Biomarker Outcomes from the Phase 1b/2a Safety Trial of the Anti-Aβ Oligomer Drug CT1812 in Alzheimer's Patients

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ABSTRACT

Background: CT1812 is the only therapeutic candidate demonstrated to displace Aβ oligomers from synaptic receptor sites and clear them from the brain into the cerebrospinal fluid, restoring normal cognitive performance in aged transgenic mouse models of AD. Chronic treatment of aged transgenic mice with efficacious doses of CT1812 significantly reduces inflammatory protein expression in CSF, and normalizes Alzheimer's disease-related protein expression in CSF and plasma as measured by LC/MSMS. CT1812 appears safe and well tolerated with multiple doses up to 560 mg/day in healthy elderly volunteers (ClinicalTrials.gov NCT02570997). To further the clinical development of CT1812, we completed a clinical trial in mild to moderate Alzheimer's patients to evaluate protein biomarkers as well as safety (ClinicalTrials.gov NCT02907567).
Methods: A multi-center, double-blind, placebo-controlled parallel group trial was performed to evaluate the safety, tolerability and pharmacokinetics of three doses of CT1812 (90, 280 and 560 mg) or placebo (N = 4 or 5 patients/group) given once daily for 28 days to Alzheimer's patients (MMSE 18-26). Plasma and CSF protein expression were measured by LC/MSMS in samples drawn prior to dosing (Day 0) and at end of dosing (Day 28) and were compared within each patient and between dosing groups.
Results: LC/MSMS analysis resulted in the identification and relative quantitation of 911 CSF proteins and 1965 plasma proteins across all subjects. Changes in expression of specific proteins were observed in both CSF and plasma following treatment with study drug. Multiple proteins were upregulated in the CSF in response to drug. These include proteins previously linked to AD and proteins involved in axon guidance/CNS development, all of which could be expected to increase with disease reversion. The relationship between protein function and disease, and association with therapeutic target receptor pathways will be reported in detail, along with additional CSF outcomes including Aβ 40, 42 tau and p-tau.
Conclusions: Treatment of Alzheimer's patients with study drug once daily for 28 days results in protein expression changes in plasma and CSF as measured by LC/MSMS. Along with safety and clinical outcomes, the protein expression outcomes will help guide the future development of CT1812. Additional trials include an indwelling lumbar catheter study to detect changes in Aβ oligomers in CSF, a PET study to assess synaptic density after treatment with CT1812 and a longer term Phase 2 efficacy trial.

METHODS

COG0102 clinical trial: This trial (ClinicalTrials.gov NCT02907567) was a randomized, double-blind, placebo-controlled clinical trial in mild to moderate Alzheimer's patients (MMSE 18-26). The trial was conducted at six sites in Australia. Patients were randomized into placebo (n = 5) or CT1812-treated groups: 90 mg (n = 4), 280 mg (n = 5) or 560 mg (n= 5) and treated once daily for 28 days. Clinical chemistry was done at Sydpath (Sydney, AU). Pharmacokinetics were done at CPR Pharma Services (Adelaide, AU). Cerebrospinal fluid samples were taken at screening and day 21-28 of treatment and aliquoted. Plasma samples were taken at screening or Day 1 (one sample) and day 14-28, then spun down to separate plasma and aliquoted. One set of CSF samples was analyzed for AD biomarkers; protein measurements (Aβ42, Aβ40, total tau, tau phosphorylated at threonine 181 (phosphorylated tau), NFL and neurogranin) were performed using commercially available ELISA assays from Fujirebio according to the manufacturer's instructions (INNOTEST β-AMYLOID(1-42), INNOTEST β-AMYLOID(1-40), INNOTEST hTau Ag, INNOTEST PHOSPHO-TAU(181P), Uman Diagnostics NFL assay). Neurogranin was measured with a sandwich ELISA method using antibodies Ng2 and Ng22 developed at the University of Gotteborg (Kvartsberg et al. 2015). Identical aliquots of CSF, and plasma were analyzed for protein expression changes via LC/MSMS at Caprion (see below).

Neurogranin expression study in vitro: 0.5uM synthetic Aβ oligomers were added to DIV21 hippocampal/cortical cultures (described in Izzo et al., 2014 a,b) for 1 hour prior to 4.8 nM of CT1812, then incubated for 24 hours. Neurons were fixed in 3.75% formaldehyde and stained with full-length neurogranin (Abcam catalogue# ab23570, used in combination with novel Ng7 to detect full length neurogranin in AD patient brain IP; Kvartsberg et al. 2015 Blennow lab), MAP2 (Millipore), and 4G8 (Biolegend) antibodies. Imaging was performed on Cellomics VX automated microscope with a 20X, 0.75 NA objective and analyzed using a compartmental analysis algorithm to measure staining in the nucleus versus cytoplasm.

