Utility of human iPSC-derived neuronal model for evaluating synaptic binding of amyloid beta (Aβ) oligomers

Elizabeth Johnson[1], Hannah Maxwell[2], Miriam Scarpa[2], James Smith[2], Aitor Martinez-Zarate[2], Omar De Leon Velez[3], Kirsten Viola[3], James Cruse[4], Sanofar Jainul Abdeen[4], William Klein[3], Jasna Jerecic[1], Erika Cline[1]

(1) Acumen Pharmaceuticals Inc., Newton, MA, United States of America, (2) Talisman Therapeutics Limited, Cambridge, United Kingdom, (3) Northwestern University, Evanston, IL, United States of America, (4) B2S Life Sciences, Franklin, IN, United States of America

Objectives

- *Objective: Evaluate brain-derived Aδ oligomer (AδO) binding and antibody-based neutralization of AδO binding in human induced pluripotent stem cell (iPSC)-derived neurons.*
- Human iPSC-derived neurons present a biological model for the study of neurodegenerative diseases, with potential to reduce attrition rates seen in drug development due to species differences.
- AβO binding to synapses and downstream synaptic dysfunction have been demonstrated robustly in primary rodent neurons and transgenic animal models of Alzheimer's disease (AD).
- In this study, we examine synaptic binding of synthetic and human AD brain-derived AβOs to iPSC-derived neurons and evaluate the utility of this model for characterizing inhibitory monoclonal antibody therapies.
- Here we use the AβO-selective antibody sabirnetug, which is currently being evaluated for safety and efficacy in the ongoing phase 2 ALTITUDE-AD study (NCT06335173).

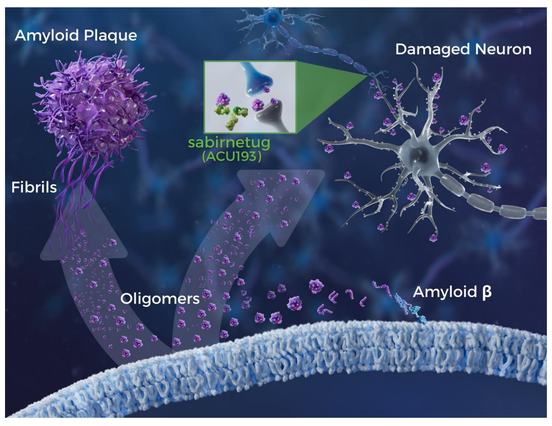


Figure 1. Sabirnetug targets soluble oligomers of amyloid beta. Amyloid beta peptides aggregate to form insoluble fibrils and various soluble conformers, including oligomers, which are targeted by sabirnetug.

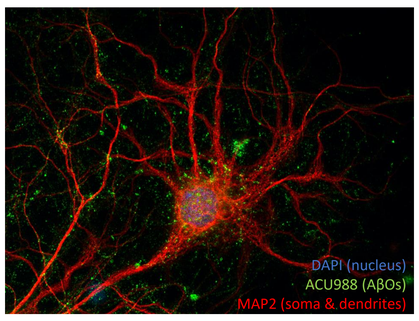
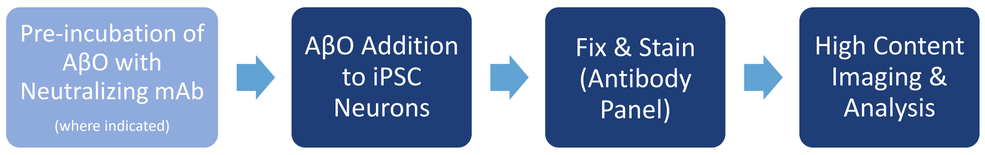


Figure 2. Binding of AβOs to primary rodent neurons. The image at left (data on file, Klein lab) shows punctate binding of synthetic AβOs (ADDLs prepared according to Lambert et al. 2001; labeled with ACU988, green) to rodent neurons (anti-MAP2, red) along dendrites & around cell body. AβOs have been previously demonstrated to bind dendritic spines in culture (Lacor et al. 2004).

