PROTEOMIC ANALYSIS OF CSF IN A PHASE 2 CLINICAL TRIAL FOR AD TO IDENTIFY PHARMACODYNAMIC BIOMARKERS OF THE S2R MODULATOR CT1812

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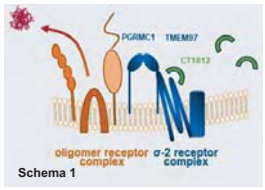
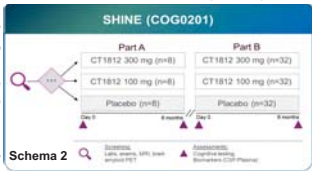
INTRODUCTION

The sigma-2 receptor (S2R) is encoded by TMEM97, a four-domain transmembrane protein that forms a complex with progesterone receptor membrane component 1 (PGRMC1). CT1812 is a highly brain-penetrant small molecule modulator of S2R, that displaces Aβ oligomers bound to neuronal synapses[1] (**Schema 1**). In preclinical studies, CT1812 protects synapses, facilitates their restoration and improves cognitive performance in transgenic Alzheimer's disease (AD) mice[1]. CT1812 is in clinical development for AD.



Schema 1

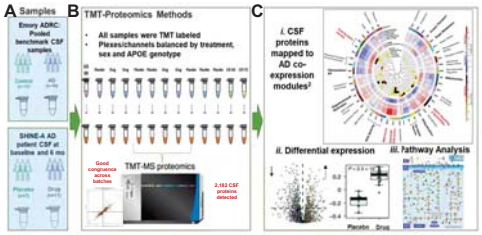
COG0201, the SHINE study, is a randomized, double-blind, placebo-controlled Phase 2 clinical trial designed to enroll ~120 patients with mild-to-moderate AD to evaluate the safety and efficacy of CT1812. Participants are divided equally in two CT1812 dose groups (100 mg, 300 mg) and placebo for once 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. No subjects were withdrawn from the study due to treatment-emergent adverse events and there were no SAEs attributed to study.



Schema 2

METHODS

Tandem-mass tag mass spectrometry (TMT-MS) followed by unbiased quantification of CSF proteomes was conducted on all treatment-compliant patients for which CSF at baseline and end of study CSF was collected (N=18; **Schema 3A, B**). CSF proteomes were compared to within-study pooled AD and age-matched non-demented control CSF reference standards from the Emory Alzheimer's Disease Research Center (ADRC) to compare protein levels in the SHINE-A cohort with well-characterized AD CSF and to assess treatment effects through differential expression and pathway analyses (**Schema 3C**).



Schema 3. Following sample **(A)** analysis via TMT-proteomics **(B),** differentially expressed proteins **(C*ii*)** were mapped to a previously generated protein co-expression networks **(C*i*)** built from 516 brain samples with healthy individuals, asymptomatic and symptomatic AD patients (Johnson et al. 2022 *Nat Neuro*) NCT03507790. **C*iii)*** Pathway analysis was also performed on DE proteins using Metacore, STRING, and GO terms.

Proteomics Method Shows High Congruence with Clinically Validated Assays & Allows Benchmarking of SHINE-A Cohort

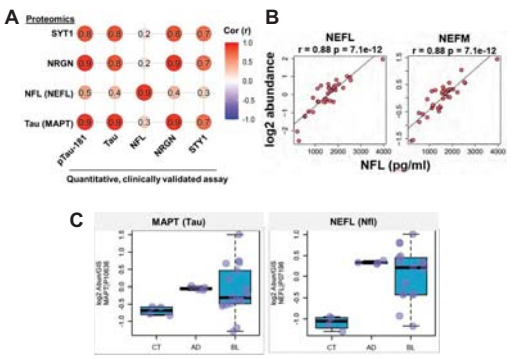


Fig 1. **A)** Pearson correlation analysis of AD core biomarkers from clinically validated assays[3] to that in the TMT proteomics dataset. **B)** Top two most highly correlated proteins with NFL quantitative assay (Uman Diagnostics; pg/ml). **C)** AD core biomarkers[3] from CSF proteomes from SHINE-A at baseline (BL) were compared to pooled AD and non-demented control (CT) CSF reference standards (Emory ADRC).

Pharmacodynamic (PD) Biomarkers of CT1812 (126) Identified, & Mapping to the Brain Network Supports Role at Synapses

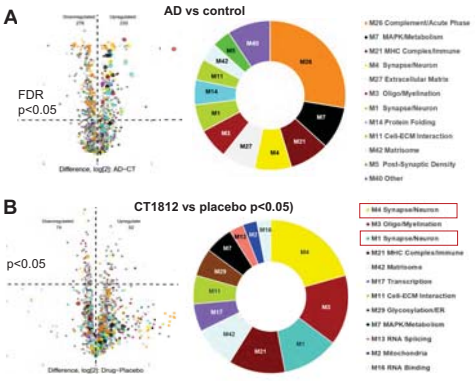


Fig 2. Differential expression analysis of CSF from AD vs control (**A, left**) and AD patients given CT1812 vs placebo (**B, left**). Differentially expressed proteins for each comparison were mapped to the AD co-expression modules[2] (Schema 3C, top panel; top 12 shown here **(A,B right**)).

