Synaptic intervention in Alzheimer's disease: soluble Aβ oligomer directed ACU193 monoclonal antibody therapeutic for treatment of early Alzheimer's disease

New therapies and clinical trials

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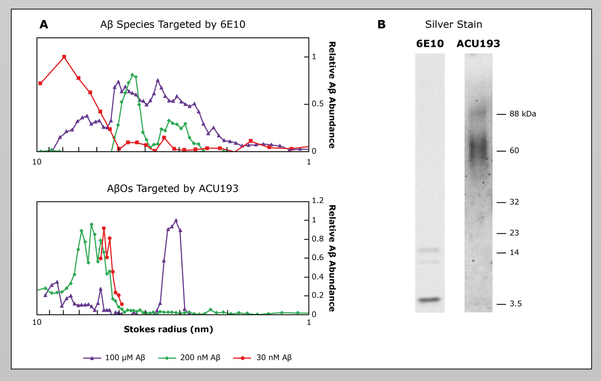
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Abstract

The number of patients with Alzheimer's disease (AD) is increasing worldwide as the population ages, and thus attempts to develop disease modifying treatments for AD have been unsuccessful, despite the realization that the time between the onset of pathological changes and the onset of clinical symptoms may be up to 20 years. The efforts to develop disease modifying therapies have focused mainly on the monomeric Aβ peptide and deposited amyloid plaques, and more recent efforts have begun to target tau and neurofibrillary tangles. Studies of soluble amyloid β oligomers (AβOs) (also known as Aβ-derived diffusible ligands, AβOs) more than two decades ago showed synaptotoxic properties and functional impairment of neurons, suggesting a role of AβOs as a potential cause for synaptic dysfunction and neurodegeneration in AD. With that understanding, ACU193, a humanized monoclonal antibody, was developed using soluble Aβ oligomers as the antigen. ACU193 binds a broad spectrum of small to large soluble Aβ oligomers. ACU193 showed preferential affinity for AβOs vs monomeric Aβ and Aβ plaque, and no visible binding to vascular amyloid was present. In vitro studies have shown that ACU193 and its murine analogue ACU3B3 restored long term potentiation (LTP) in rat hippocampal slices incubated with Aβ1-42. AβO toxicity can be measured in vitro by an increase in intracellular synaptic calcium concentration, and this increase is blocked by ACU193 administration. Further, subchronic administration of ACU3B3 to transgenic J20 (Swedish and Indiana mutations) and hAPPSL (Swedish and London mutations) mice, which overexpress the mutated human amyloid precursor protein, reduced a variety of memory and behavioral deficits seen in these animals. The differentiation in binding specificity of ACU193 compared to other previously studied monoclonal antibodies may provide enhanced efficacy in the clinic. IND-enabling toxicology studies for ACU193 are planned, and a Phase 1 trial is expected to begin in 2020.

ACU193 selectivity

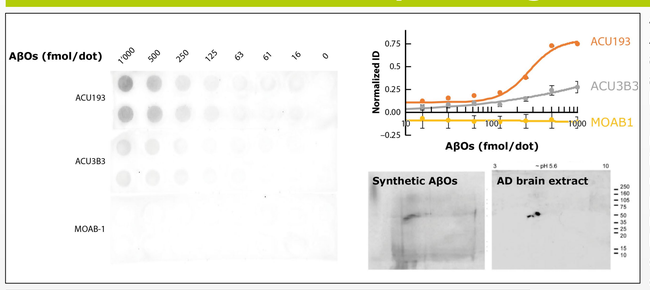
ACU193 targets a broad range of soluble oligomeric Aβ species



