P075

PROTEOMIC ANALYSIS OF PLASMA IN A PHASE 2 CLINICAL TRIAL IN ALZHEIMER'S PATIENTS TO IDENTIFY PHARMACODYNAMIC BIOMARKERS OF THE S2R MODULATOR CT1812

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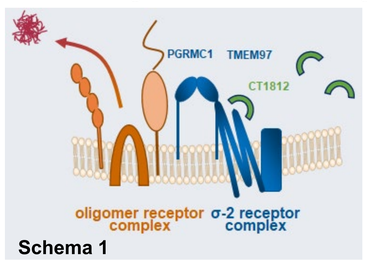
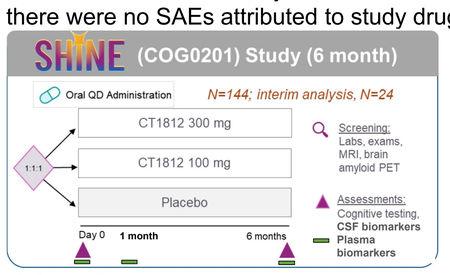
INTRODUCTION

The sigma-2 receptor (S2R) is encoded by TMEM97, a four-domain transmembrane protein that complexes with progesterone receptor membrane component 1 (PGRMC1). CT1812 is a brain-penetrant small molecule modulator of S2R, that displaces Aβ oligomers (AβO) from neuronal synapses[1] (**Schema 1**) and rescues AβO-induced neuronal trafficking deficits[1]. CT1812 protects synapses in neuronal cultures and rescues cognitive deficits in transgenic Alzheimer's disease (AD) mice[1].



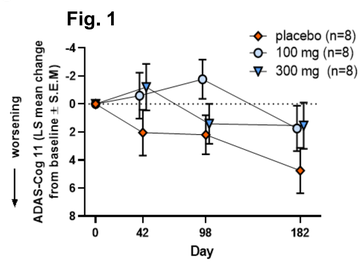
Schema 1

COG0201 (SHINE; NCT03507790), is a randomized, double-blind, placebo-controlled Phase 2 clinical trial designed to enroll 144 patients with mild-to-moderate AD to evaluate the safety and efficacy of CT1812. Participants are divided equally in two CT1812 dose groups and placebo for once daily oral dosing for 6 mo (**Schema 2**). Endpoints include safety, cognitive function, as measured by the AD Assessment Scale-Cognitive Subscale 11-item version (ADAS-Cog-11), and biomarker evidence of disease modification. An interim analysis of the first 24 patients was conducted (SHINE-A), which showed a non-significant, but clinically meaningful 3 pt difference in ADAS-Cog 11 (**Fig 1**). No subjects were withdrawn from the study due to treatment-emergent adverse events and there were no SAEs attributed to study drug.



Schema 2

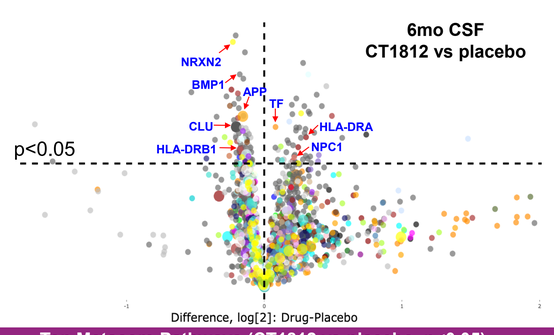
Fig. 1



Tandem-mass tag mass spectrometry (TMT-MS) followed by quantification of CSF proteomes change from baseline was performed on treatment-compliant patients for which a baseline and end of study CSF sample was taken (N=18) to assess treatment effects (CT1812 vs placebo) through differential expression and pathway analyses[2] (**Fig 2A,B**). Candidate pharmacodynamic biomarkers were identified (**Fig 2A**) and pathways affected illuminated that pointed to a role of CT1812 in regulating Aβ biology and neuronal signaling. (**Schema 4**).