LC/MSMS biomarker discovery: Matched screening and day 28 plasma and CSF samples were analyzed by LC/MSMS at Caprion Biosciences Inc. Plasma was depleted of high and medium abundance proteins using a commercial immunoaffinity column, IgY14-Supermix (Sigma). CSF was depleted of only 14 high abundance proteins using MARS-14 (Agilent). All samples were digested with trypsin and plasma samples were fractionated by strong cation exchange chromatography (3 fractions). Both CSF and plasma samples were analyzed using a nanoAcquity UPLC coupled to a Q Exactive MS. Peptide separation was achieved with a nanoAcquity Symmetry UPLC Trap column and nanoAcquity UPLC BEH300 analytical column. The 12 most intense peaks per survey scan with charge states 2-8 fragmented and scanned with a mass range from 200 to 2000 m/z at a resolution of 17,500. Raw spectrometer data files for each LC-MS run were aligned independently using Elucidator software (Rosetta Biosoftware). The MS/MS spectra were matched to corresponding peptide sequences found in the Uniprot Human protein database (January 2017) using Mascot software, allowing for up to 2 missed cleavages, a peptide tolerance of 20ppm, and an MS/MS tolerance of 0.05Da. Outlier detection was performed by determining the average log-intensity of all isotope groups (IG) over injection order for all samples. Samples with an average value greater than 2 standard deviations from the mean were flagged for investigation. Following data transformation and normalization, expression analysis of the identified isotope groups was performed and the statistical significance of each comparison was assessed with a parametric, linear mixed model (LMM) and a non-parametric Wilcoxon ranked test (ranked p-values). Expression analysis was also performed at the peptide and protein levels, which used the same methodology as above, but applied to peptide/protein intensities, derived by rolling-up the corresponding isotope group intensities. Isotope groups not detected in at least half of the samples in either of the two groups being compared were not used for the roll-up.

Heat map analysis: The difference in protein abundance between day 28 and baseline for each sample was normalized for graphical comparison by converting to z-score. First, the mean and standard deviation (SD) of day 28 – baseline values were calculated across all paired samples for each protein. Then, for each patient's paired sample fold change, the difference from the mean was determined and divided by the SD to derive the z-score. These values, normalized by SD to the same scale, allow direct comparison of relative change from baseline in response to treatment for each protein in each subject.

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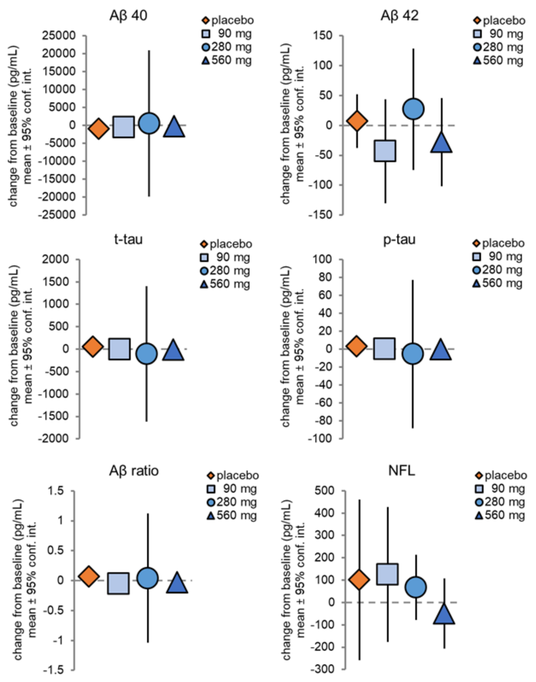
References

1. Izzo NJ, et al. Alzheimer's therapeutics targeting Amyloid beta 1-42 oligomers I: Abeta 42 oligomer binding to specific neuronal receptors is displaced by drug candidates that improve cognitive deficits. PLoS ONE 10: e0111898, 2014.
2. Izzo NJ, et al. Alzheimer's therapeutics targeting Amyloid beta 1-42 oligomers II: Sigma-2/PGRMC1 receptors mediate Abeta 42 oligomer binding and synaptotoxicity. PLoS ONE 10: e0111899, 2014.

