties in wild-type and ARTE10 mice.
- The goal of this study is to support the selection of a lead EBD candidate for clinical development for Alzheimer's disease.

Methods

In vitro & *in vivo* methods

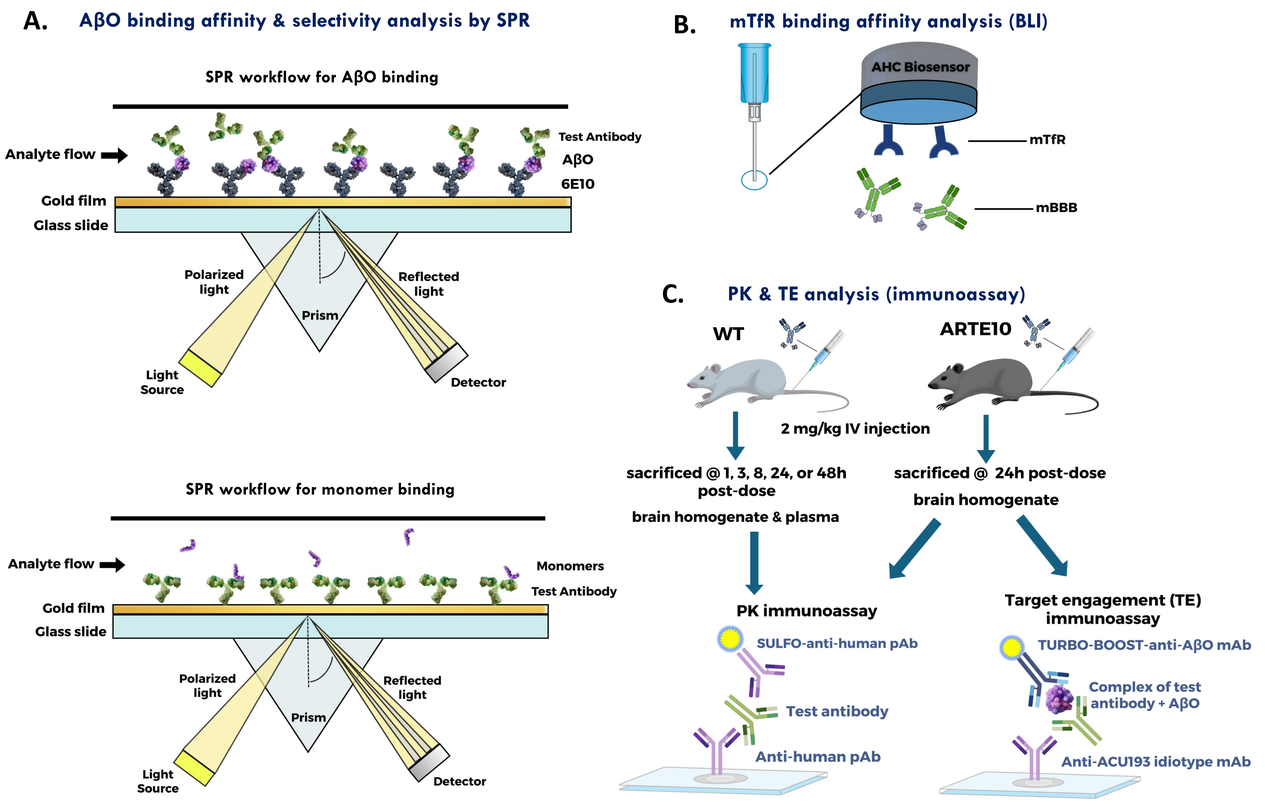
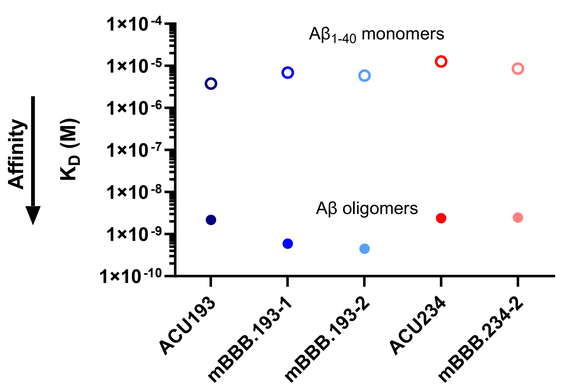