Fig 2. CSF Proteomic Analysis Identified Pharmacodynamic Biomarkers and Pathways Impacted by CT1812

A



B

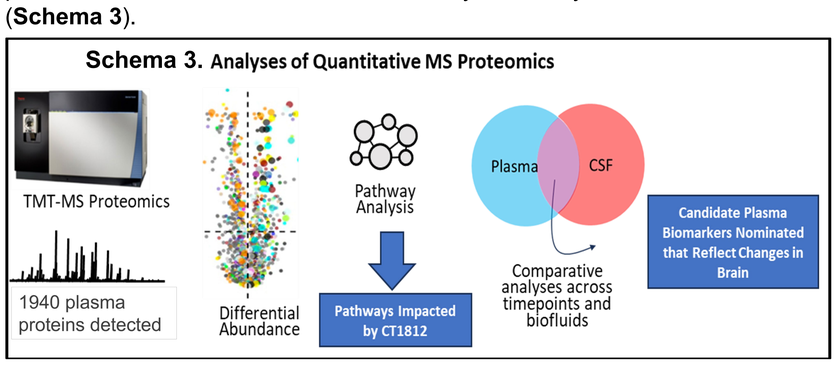
Top Metacore Pathways (CT1812 vs placebo, p≤0.05)	p-value
Protein folding and maturation: Amyloid precursor protein processing	9.14e-38
Gamma-secretase proteolytic targets	1.66e-10
Protein folding and maturation: Regulation of amyloid precursor protein processing	3.33e-09
Gamma-secretase regulation of neuronal cell development and function	2.53e-06
Neurophysiological process: ACM1, ACM3, and ACM5 signaling in the brain	2.01e-04
Cell adhesion: Desmosomes	2.86e-04
Aberrant lipid trafficking and metabolism in age-related macular degeneration pathogenesis	7.76e-04

The present study presents new findings from an unbiased assessment of plasma proteomes from the first 24 patients of SHINE to a) identify plasma pharmacodynamic biomarkers of CT1812, and b) to compare findings to that in CSF to identify pharmacodynamic plasma biomarkers of CT1812 that may reflect changes in brain.

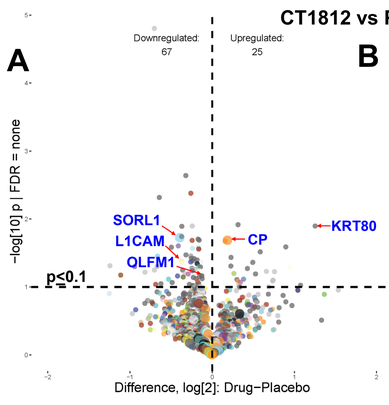
METHODS

Tandem-mass tag mass spectrometry (TMT-MS) proteomics was performed on baseline, 1 mo (N=22), and end of study (6 mo; N=21) plasma taken from the first 24 participants that completed the SHINE 6 mo trial (SHINE-A) and were treatment-compliant to test the effects of CT1812 (given orally, once daily) compared to placebo in mild to moderate AD patients. Treatment effects were assessed through differential abundance analyses using two statistical levels (p≤0.1, p≤0.05) followed by pathway analyses (MetaCore, v23.3.71400). Plasma proteomes were compared across timepoints and to a previous CSF proteomics analysis of SHINE-A to determine whether any plasma and CSF biomarkers were commonly altered by CT1812 within cohort (**Schema 3**).

Schema 3. Analyses of Quantitative MS Proteomics



Pharmacodynamic Plasma Biomarkers of CT1812 Identified After 1 Month of Treatment



B

Top 5 most robust ↑ changes

Protein	Uniprot ID	Log2 FC	p-value
KRT80	Q6KB66	1.25	1.27E-02
CTSW	P56202	0.90	2.57E-02
LILRB2	Q8N423	0.59	3.05E-02
ANGPTL2	Q9UKU9	0.49	4.35E-02
SCGB1A1	P11684	0.32	1.21E-02