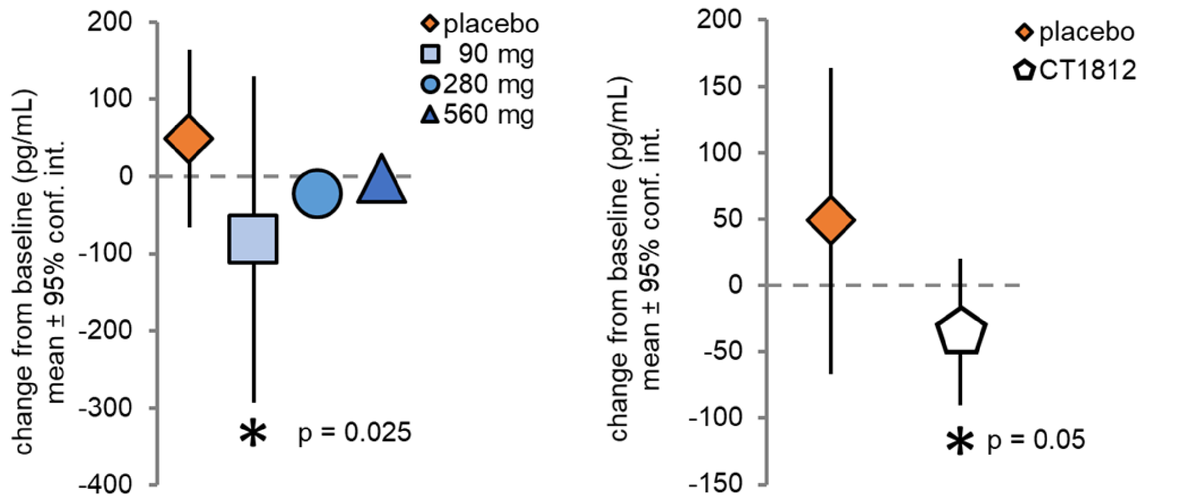
AD CSF BIOMARKERS

Aβ, tau, and NFL



- **No change in most biomarkers**

Neurogranin



- **Neurogranin (synaptic damage marker elevated in Alzheimer's CSF) *reduced* 33% in 90 mg dose group, 17.6% in the pooled CT1812-treated group**
- **Reduction consistent with a positive effect on synapses, CT1812's mechanism of action, and preclinical studies**

BIOFLUID LC/MSMS ANALYSIS

CSF

CSF: Treated (day 28 - Baseline) vs. Placebo (day 28 - Baseline)

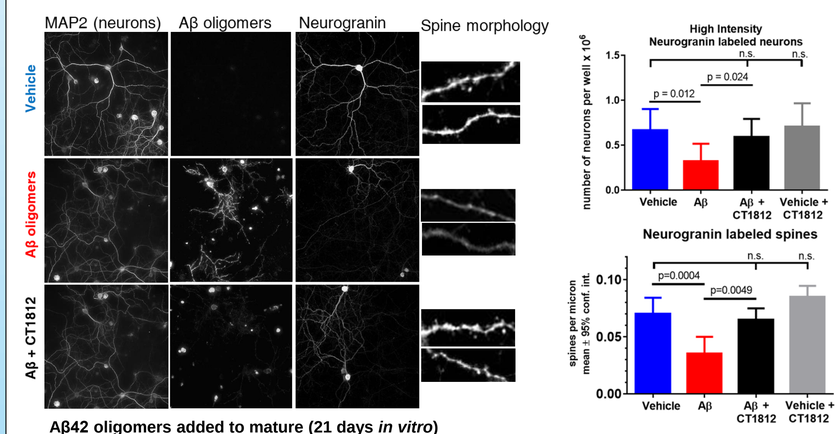
PROTEIN FUNCTION	Fold Change (Treated vs. Placebo)	p-value	rank p-value
enzyme inhibitor	0.81	**0.038**	0.148
vitamin transport	0.84	**0.002**	**0.003**
receptor ligand	0.91	0.060	**0.020**
receptor ligand	0.69	**0.050**	0.148
hydrolase	1.24	0.080	**0.034**
lipopeptide binder	1.20	**0.046**	**0.034**
cell adhesion	0.81	**0.048**	0.076
tyrosine kinase receptor ligand	0.93	**0.029**	**0.034**
tyrosine kinase receptor	0.93	**0.035**	0.076
growth factor receptor	1.83	**0.050**	0.260
synaptic plasticity	1.41	**0.019**	**0.020**
phosphorylation enzyme	1.26	0.089	**0.011**
cell adhsion	1.23	**0.029**	**0.034**
enzyme inhibitor	0.79	**0.047**	**0.020**
synaptic plasticity	1.22	**0.026**	**0.034**
immune receptor	1.25	**0.003**	**0.003**
receptor ligand	> 10	**0.020**	**0.006**
protein lipidation	0.71	**0.036**	0.106
growth factor receptor ligand	1.27	**0.046**	0.050
protein folding enzyme	1.21	**0.017**	**0.006**
protein folding enzyme	1.44	**0.000**	**0.003**
gluconeogenesis	0.81	**0.018**	**0.011**
axon guidance	0.82	**0.042**	0.106
axon guidance	1.62	**0.042**	0.076
chemotaxis	1.35	**0.046**	**0.034**
axon guidance	1.58	**0.046**	0.050
synaptic transmission	0.63	**0.011**	**0.011**
cell adhesion	0.91	**0.029**	**0.011**
gluconeogenesis	0.87	**0.044**	0.076