Figure 3. Characterization of the fusion proteins. Three different fusion proteins comprising ACU193 and ACU234 fused to antibody fragments targeting the mTfR (see Figure 2) were prepared and characterized using *in vitro* and *in vivo* experimental approaches. A) The binding affinities of each fusion protein to synthetic AβOs[4] and $Aβ_{1-40}$ monomers were measured by surface plasmon resonance (SPR). To measure AβO binding, the pan-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 immobilized onto an IgG capture chip and the monomers were titrated. B) The binding affinities of each fusion protein to mTfR were measured by bio-layer interferometry (BLI). C) Plasma and brain pharmacokinetics (PK) were measured in wild-type (WT) mice following a single 2 mg/kg IV injection using immunoassays. Subsequently, PK and target engagement (TE), measured as the drug-AβO complex, were assessed in the brain of the ARTE10 (Thy1-PSEN1^{M146V}, -APP^{Swe}) AD mouse model following a single 2 mg/kg IV injection. The TE assay used the MSD S-PLEX® platform, with anti-ACU193 idiotype mAb (1H1) and TURBO-BOOST®-anti-AβO mAb (2B4.6) used as capture and detection antibody, respectively. The PK assay used an MSD®ECL assay with anti-human antibodies for capture and detection.

Results

All EBD fusion proteins had AβO affinity & selectivity comparable to sabirnetug (ACU193)

Selectivity for AβOs over $Aβ_{1-40}$ monomers



- All analyzed antibodies and fusion proteins had comparable binding affinity to AβOs.
- All had comparable low binding affinity to $Aβ_{1-40}$ monomers.
- All had similar selectivity for AβOs vs $Aβ_{1-40}$ monomers.

Figure 4. SPR analysis of the interactions between ACU193, ACU234, and fusion proteins with synthetic AβOs and $Aβ_{1-40}$ monomers. Summary of binding affinity data (K_D) to AβOs and $Aβ_{1-40}$ monomers for ACU193, ACU234, and the fusion proteins determined by SPR.

Results

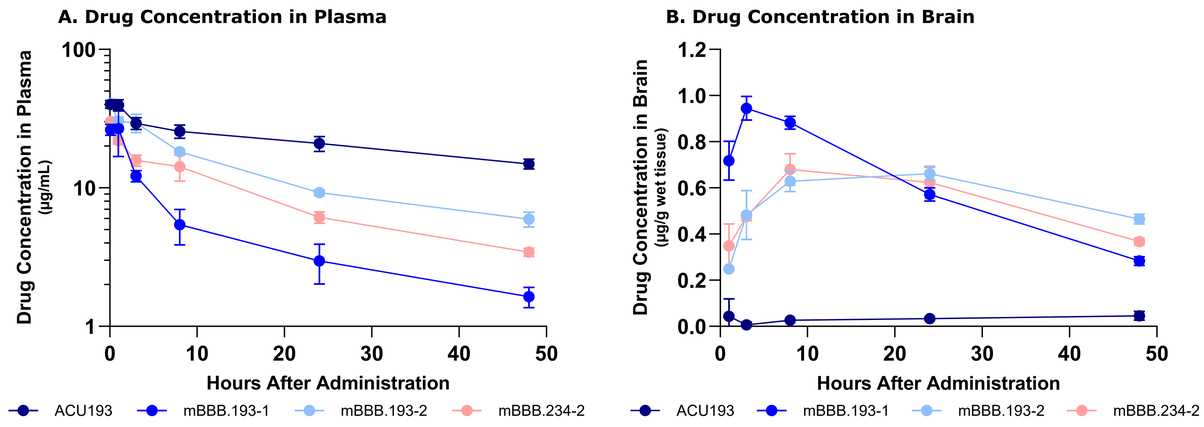
mBBB.193-1 utilizing scFv #1 had pM affinity for mTfR, while mBBB.193-2 and mBBB.234-2 with scFv #2 had low nM affinities

No.	mBBB.193-1	mBBB.193-2	mBBB.234-2
Affinity to mTfR*	WS1 (Test No. BTV-25025) **0.33 nM**	WS1 (Test No. BTV-25025) **5.34 nM**	WS1 (Test No. BTV-25025) **3.52 nM**
Sensor gram	Kon (1/Ms): 3.71E+05 Kdis(1/s): 1.24E-04	Kon (1/Ms): 8.54E+05 Kdis(1/s): 4.56E-03	Kon (1/Ms): 1.05E+06 Kdis(1/s): 3.68E-03

Figure 5. Analysis of binding affinity to mTfR. The affinity was assessed by the biolayer interferometry (BLI) kinetic measurement technique using nickel capture to immobilize mTfR. K_D = affinity constant; K_{on} = association rate; K_{dis} = dissociation rate. Representative BLI traces for each construct are shown.

- mBBB.193-1 demonstrated the highest affinity for mTfR.
- mBBB.193-2 and mBBB.234-2 had 10- to 15-fold lower affinities.
- The higher binding affinity of mBBB.193-1 was driven by slower dissociation rate.
- The mTfR affinity corresponded to the type of TfR-binding construct used.

