Target Engagement in INTERCEPT-AD: Development of a Novel Assay Measuring Sabirnetug (ACU193)-Amyloid Beta Oligomer Complexes in Human CSF

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Introduction

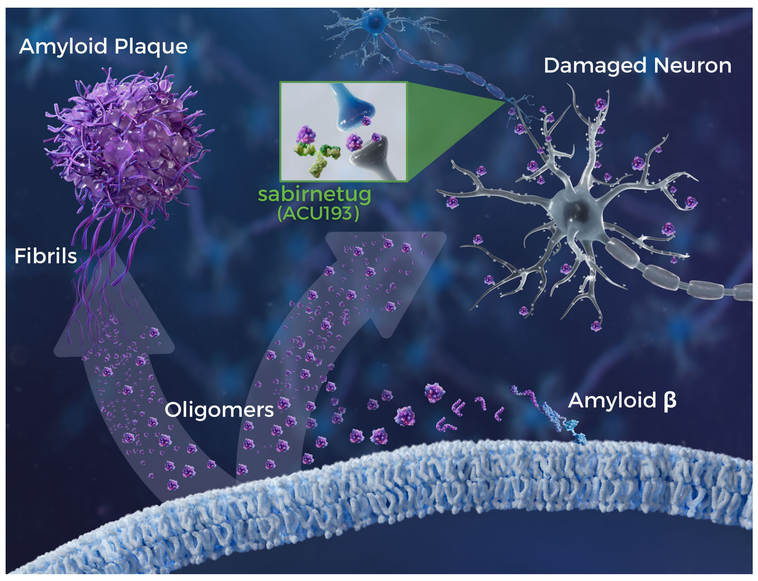

Figure 1. Sabirnetug (ACU193) is highly selective for soluble, synaptotoxic amyloid β oligomers (AβOs). In human neuronal membranes, Aβ monomers are cleaved from the amyloid precursor protein. Due to high hydrophobicity, Aβ monomers readily aggregate to oligomers (including protofibrils) or to larger insoluble amyloid fibrils, the primary component of amyloid plaques. The solubility of AβOs enables them to diffuse in the cellular milieu and bind to neuronal synapses. Their synaptic toxicity elicits downstream effects such as tau hyperphosphorylation, calcium dysregulation, and inhibition of long-term potentiation, ultimately resulting in the neuronal degeneration and cognitive impairment associated with Alzheimer's disease (AD).[1] **Sabirnetug is > 500-fold selective for AβOs over Aβ monomers[2] and > 85-fold selective for AβOs over Aβ fibrils.[3]**

GOAL: Aβ-targeting immunotherapies have traditionally used plaque reduction (assessed by PET) as a measure of target engagement. **Because sabirnetug selectively targets AβOs, we aimed to develop a novel target engagement assay to measure the sabirnetug-AβO complex in CSF.**

(1) Cline et al., 2018, J Alzheimers Dis, 61(s1): S567-S610. (2) Krafft et al., 2022, Front Neurosci, 16: 848215. (3) Data on file