Plasma

PLASMA: Treated (day 28 - Baseline) vs. Placebo (day 28 - Baseline)

PROTEIN FUNCTION	Fold Change (Treated vs. Placebo)	p-value	rank p-value
ECM biosynthesis enzyme	0.52	**0.001**	**0.004**
enzyme inhibitor	0.62	**0.003**	**0.004**
immune signaling	0.59	**0.004**	**0.042**
DNA binding protein	2.02	**0.004**	**0.002**
synpatic plasticity	0.58	**0.005**	**0.042**
proteolysis	2.09	**0.006**	**0.001**
ECM biosynthesis enzyme	1.31	**0.007**	0.078
immune signaling	1.49	**0.008**	**0.030**
intracellular signaling	< 0.1	**0.008**	0.058
innate imune system	1.99	**0.009**	**0.020**
neuronal signal transduction	1.44	**0.012**	0.103
nervous system development	1.39	**0.013**	**0.020**
synaptic transmission	0.45	**0.014**	**0.042**
exosome protein	1.46	**0.014**	**0.030**
phosphatase enzyme	0.43	**0.014**	**0.013**
phosphatase regulator	2.42	**0.015**	**0.008**
ECM signaling	1.30	**0.015**	**0.042**
integral membrane protein	0.62	**0.016**	**0.020**
ECM signaling	0.56	**0.019**	0.103
kinase	0.31	**0.019**	**0.030**
calcium binding protein	0.47	**0.020**	**0.013**
phosphatase enzyme	0.43	**0.020**	**0.042**
integral membrane protein	0.26	**0.021**	**0.030**
growth factor signaling	1.46	**0.021**	0.103
immune signaling	0.24	**0.022**	0.058
calcium binding protein	0.65	**0.022**	**0.042**
glycoprotein	1.61	**0.023**	0.058
nuclear protein	0.40	**0.023**	**0.042**
cholesterol homeostasis	1.42	**0.023**	0.058
protein kinase	0.43	**0.024**	**0.020**

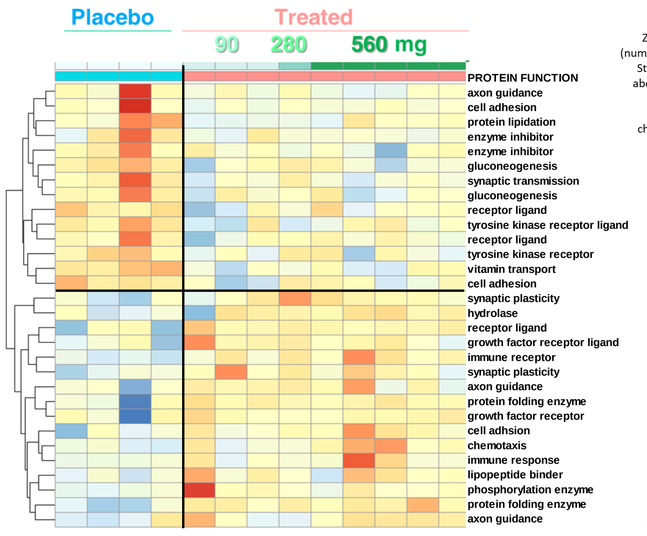
In vitro studies: Neurogranin



Aβ42 oligomers added to mature (21 days *in vitro*) hippocampal/cortical cultures (500 nM, 1 hour) followed by CT1812 (4.8nM) or vehicle treatment (24 hour)