Unbiased Pathway Analysis of Differentially Abundant Proteins Implicates CT1812 in Regulating Amyloid Biology

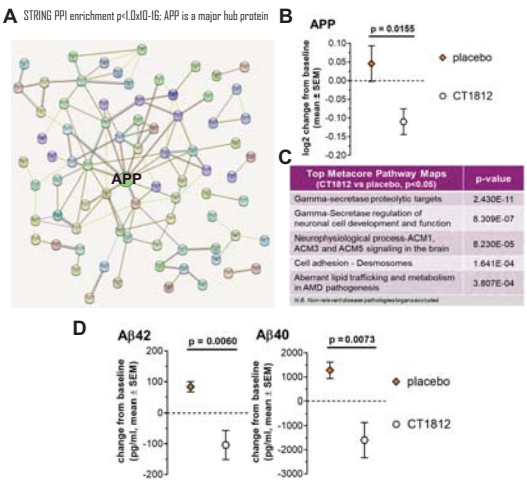
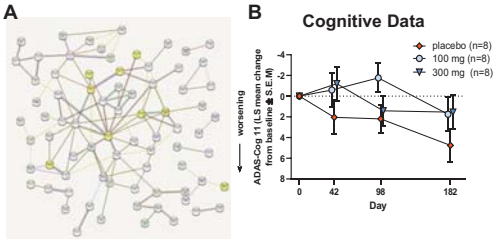


Fig 3. A) Differentially abundant proteins in CSF from CT1812 vs placebo treated AD patients are highly interconnected (STRING analysis) and APP is a hub gene that is significantly lower in CT1812 vs placebo patients **(B)**. **C)** Metacore pathway mapping shows amyloid pathways as the top most significantly associated with CT1812 treatment vs placebo (p<0.05). **D)** Statistically significant lowering of Aβ40 and Aβ42, as assessed via Lumipulse, by CT1812 vs placebo.

Candidate PD Biomarkers Linked to Changes in Cognition Identified from SHINE-A CSF Proteomics Analyses



C

GO term ID	GO Biological Process	strength	FDR p-value
GO:0045055	Regulated exocytosis	0.67	1.88E-12
GO:0016192	Vesicle-mediated transport	0.46	5.81E-12
GO:0032940	Secretion by cell	0.58	9.68E-12
GO:0046903	Secretion	0.55	9.68E-12
GO:0002444	Myeloid leukocyte mediated immunity	0.71	2.41E-11
GO:0002376	Immune system process	0.39	3.73E-11

Fig 4. A) Several PD biomarkers (CT1812 vs placebo; p<0.05) are associated with GO term "cognition" (in yellow; GO:0050890; STRING). **B)** SHINE-A ADAS-Cog-11 cognitive score data which showed a non-significant but clinically meaningful (3-point difference) from placebo. **C)** Top biological processes from gene ontology of protein log2 abundance significantly correlated (Pearson, p<0.05) with ADAS-Cog11 change from baseline scores.

Candidate Disease Modification Biomarkers Identified: Proteins Dysregulated in AD Normalized with CT1812

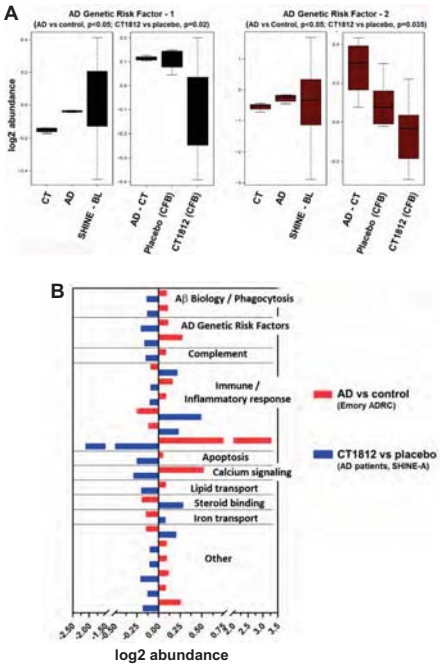


Fig 5. Within-study comparisons to CSF protein levels in reference standards (ADRC AD and control (CT)) enabled comparison of the SHINE-A AD cohort to well-characterized AD and non-demented control CSF **(A,B). A)** Box plots illustrate two proteins significantly increased in AD compared to control CSF that are significantly downregulated in CT1812 vs placebo (SHINE-A). **B)** 22 proteins are significantly (p<0.05) normalized towards control with CT1812 (log2 change in abundance in AD vs control (red) and CT1812 vs placebo (blue)).

CONCLUSIONS

- **Strong correlations with clinically validated assays for core AD biomarkers validate TMT-MS proteomics as a quantitative method**
- **Brain module association and pathway analysis corroborate the mechanism of action of CT1812 in regulating synapses and AD biology**
- **Comparisons to reference CSF standards illuminate proteins disrupted in, or genetically linked to, AD that were normalized by CT1812**
- **Pharmacodynamic biomarkers of CT1812 were identified, including that which may reflect disease modification and cognitive improvement**

Overall, data provide additional support that the S2R modulator CT1812 may be a promising therapeutic approach to AD

REFERENCES

1. Izzo et al. Preclinical and clinical biomarker studies of CT1812: A novel approach to Alzheimer's disease modification. Alz & Dementia 2021.

2. Johnson et. al. Large-scale deep multi-layer analysis of Alzheimer's disease brain reveals strong proteomic disease-related changes not observed at the RNA level. Nat Neurosci. 2022.

3. Blennow et al. Fluid biomarker-based molecular phenotyping of Alzheimer's disease patients in research and clinical settings. Prog Mol Biol Transl Sci. 2019;168:3-23.

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