Methods



Antibody Panel		
Antibody	**Source**	**Antigen/Epitope**
3D6	ADx	Aβ N-terminus
ACU988	Acumen	AβO
Anti-drebrin	Abcam	Actin-binding protein, localized in synapses (postsynaptic)
Anti-MAP2	Abcam	Microtubule-associated protein 2, in neuronal soma/ dendrites

Figure 3. Method to detect binding of soluble Aβ species to human iPSC-derived excitatory neurons. Human iPSC-derived neurons from non-demented control donors were generated and matured to ~70 days *in vitro*. Exogenous AβOs (synthetic ADDLs at 500 nM or soluble human AD brain extract) were applied for 0.5-4 h. For neutralization studies, AβOs were first preincubated with sabirnetug for 1 h. Immunofluorescent co-labeling used antibodies (antibody panel). Cell imaging and image analysis were performed using OPERA-Phenix high content imaging platform and automated image analysis algorithms for spot quantification and colocalization. Fields of view were filtered to remove areas with extremely high or low cell density. Colocalization was defined as being within 1 µm. Total number of spots, and spots within each class were calculated, and expressed as a mean per field of view.

Results

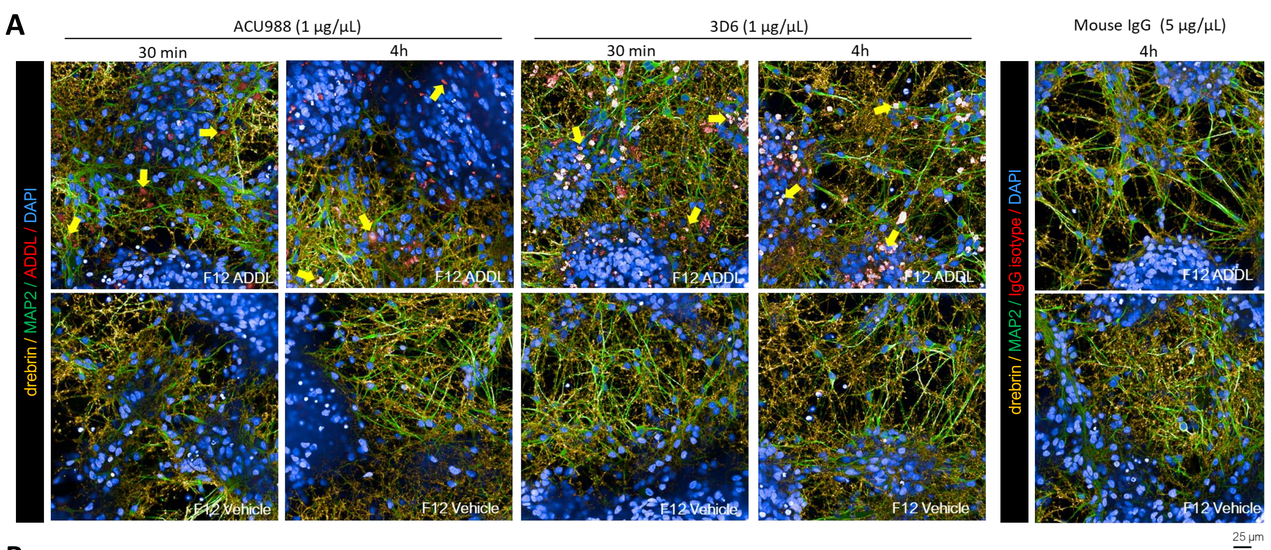
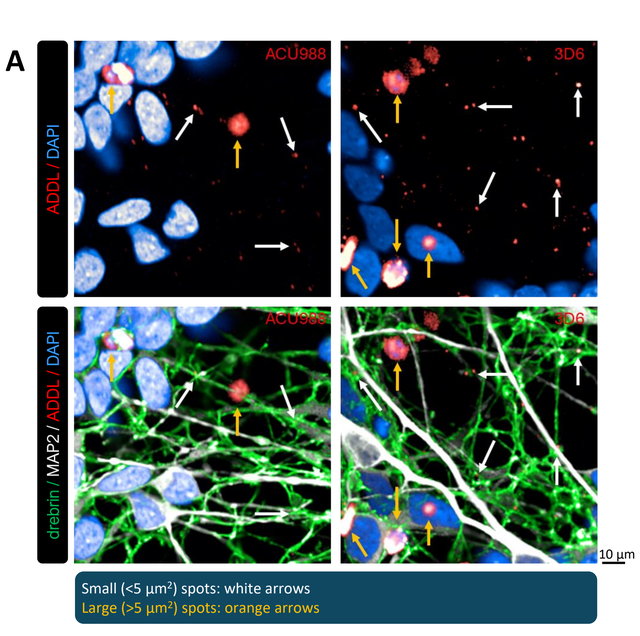