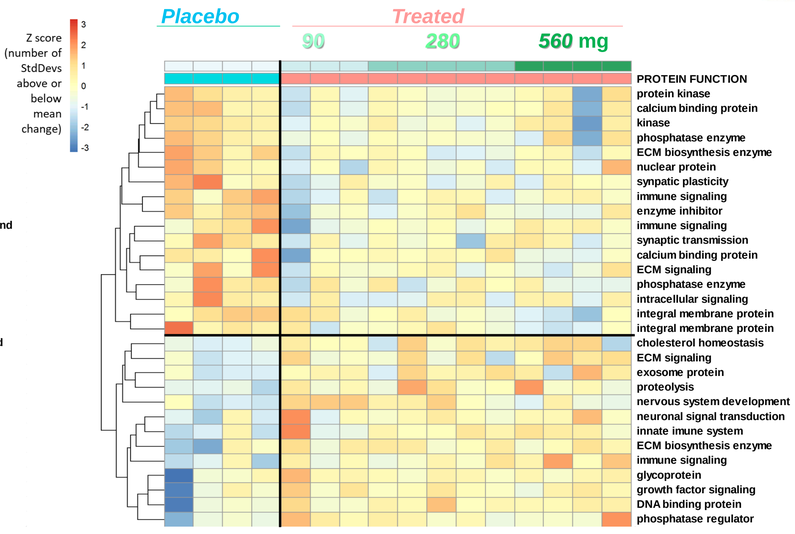
- **Aβ oligomer treatment decreases full length neurogranin protein expression in neurons**
- **Consistent with increase in cleaved, secreted forms of neurogranin in Alzheimer's CSF**
- **CT1812 treatment following Aβ42 oligomer exposure restores neurogranin expression**
- **Consistent with positive effect on synapses**

CSF

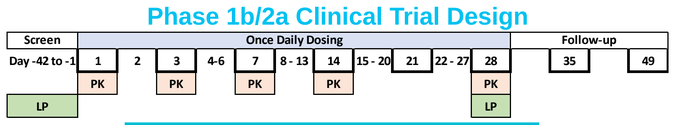


- **30 proteins changed differentially in CT1812-treated vs. placebo patients (p ≤ 0.05, i.e., higher or lower expression vs. placebo)**
- **Several play key roles in synaptic plasticity and are dysregulated in AD brain:**
  - **Synaptotagmin-1, a synaptic damage marker elevated in Alzheimer's CSF**
  - **Expression decreased 59% in CT1812-treated vs. placebo**
  - **Consistent with positive effect on synapses and CT1812's mechanism of action**

Plasma



Phase 1b/2a Clinical Trial Design



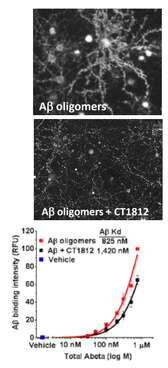
	Placebo	90 mg	280 mg	560 mg
Age	64.4 ± 8.9	71.8 ± 7.7	71.8 ± 12.4	73 ± 6.8
Sex M/F	2/3	3/1	3/2	1/4
MMSE	21.2 ± 3.1	24 ± 3.4	21.4 ± 2.6	21.8 ± 3

Conclusions:

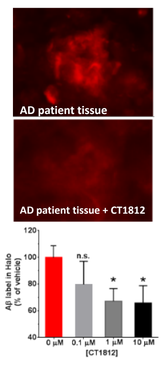
1. **CT1812 is safe and well tolerated across all doses, no SAEs**
2. **PK: Greater than 80% estimated brain receptor occupancy achieved at all doses (threshold needed to demonstrate efficacy in preclinical studies)**
3. **The concentrations of 30 CSF proteins changed differentially in the CT1812 treatment group versus placebo (p<0.05).**
4. **CSF synaptic damage markers decreased (neurogranin and synaptotagmin), consistent with a positive synaptic effect and CT1812's mechanism of action**
5. **Further studies with CT1812 are planned**

CT1812 mechanism of action: displacement of Aβ oligomer binding and clearance into the CSF

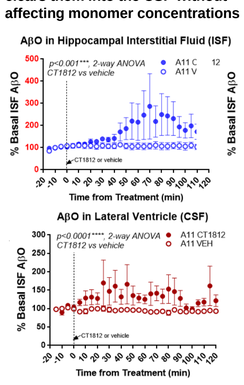
CT1812 displaces Aβ oligomers from neurons *in vitro*…



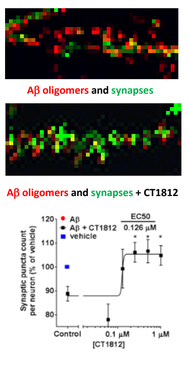
…and from AD patient neocortical tissue sections.



….as well as the hippocampus of living transgenic AD mice, and clears them into the CSF without affecting monomer concentrations



CT1812 restores synapse number to normal…



…and restores cognitive performance to normal in transgenic AD mice at brain concentrations >80% receptor occupancy (Minimum Efficacious Dose)