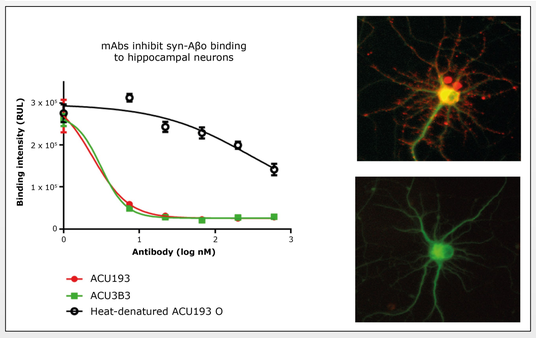
ACU193 targets a broad range of soluble AβO species with distinct conformations from 6E10 targets. Analysis under non-denaturing (A: size exclusion chromatography with dot immunoblot detection) and denaturing (B: SDS-PAGE with Western blot detection) conditions shows that ACU193 targets a broad range of AβO conformations. For non-denaturing analysis (A), AβOs were formed at 100 μM (purple), 200 nM (green), or 30 nM (red) Aβ; for denaturing analysis (B), AβOs were formed at 100 μM Aβ. Modified from Cline, et al., 2019, J Neurochem, 148(6): 822-36.

ACU193 Functional and behavioral efficacy

ACU193 and its murine precursor ACU3B3 bind a synthetic preparation that is structurally homologous to brain-derived AβOs

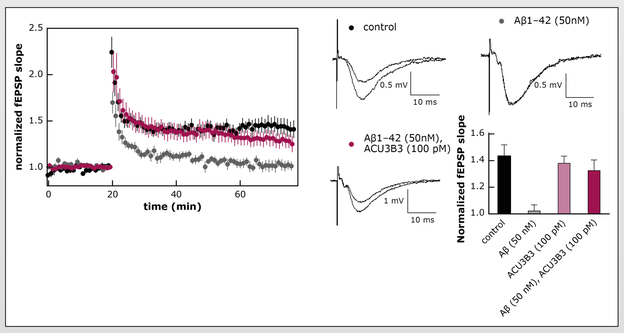


ACU193 and its murine precursor ACU3B3, but not MOAB-1, detect a synthetic AβO preparation previously shown to be structurally homologous to brain-derived AβOs. AβOs were prepared at 100 μM in Ham's F12 and titrated onto nitrocellulose for detection via dot immunoblotting (left). All primary antibodies (indicated left of blots) were utilized at 1 μg/mL. Quantification was done in ImageJ; non-linear (y = D + [(A–D)/(1+(x/C)^B)]) or linear (MOAB1, only) regression was fit to the data in Excel (top right). Bottom right) 2D electrophoresis of this synthetic AβO preparation compared to AβOs present in AD brain extract (modified from Gong, et al., 2003, PNAS, 100(18):10417-22).



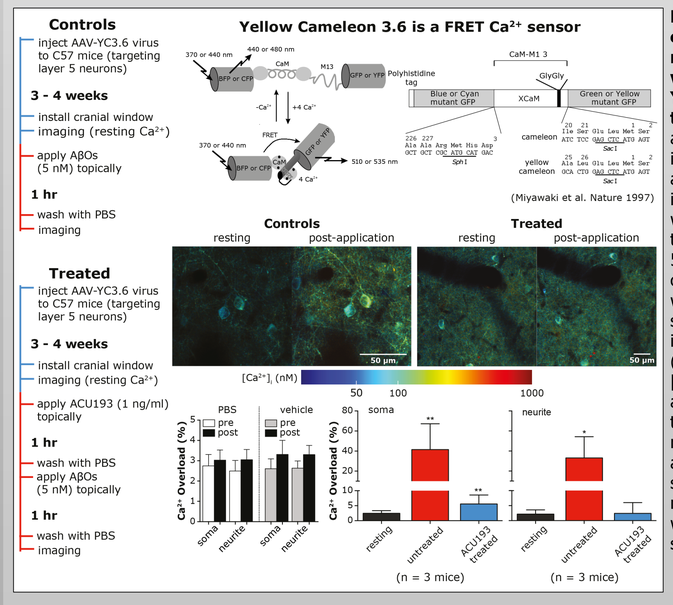
Biotinylated AβOs (500nM) were incubated with increasing concentration of ACU193 and the murine precursor ACU3B3 on DIV21 primary hippocampal neurons. Heat denatured ACU193 was used as control (left). Binding inhibition was detected using Streptavidin-AP antibody and Tropix CPD Star substrate luminescence. (A) Fluorescent microscopy shows synaptic binding of biotinylated AβOs (red) in MAP2 positive rat primary hippocampal neurons (DIV21; green) in vehicle treated neurons. (B) ACU193 inhibition and lack of synaptic AβO binding in MAP2 positive primary hippocampal neurons (green).