Methods

INTERCEPT-AD Trial Design

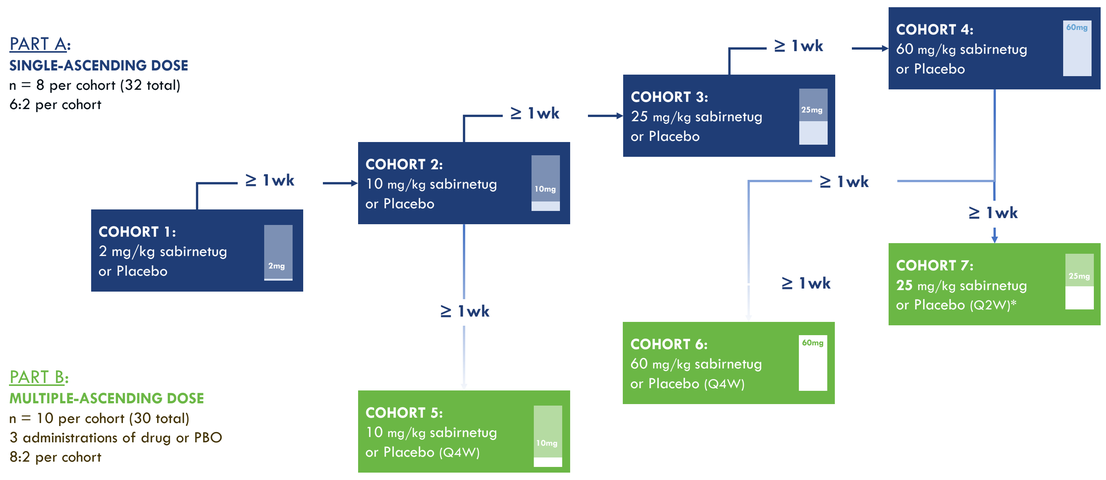

Figure 2. INTERCEPT-AD was a Phase 1 clinical trial testing the safety, pharmacokinetics, and pharmacodynamics **of sabirnetug (ACU193) in mild cognitive impairment and mild dementia due to AD** (NCT04931459). The dosing regimen and sample sizes are shown here. CSF was drawn from each study participant for assessment of central target engagement [as well as pharmacokinetics and biomarker responses] at two timepoints: before the first dose and 7-21 days after the last dose. *Q2W = every 2 weeks; Q4W = every 4 weeks.*

Target Engagement assay format on MSD S-Plex (Turbo) Immunoassay Platform

Figure 3. The target engagement assay for sabirnetug was designed to measure the sabirnetug-AβO complex in CSF. The assay was designed on the ultrasensitive MSD S-PLEX (aka Turbo) immunoassay platform. The sabirnetug specificity of the anti-idiotypic (anti-ID) capture antibody and the AβO-selectivity of the detection antibody yielded an assay highly specific and for the sabirnetug-AβO complex in CSF. A schematic presentation of the assay format is shown to the left.