Figure 4. Synthetic AβOs bind to synaptic sites of human iPSC-derived neurons. Similar binding is observed in rodent neuron culture systems. Qualitatively, slightly less-dense binding is observed in human neuron cultures compared to rodent culture systems. This may represent a phenotype in which long-term exposure of low levels of AβOs is important. **A.** Immunostaining with an anti-Aβ antibody (3D6) and an anti-AβO antibody (ACU988) after synthetic AβO (ADDLs, 500 nM) application to iPSC-derived neurons (30 min, 4 h) demonstrates rapid binding of AβOs (yellow arrows) as puncta colocalized with the postsynaptic marker drebrin, which is consistent with literature findings in rodent cultures. No puncta are detected after Ham's F12 vehicle application or by murine IgG immunostaining (right panel). **B.** Exogenous AβO (ADDLs, 500 nM) binding to human iPSC neurons detected by both ACU988 and 3D6 increases slightly with longer incubation time (4 h vs 30 min), as shown by quantification of ADDL spot count colocalized with drebrin. Data are plotted as mean ± SD from n=3 wells per condition, 74 fields of view per well.



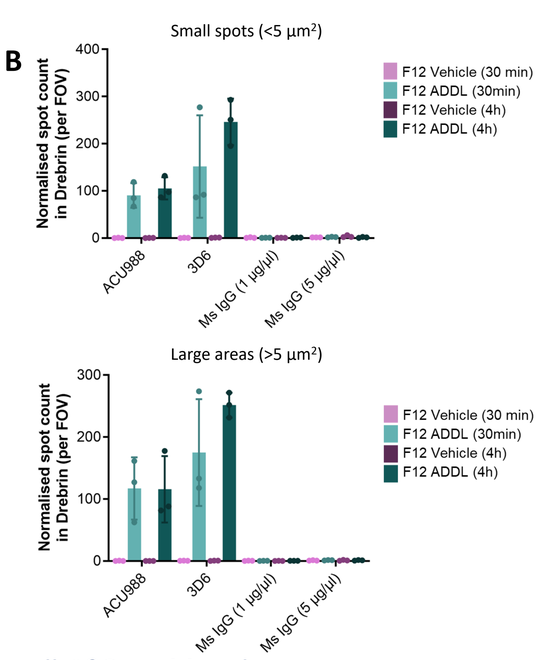


Figure 5. Imaging analysis identifies distinct large and small AβO-positive clusters. Large and small oligomer-positive spots observed in this study are also observed in rodent culture. Small spots correspond to the typical sizes reported for oligomers. Large spots may represent larger aggregates, and their tendency to bind in or near cell soma may reflect preferential binding or a mechanism of further aggregation in a cellular environment. **A.** After 1 h application to neurons, AβO (ADDLs, 500 nM) binding appears as small punctate spots along dendrites (white arrows) & large spots, in some cases located closer to cell soma (orange arrows). Both types of spots colocalize with the actin-binding, synaptic protein drebrin. **B.** Quantification of small and large ADDL-positive puncta shows comparable detection of small and large spots with ACU988 & 3D6. Data plotted as mean ± SD from n=3 wells per condition, up to 45 fields of view per well.

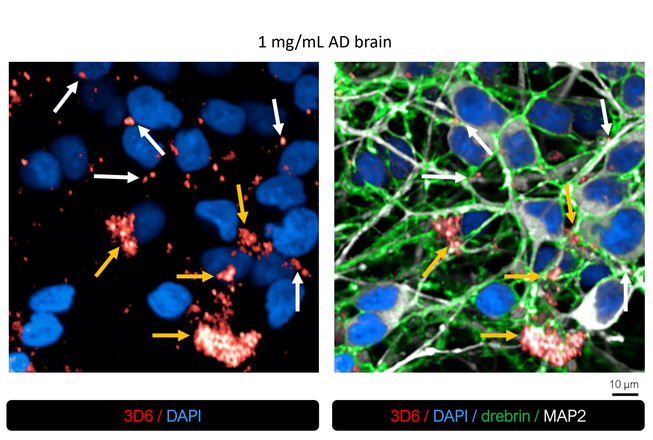


Figure 6. AD brain-derived AβOs demonstrate synaptic binding in human iPSC-derived neurons. Brain tissue samples were kindly provided by the Mesulam Center for Cognitive Neurology and Alzheimer's Disease at Northwestern University Feinberg School of Medicine and correspond to one individual each with AD (63 yo M) and without AD (75 yo F). Soluble extracts were prepared in Ham's F12 media. Human iPSC-derived neurons were incubated for 1 h with soluble extracts from AD or non-AD brain (1, 0.3, and 0.1 mg/mL total protein), 500 nM F12 ADDLs (positive control), or F12 vehicle (negative control). Staining with 3D6 antibody after treatment with AD brain extract demonstrates large (orange arrow) and small (white arrow) Aβ-positive puncta, which are colocalized with drebrin (right panel, spots <5 µm²; similar results for spots >5 µm² not shown). Significantly higher spot count is observed following application of AD brain extract compared to the negative controls and non-AD brain (right panel). Data are plotted as mean ± SD from n=3-4 replicate wells, up to 45 fields of view per well. Stars indicate significance in 1-way (purple) or 2-way (green) ANOVA with Tukey's multiple comparisons test (α=0.05). *** indicates p < 0.001, **** indicates p < 0.0001.