Top 5 most robust ↓ changes

Protein	Uniprot ID	Log2 FC	p-value
STAT3	P40763	-1.24	4.87E-02
SEZ6L	Q9BYH1	-0.70	1.58E-05
KSR1	Q8IVT5	-0.67	4.95E-02
PDCD6IP	Q8WUM4	-0.66	3.53E-02
MSLN	Q13421	-0.64	4.82E-03

Fig 3. Differential expression analysis (CT1812 vs placebo) of plasma 1 mo change from baseline in AD patients. **A)** Volcano plot illustrates differentially abundant proteins (92) at p≤0.1, with proteins of interest labeled with red arrows. **B)** Top-most robustly increased (red) and decreased (blue) changes in CT1812 vs placebo are listed (p≤0.05).

Early Effects (1 mo) of CT1812 in Regulating Inflammation, β-Catenin Signaling, and Lipoprotein and Amyloid Biology

A

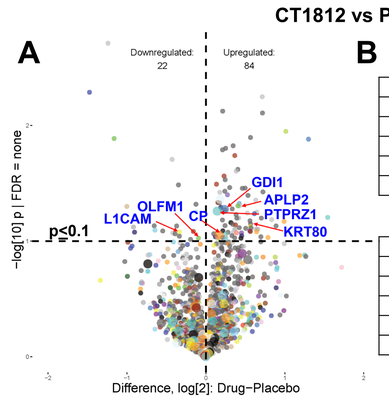
Top Metacore Pathway Maps (CT1812 vs placebo; p≤0.1)	p-value
Cell adhesion – Desmosomes	8.94E-07
Cytoskeleton remodeling – Keratin filaments	1.29E-05
Signal transduction – Anti-inflammatory and cardioprotective Adiponectin signaling	2.60E-05
Signal transduction – Angiotensin II/AGTR1 signaling via Notch, Beta-catenin and NF-kB pathways	2.76E-04
Signal transduction – NOTCH signaling in tissue homeostasis	3.19E-04
N.B. non-relevant disease pathologies/organs excluded.	

B

Top Metacore Pathway Maps (CT1812 vs placebo; p≤0.05)	p-value
Development -NOTCH signaling in the nervous system	2.28E-05
Regulation of immune cell differentiation by Notch signaling	1.01E-04
Signal transduction – Angiotensin II/AGTR1 signaling via Notch, Beta-catenin and NF-kB pathways	4.28E-04
Gamma-secretase proteolytic targets	4.61E-04
Development – NOTCH signaling activation	4.96E-04
N.B. non-relevant disease pathologies/organs excluded.	

Fig 4. Differentially abundant proteins at either p≤0.1 (**A**) or p≤0.05 (**B**) in 1 mo plasma were analyzed for pathway enrichment using Metacore.

Pharmacodynamic Plasma Biomarkers of CT1812 Identified After 6 Months of Treatment



B

Top 5 most robust ↑ changes

Protein	Uniprot ID	Log2 FC	p-value
PSMD7	P51665	1.30	1.31E-02
PSMB9	P28065	1.01	1.12E-02
PLA2G2A	P14555	0.72	3.75E-02
IGLV5-52	A0A0A0MRZ9	0.72	7.79E-03
DCD	P81605	0.71	5.64E-03

Top 5 most robust ↓ changes

Protein	Uniprot ID	Log2 FC	p-value
TMX3	Q96JJ7	-1.47	5.16E-03
NLRX1	Q86UT6	-1.24	1.94E-03
OXCT1	P55809	-1.16	1.30E-02
NBL1	P41271	-0.43	1.96E-02
ST8SIA4	Q92187	-0.35	3.26E-02

Fig 5. Differential expression analysis (CT1812 vs placebo) of plasma 6 mo change from baseline in AD patients. **A)** Volcano plot illustrates differentially abundant proteins (106) at p≤0.1, with proteins of interest labeled with red arrows. **B)** Top-most robustly increased (red) and decreased (blue) changes in CT1812 vs placebo are listed (p≤0.05).