In WT mice, all EBD fusion proteins showed increased brain exposure compared to sabirnetug (ACU193)



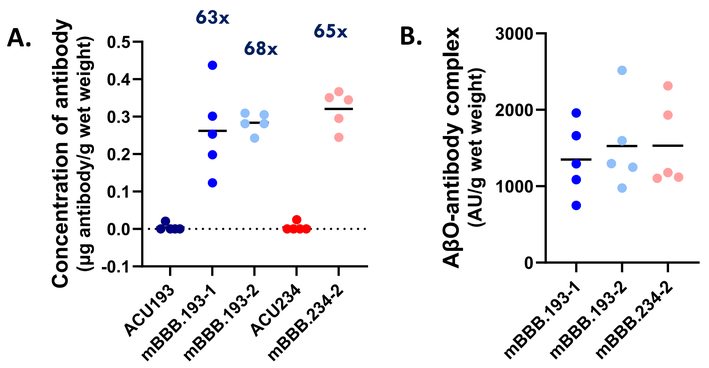
Plasma PK Parameters	
Test Article	$T_{1/2}$ (h)
ACU193	51.1
mBBB.193-1	23.5
mBBB.193-2	25.5
mBBB.234-2	19.7

Brain PK Parameters			Fold-differences	
Test Article	C_{max} (µg/mL)	AUC_{INF} (h*ug/mL)	C_{max}/ C_{max} (ACU193)	AUC_{INF}/ AUC_{48h} (ACU193)
ACU193	0.0453	1.57*	1.0	1.0*
mBBB.193-1	0.945	38.6	20.9	24.6
mBBB.193-2	0.661	87.1	14.6	55.5
mBBB.234-2	0.679	50.2	15.0	32.0

Figure 6. PK analysis of the fusion proteins in WT mice. A) Drug concentrations were measured in plasma at 5 min, 1, 3, 8, 24, or 48 h post-dose. The PK measurement was performed using anti-human antibodies for capture and detection. N=3 mice/construct/time point. Half-life ($T_{1/2}$) was estimated using noncompartmental analysis using the analysis software "Moment.xls ver. 971107. B) Drug concentrations were measured in brain homogenates at 1, 3, 8, 24, or 48 h post-dose. Maximum concentration (C_{max}) and area under the curve (AUC) were estimated using a noncompartmental analysis. *AUC_{INF} could not be extrapolated for ACU193 due to low concentration and flat slope, therefore AUC_{48h} is reported for ACU193 instead.

- Higher brain exposure was observed for all fusion proteins compared to ACU193.
- For mBBB.193-1, greater affinity to mTfR corresponded with a more rapid brain accumulation, the highest maximum brain exposure (**21-fold** higher concentration than ACU193 at C_{max}), and more gradual clearance from the brain and plasma.
- mBBB.193-2 and mBBB.234-2 showed a more gradual brain accumulation, the greatest cumulative exposure (**32- to 55-fold** higher AUCs than ACU193), and more gradual clearance from the brain and plasma.

In ARTE10 mice, EBD fusion proteins showed increased brain exposure compared to sabirnetug (ACU193) and engaged AβO target in the brain



- As observed in the WT mice, all three fusion proteins were detected in the brain homogenates at comparable levels 24 h after dosing.
- The brain exposure of the three fusion proteins was **63- to 68-fold** higher compared to ACU193 and ACU234.
- All three fusion proteins crossed the blood-brain barrier and engaged their intended AβO targets to a similar extent at 24h post-injection.

Figure 7. Drug concentrations and target engagement in ARTE10 mouse brains 24 h post-injection. A) The drug concentrations were measured in the detergent-soluble brain homogenates at 24 h post-dose. N=5 mice/construct. Any sample below limit of quantitation (4/5 animals in each of ACU193- and ACU234-treated groups) are reported as 0. Lines indicate mean values, which were used in calculation of fold-changes. B) Target engagement was measured in the water-soluble brain homogenates at 24 h post-dose. N=5 mice/construct; lines indicate mean values.

EBD RESEARCH HIGHLIGHTS

- The J-Brain Cargo technology showed promising increases in brain exposure of sabirnetug and ACU234 using murine scFvs targeting the TfR.
- The fusion of the AβO-selective antibodies sabirnetug and ACU234 to scFvs did not alter the AβO/monomer selectivity profile.
- The EBD constructs showed differential pharmacokinetic properties and elevated brain exposure levels in both wild-type and ARTE10 mice.

References: 1. Sonoda, et al., Mol Ther. 2018; May 2;26(5):1366-1374. 2. Pizzo, et al., Science 2025; Aug 7;389(6760):611-612. 3. Alzheimer's Research forum 2023 Nov 11 https://www.alzforum.org/news/conference-coverage/unlocking-blood-brain-barrier-boosts-immunotherapy-efficacy-lowers-aria. 4. Lambert, et al., J Neurochem 2001; 79 (3):595-605.