Reversal of AβO induced loss of LTP in rat hippocampal slices



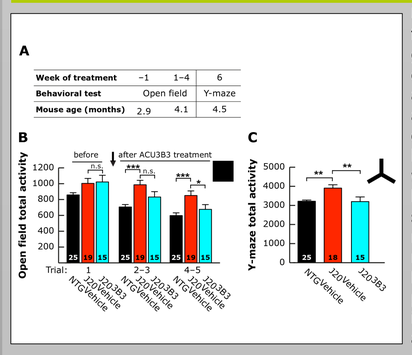
Effects of ACU3B3 (100pM) on AβO induced synaptic toxicity. Application of ACU3B3, the murine precursor of ACU193 (100pM) significantly reversed the synaptotoxic effects of AβO (50nM) (**p=0.0038). The diagram shows control LTP, LTP in the presence of AβO (50nM) and LTP with ACU3B3 (100pM) + AβO (50nM). In the presence of ACU3B3 (100pM) and AβO (50nM) field post synaptic potentials (fEPSPs) were potentiated to 132.5 ± 8.0% (mean ± SEM). The insets show representative fEPSP traces of the respective experiments before and after high frequency stimulation (HFS). The bar diagram shows the mean value of the normalized fEPSP slope of the. measurements taken from minute 50-60 after HFS. Control LTP in the presence of ACU3B3 (100pM) showed a potentiation of 138.0± 5.4%.

Reversal of AβO-triggered dysregulation of cytosolic calcium concentration [Ca^{2+}]



Multiphoton microscopy to measure changes in neuronal [Ca2+]i in adult WT mice that had been virally transduced with the ratiometric calcium sensor YC3.6, targeted to layer 5 neurons in the somatosensory cortex. Imaging was accomplished through a cranial window installed above the injection site 3–4 weeks after viral vector injection. Pre-treatment imaging to establish baseline calcium levels was carried out first, followed by opening of the cranial window and direct application of 5 nM AβOs to the cortical surface. After a one-hour incubation, the brain surface was washed three times with phosphate-buffered saline (PBS), a new coverslip window was installed, and the animal was re-imaged (post-treatment) to determine changes in [Ca2+]i. A fluorescent angiogram was used as a reference, enabling alignment of pre-treated regions with the post-treatment regions in all the experiments. To assess antibody blocking capability, the cortical surface of the brain was pre-treated with 1 ng/mL ACU193, followed by three PBS washes prior to final exposure of the cortical surface to AβOs.

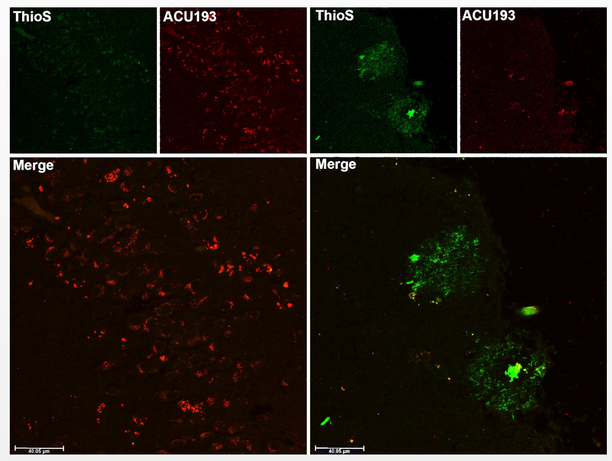
Reversal of behavioral deficits in young hAPPJ20 mice



Untreated hAPP J20 mice (n = 62 naive J20 mice) were tested in the open field (trial 1) and randomly assigned to vehicle or ACU3B3 experimental groups. Thereafter mice were monitored for open field activity 24h after each weekly injection (Fig. A). Open field activity data are presented as average of trials 2-3 (middle bars) or 4-5 (right bars). One-way ANOVA and Bonferroni test for open field trials 2–5 (after ACU3B3 treatment) confirmed that ACU3B3 treatment reduced the hyperactivity phenotype in hAPPJ20 mice at 10 mg/kg. *p<0.05, **p<0.01, and ***p<0.001 by one-way ANOVA and Bonferroni test. After 6 weeks of ACU3B3 treatment, total locomotor activity was assessed in the Y-maze (Fig. B). ACU3B3 treatment significantly prevented the hyperactive phenotype of hAPPJ20 mice at 10 mg/kg. Bars represent mean + SEM. Numbers in bars represent the number of mice. *p<0.05, and **p<0.01 by one-way ANOVA and Bonferroni test. In summary, the AβO selective antibody ACU3B3, murine precursor to the clinical development candidate ACU193 significantly improves behavioral deficits in young hAPPJ20 mice during early stages of amyloid deposition.