Results

Antibody pair chosen for optimal detection of endogenous CSF AβOs

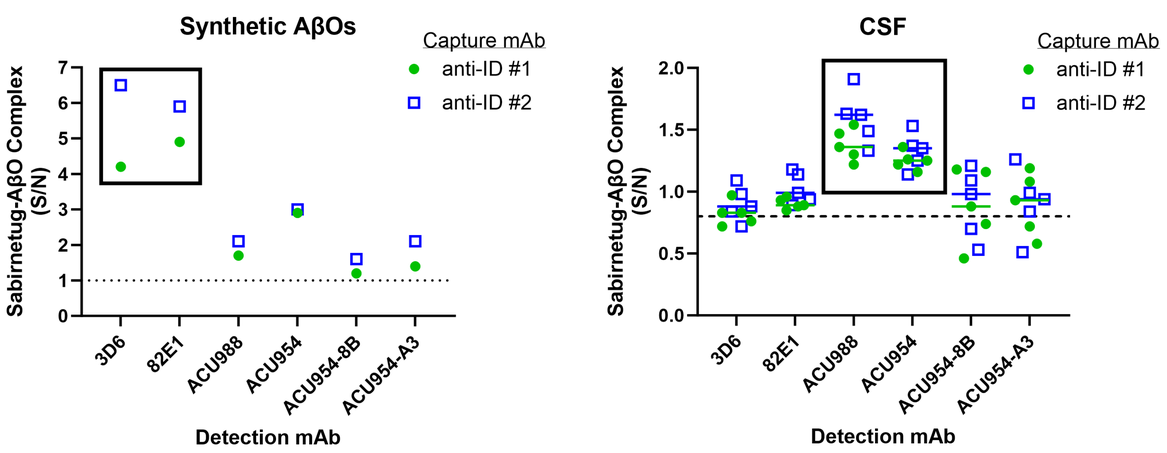

Figure 4. Assay comparison of sabirnetug complexed with synthetic AβOs or CSF AβOs highlights importance of including biological samples in assay development. The detection antibody had a significant impact on detection of synthetic AβOs (ADDLs > 50 kDa used here)[4] or CSF AβOs (sabirnetug spiked into human CSF) that appears to be most impacted by AβO selectivity rather than linear epitope of the antibody, suggesting conformational epitopes of the ACU-series antibodies (data not shown). n = 5 CSF samples. The CSF was a kind gift from Kaj Blennow (University of Gothenburg). S/N = signal/noise; mAb = monoclonal antibody.
(4) Savage et al., 2014, *J Neurosci*, 34(8): 2884-2897

Assay is specific for sabirnetug complexed with AβOs over Aβ monomers

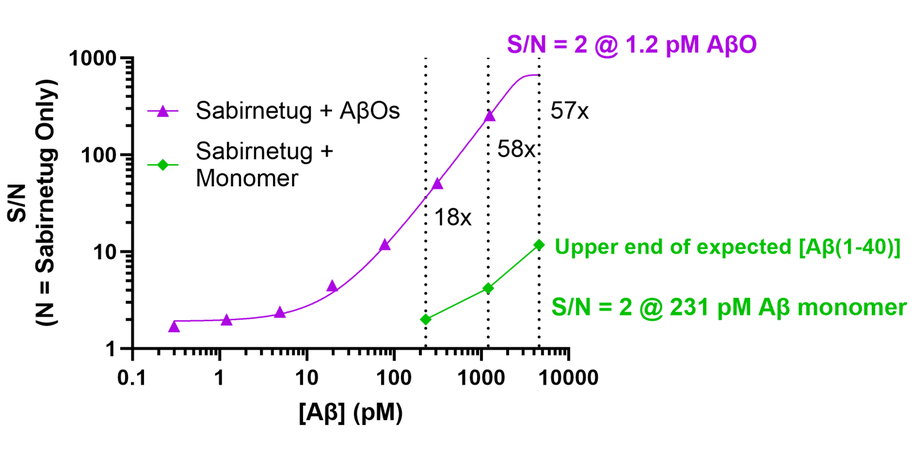

Figure 5. ACU988 was chosen as detection antibody to maximize detection of endogenous AβOs (Figure 4). Assay response to sabirnetug pre-incubated with oligomeric Aβ(1-42) or monomeric Aβ(1-40) was assessed at multiple concentrations (above) showing a 18-58x higher response for AβOs vs. monomer at matching concentrations and a 193x difference in the lower limit of detection (defined at S/N = 2). Note the interpretation of this data is complicated by the fact that Aβ(1-40) does form ACU988-immunoreactive oligomers in the time frame of the experiment (data not shown). By itself, sabirnetug has been shown to be > 500x selective for AβOs over monomer using experimental setups prohibiting monomeric aggregation.[2] S/N = signal/noise.