Transport and Endosomal Trafficking Pathways, in Addition to Similar Pathways at 1 mo, are Altered at 6 mo by CT1812

A

Top Metacore Pathway Maps (CT1812 vs placebo; p<0.1)	p-value
Development – NOTCH signaling activation	5.30E-05
Immune response – Antigen presentation by MHC class I, classical pathway	1.46E-04
Notch signaling in oligodendrocyte precursor cell differentiation in multiple sclerosis	3.92E-04
Gamma-secretase regulation of angiogenesis	4.34E-04
Cell adhesion – Cell-matrix glycoconjugates	8.76E-04
N.B. non-relevant disease pathologies/organs excluded.	

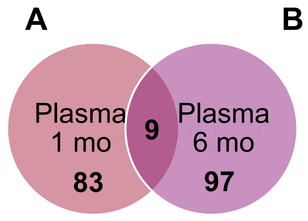
B

Top Metacore Pathway Maps (CT1812 vs placebo; p≤0.05)	p-value
Immune response – Antigen presentation by MHC class I, classical pathway	1.94E-03
Transport – Macropinocytosis	1.94E-03
Ubiquitin-proteasome system in Huntington's disease	2.66E-02
Transport – CFTR sorting endosome	2.66E-02
Mitochondrial ketone bodies biosynthesis and metabolism	2.90E-02
N.B. non-relevant disease pathologies/organs excluded.	

Fig 6. Differentially abundant proteins at either p≤0.1 (**A**) or p≤0.05 (**B**) in 1 mo plasma were analyzed for pathway enrichment using Metacore.

Nine Candidate Plasma Biomarkers Identified Altered in a Similar Direction at Both 1 and 6 Months

A



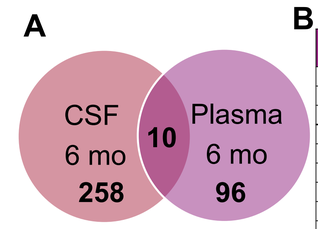
B

Protein	Uniprot ID	1mo Log2 FC	6mo Log2 FC	1mo p-value	6mo p-value
KSR1	Q8IVT5	-0.67	-0.92	4.95E-02	7.55E-02
PDCD6IP	Q8WUM4	-0.66	-0.65	3.53E-02	8.03E-02
L1CAM	P32004	-0.37	-0.33	4.22E-02	8.32E-02
ACAN	P16112	-0.17	-0.12	2.63E-02	5.59E-02
OLFM1	Q99784	-0.13	-0.09	6.81E-02	9.42E-02
CP	P00450	0.19	0.17	2.05E-02	8.85E-02
SCGB1A1	P11684	0.32	0.21	1.21E-02	9.32E-02
KRT9	P35527	0.66	0.65	5.90E-02	5.61E-02
KRT80	Q6KB66	1.25	0.57	1.27E-02	7.10E-02

Fig 7. Comparisons between 1 mo and 6 mo plasma reveal a subset of commonly changed proteins. **A)** Venn diagram illustrates overlapping proteins between 1 mo and 6 mo plasma (CT1812 vs placebo (p≤0.1)). **B)** Listed are overlapping significant (p≤0.1) proteins changed with CT1812 treatment vs placebo (red: increased; blue: decreased).

Ten Proteins Are Significantly Regulated in CT1812-treated Patients Across 6 Month Plasma and CSF