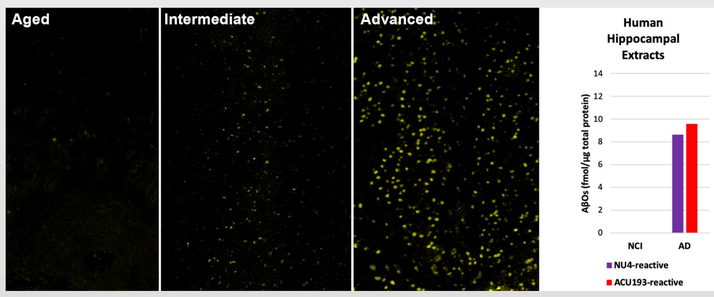
ACU193 in AD pathology

ACU193 shows minimal co-localization with ThioS-positive plaques



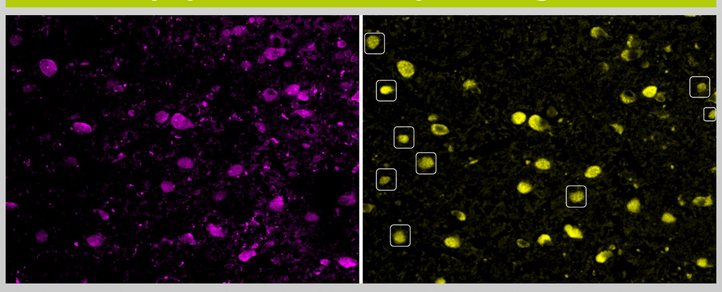
In human AD tissue samples, ACU193 shows minimal co-localization with plaques. In the advanced AD hippocampus, ACU193 detects AβO pathology (left panels) that is distinct from ThioS positive plaques (right panels). In the cell-dense regions of the dentate gyrus, the size of AβO deposits is smaller than a typical cell soma. In the neurite rich region of CA4, smaller, synaptic bouton-sized particles are observed (lower left corner). Imaged with a Leica SP5 confocal microscope. Scale bars = 40μm

ACU193 provides disease-relevant signal by both immunofluorescence and Erenna®



In human tissue samples, ACU193 shows a disease state-relevant signal both with immunofluorescence and with the ultra-sensitive Erenna® assay. Immunofluorescence labeling of human brain tissue (10mm) from aged (NCI, 76 y, F), intermediate AD (AD path-ADNC intermed, 100 y, F), and advanced AD (AD-ADNC high, 79 y, F) patients shows an increase in ACU193-positive pathology that corresponds with the disease state (first 3 panels). (Imaged with a BioTeck automated LionHeart microscope). On the far-right panel, ACU193 detects AβOs in soluble hippocampal extracts from an AD (41 y, F, PSEN1 mutation), but not a cognitively normal (NCI, 76 y, F) patient, as assayed using the Erenna® SMC™ platform (Singulex, EMD Millipore) with the anti-AβO antibodies NU4 (purple) and ACU193 (red).

ACU193 labels all pTau-positive neurons and a subpopulation that is pTau-negative



In human tissue samples, ACU193 detects AβOs in all neurons that are pTau positive, but also occurs in neuron that are pTau negative. On the left, a population of neurons is labeled with AT8, a marker for AD pTau. On the right, the same section is stained with ACU193. All pTau positive neurons are also positive for ACU193, but a subset of ACU193 positive hippocampal neurons are negative for pTau (shown in boxes). Results suggest that ACU193 detects AD pathology that may precede hyperphosphorylation of Tau. Images captured by a BioTeck automated LionHeart microscope.

Acknowledgements

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