Sensitivity

Reproducibility

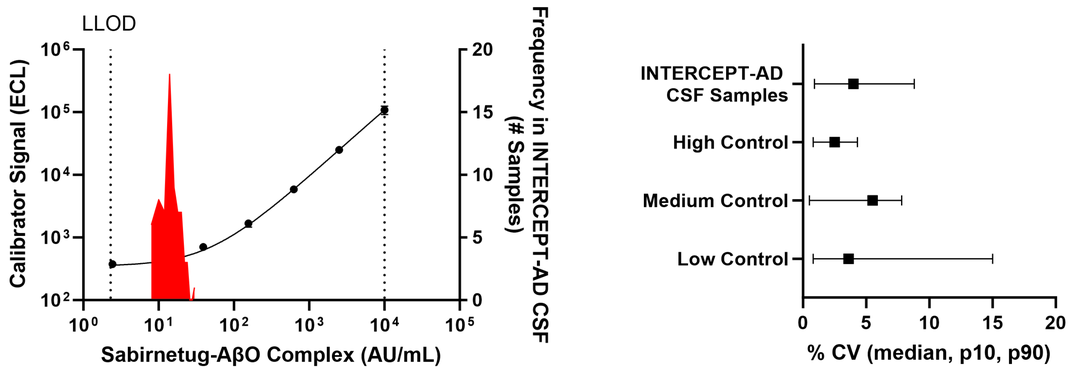

Figure 6. Assay achieves sufficient sensitivity to measure target engagement in INTERCEPT-AD CSF with reproducibility at < 20 % CV. Sensitivity, (left) The amount of sabirnetug-AβO complex in INTERCEPT-AD CSF was semi-quantitated against a calibrator of 900 pM sabirnetug (constant) and 10,000-2.44 pM AβOs (stabilized Aβ1-42 oligomers purchased from GBS Leiden); 1 AU/mL = 900 pM sabirnetug + 1 pM AβOs; AU = arbitrary units. 4PL non-linear regression was applied to the data (black), achieving a lower limit of detection (LLOD) of 2.31 AU/mL. Observed values in INTERCEPT-AD CSF samples plotted in red. **Reproducibility, (right)** % coefficient of variation (CV) of samples and controls (i.e., sabirnetug-AβO complexes in CSF) prepared at high, medium, low concentrations. P10, p90 = 10th & 90th percentile, respectively. INTERCEPT-AD samples run in duplicates.

INTERCEPT-AD Results

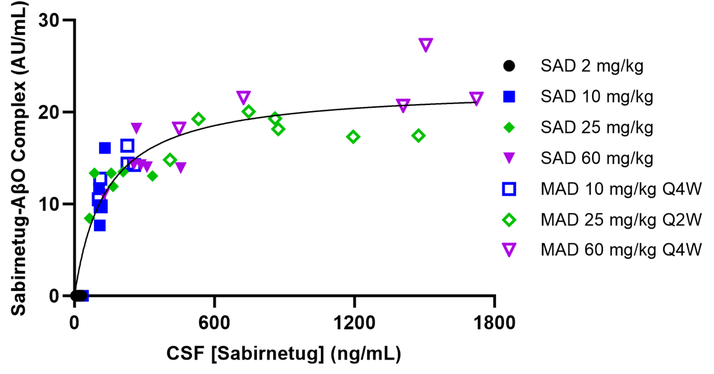

Figure 7. Application of assay to INTERCEPT-AD CSF shows dose-dependent target engagement at ≥ 10 mg/kg, approaching saturation at 25 mg/kg Q2W. The assay had sufficient sensitivity to measure the sabirnetug-AβO complex in all dose groups except for 2 mg/kg SAD (single ascending dose). An Emax model was applied to the data showing saturation at 22.71 AU/mL sabirnetug-AβO complex and half maximal response (EC50) at 136 ng/mL sabirnetug in CSF (data from a pharmokinetic assay). Results from this target engagement Emax model were presented by Erika Cline in a virtual poster at CTAD, October 2023.

RESEARCH HIGHLIGHTS

- A novel immunoassay was developed for the AβO-selective antibody sabirnetug, measuring sabirnetug-AβO complexes in CSF
- Comparison of assay performance with synthetic AβOs vs. CSF AβOs during assay development enabled optimization of the assay for detection of CSF AβOs
- The resulting assay proved specific for AβOs bound to sabirnetug with sufficient sensitivity to measure target engagement in the CSF of INTERCEPT-AD participants receiving a single dose of 10 mg/kg sabirnetug or more, indicating clearance of the sabirnetug-AβO complex from the brain
- Target engagement across INTERCEPT-AD cohorts was dose-dependent and approached saturation at the highest doses administered, which proved useful in dose selection for Ph 2/3 trial of sabirnetug in AD (presented by Mirjam Trame at CTAD, October 2023 in Boston, MA, USA).

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