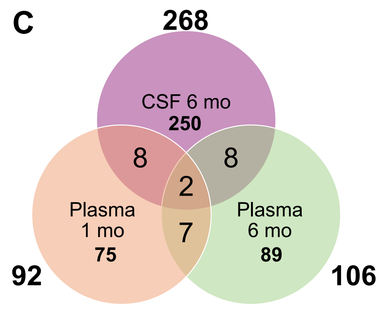
A



B

Protein	Uniprot ID	CSF Log2 FC	Plasma Log2 FC	CSF p-value	Plasma p-value
OLFM1	Q99784	-0.09	-0.09	6.82E-02	9.42E-02
HGFAC	Q04756	0.19	0.10	8.66E-02	8.64E-02
LTA4H	P09960	0.47	0.32	9.47E-02	6.91E-02
KRT80	Q6KB66	-1.54	0.57	4.75E-02	7.10E-02
PLTP	P55058	-0.17	0.15	6.05E-02	4.63E-02
OLFML3	Q9NRN5	-0.11	0.18	7.20E-02	4.35E-02
APLP2	Q06481	-0.11	0.43	3.21E-02	4.94E-02
MGAT5	Q09328	-0.11	0.17	6.38E-02	4.66E-02
BST1	Q10588	0.12	-0.37	9.03E-02	7.40E-02
DSC1	Q08554	0.30	-0.19	4.14E-02	9.66E-02

C



Protein	CSF p-value	1mo Plasma p-value	6mo Plasma p-value
KRT80	<0.05	<0.05	<0.1
OLFM1	<0.1	<0.1	<0.1

Fig 8. Comparisons between CSF and plasma reveal a subset of commonly changed proteins. **A)** Venn diagram illustrates overlapping proteins between 6 mo CSF and plasma (CT1812 vs placebo; p≤0.1). **B)** Listed are the overlapping significant (p≤0.1) proteins changed with CT1812 treatment vs placebo (red: increased; blue: decreased). **C)** Venn diagram illustrates overlapping proteins amongst 1 mo plasma, 6 mo plasma, and 6 mo CSF, with 2 proteins commonly significantly changed in all biofluids/timepoints.

CONCLUSIONS

- **Pharmacodynamic biomarkers of CT1812 were identified in plasma after 1 and 6 months of treatment**
- **Unbiased pathway analyses sheds light on pathways most significantly affected by CT1812 and supports a role for CT1812 in modulating Aβ biology, cellular trafficking, and neuroinflammation**
- **Candidate pharmacodynamic biomarkers of CT1812 identified across biofluids and timepoints revealed**
- **Differentially abundant biomarkers in CT1812 vs placebo in both plasma and CSF illuminate potential plasma biomarkers that could reflect changes occurring in the brain**

Data suggest biological effects of CT1812 may be measurable in plasma, providing a more practical means to assess biological activity in clinical trial participants

Other Posters and Presentations on CT1812 by Cognition Therapeutics

Poster LP024: RESULTS FROM: A Pilot Electroencephalography (EEG) Study to Evaluate the Effect of CT1812 Treatment on Synaptic Activity in Subjects with Mild to Moderate Alzheimer's Disease
W. de Haan, A. Caggiano, P. Scheltens, M. Grundman, E. Scheijbeler, M. Hamby, E. Vijverberg

Poster LP057: Proteomic Analysis In a Phase 2 Clinical Trial Studying CT1812 To Identify CSF and Plasma Pharmacodynamic Biomarkers and Molecular Correlates of EEG In Alzheimer's Patients
V. Di Caro, K. Pandey, B. Lizama, E. Cho, D. Duong, W. de Haan, M Grundman, N. Seyfried, A. Caggiano, E. Vijverber, M. Hamby

Presentation Friday Oct 27th, 8:45 am: LB18 - CT1812 START Study Design: Anti-Aβ Monoclonal Antibodies as Background Therapy
C. Van Dyck, R. Raman, M. Donohue, R. Rissman, M. Rafii, M. Hamby, M. Grundman, A. Caggiano, P. Aisen

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Supported by National Institute on Aging: 3R01AG058660
CT1812 is an experimental therapeutic not approved for use in any jurisdiction.

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REFERENCES
1. *Izzo et al. Preclinical and clinical biomarker studies of CT1812: A novel approach to Alzheimer's disease modification. Alz & Dementia 2021.*
2. *N. Seyfried et al.,. Proteomic Analysis of CSF in a Phase 2 Clinical Trial in Alzheimer's Patients To Identify Pharmacodynamic Biomarkers Of The S2R Modulator CT1812. AD/PD Advances in Science and Therapy; March 2022, Barcelona Spain.*

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