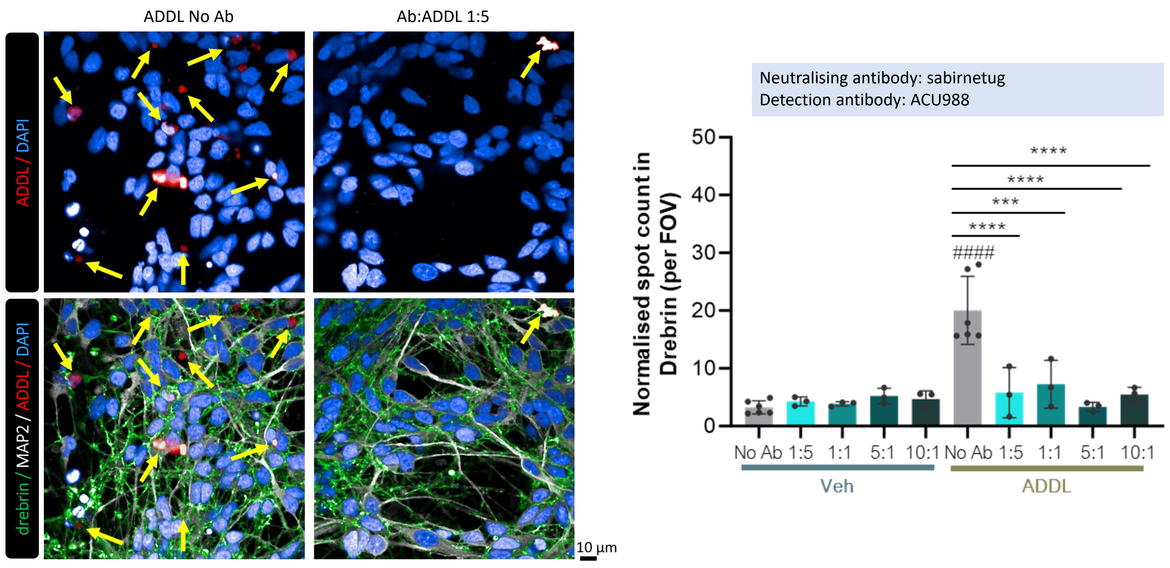


Figure 7. Sabirnetug prevents AβO synaptic binding to iPSC-derived neurons. Synthetic AβOs (ADDLs, 500 nM) or F12 vehicle were first preincubated for 1 h at 37 °C with sabirnetug at 1:5, 1:1, 5:1 and 10:1 molar ratios of antibody:ADDL (note: ADDL concentration calculated as moles of Aβ peptide), followed by application to iPSC-derived neurons for 1 h. Sabirnetug at all concentrations significantly reduced the number of <5 µm² ADDL puncta binding to synapses, detected by anti-AβO antibody ACU988. No such effect was observed after ADDL pre-incubation with a human isotype control, supporting a specific effect by sabirnetug (data not shown). Data are plotted as mean ± SD from n=3-6 replicate wells; #### indicates significance (p < 0.0001) compared to 'vehicle: no sabirnetug' condition. Stars indicate significance in 2-way ANOVA with Tukey's multiple comparisons test (α = 0.05). *** indicates p < 0.001, **** indicates p < 0.0001.

RESEARCH HIGHLIGHTS

- Both synthetic and human AD-brain derived AβOs bind to the drebrin-positive synapses of human iPSC-derived neurons.
  - Similarly, synaptic binding of both types of AβOs to rodent neuron culture is also documented in the literature.
- The drug candidate sabirnetug inhibits AβO binding to synapses. Sabirnetug is being evaluated for safety and efficacy in the ongoing phase 2 ALTITUDE-AD study (NCT06335173).
- This human iPSC-derived neuron model shows promise for evaluation of the synaptic mechanism of action of AβOs and AβO-targeting antibodies. Experiments involving longer duration of AβO exposure may be useful in developing a model for downstream effects of AβOs.