CSF Phosphoproteomics Biomarker Analysis from the Phase 2 Clinical Trial SHINE to Elucidate the Role of CT1812 in Alzheimer's Disease

Eunah Cho, PhD[1], Jill Caldwell[1], Kiran Pandey, PhD[2], Duc M. Duong, PhD[3], Nicholas T. Seyfried, PhD[3], Anthony O. Caggiano, MD, PhD[4], Valentina Di Caro, PhD[1] and Mary E. Hamby, PhD[1]

(1) Cognition Therapeutics, Inc., Pittsburgh, PA, USA, (2) Emtherapro, Atlanta, GA, USA, (3) Emory University School of Medicine, Atlanta, GA, USA, (4) Cognition Therapeutics, Inc., Purchase, NY, USA

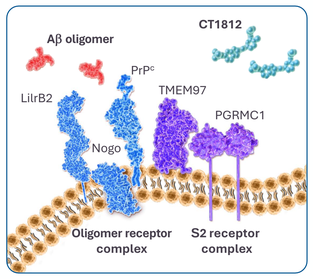
COGNITION™ Therapeutics

Sunday-797

Key takeaway: For the first time we showed that the S2R modulator, CT1812 impacts phosphorylation of AD-related proteins (e.g. APOE, CHGB) in CSF samples from Phase 2 clinical trials for Alzheimer's disease.

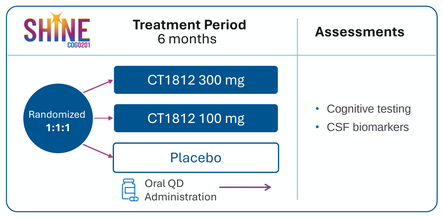
Introduction

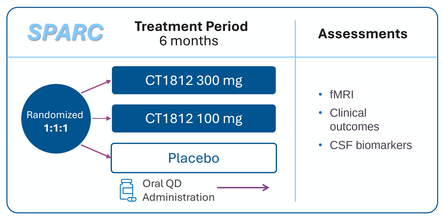
The sigma-2 receptor (S2R) modulator, CT1812, is allosteric Aβ oligomer antagonist currently in Phase 2 clinical trials[1] for Alzheimer's disease (AD). Preclinical and clinical studies have shown that CT1812 displaces Aβ oligomers from synapses[2] which may facilitate clearance of Aβ oligomers in the cerebrospinal fluid, restoring cognitive performance in a transgenic mouse model of AD[3].



To investigate the mechanism of action of CT1812 and enable biomarker discovery, a phosphoproteomic analysis of CSF samples from SHINE-A was performed. SHINE is a Phase 2 randomized, double-blind, placebo-controlled trial to assess the safety and tolerability of two doses of CT1812 for 6 months in mild-to-moderate AD patients (NCT03507790), and an interim analysis was performed on the first 24 patients enrolled (SHINE-A).

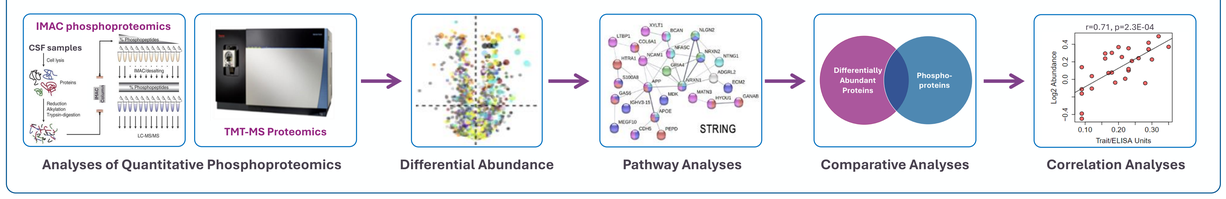
Methods





- Tandem-mass tag mass spectrometry (TMT-MS) proteomics and immobilized metal affinity chromatography (IMAC) phosphoproteomics were performed on baseline and end-of-study CSF samples from SHINE-A (N=18) and differential abundance (CT1812 vs placebo; p≤0.05) was assessed.
- STRING pathway analyses (v12.0) were conducted on total phosphoproteins.
- Pearson correlation analyses with altered phosphopeptides and CSF Aβ42 level and ADAS-Cog-11 score were performed.
- Clinical validation of findings were conducted on an independent cohort, SPARC (NCT03493282, N=23). The study populations were similar and reference Fig. 5D below.

Workflow

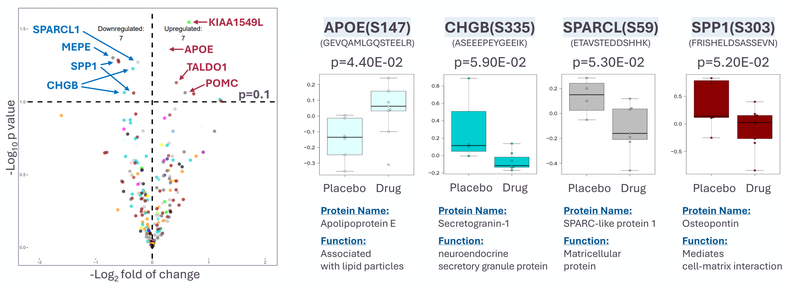


Results

CT1812 Treatment Impact on CSF Phosphoproteome

A. Top Differentially Abundant Peptides: Drug-Placebo at 6 months

B. Representative Boxplots of 8 Phosphoproteins from 14 Phosphopeptides in SHINE-A, p ≤ 0.1



- 3 peptides (3 phosphoproteins) met the significance threshold of p ≤ 0.05 for differential abundance.
- **14 peptides (8 phosphoproteins)** met the significance threshold of p ≤ 0.1 for differential abundance.

C.

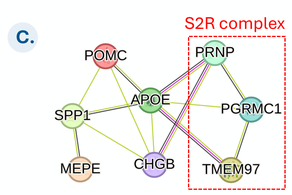


Figure 1. (A) Volcano plots to visualize the global phosphoproteomics change between CT1812-treated and placebo samples. Each data point in the scatter plot represents a protein, p≤0.1. **(B)** Identified 8 phosphoproteins, from 14 phosphopeptides, altered in SHINE-A CSF samples. Representative boxplots of the identified phosphopeptides involved AD-related proteins highlighted in blue (p≤0.1). **(C)** Protein-protein interaction map with S2R complex components via STRING (v12.0). TMEM97 (S2R), PRNP, and PGRMC1 were added to understand how they interacted with the identified proteins (list in B).

Pathways Related to Aβ and Lipoprotein are Impacted by CT1812 in CSF of Treated-Patients

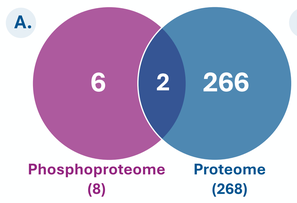
GO terms	Term description	Strength	p-value
1902998	Positive regulation of **neurofibrillary tangle assembly**	**2.34**	2.08E-02
1905908	Positive regulation of **amyloid fibril formation**	**2.21**	1.70E-03
0032805	Positive regulation of **LDL particle** receptor catabolic process	**2.16**	2.81E-02
1902947	Regulation of **tau-protein kinase activity**	**1.82**	8.80E-04
1900221	Regulation of **amyloid-beta clearance**	**1.53**	2.15E-02

N.B. Top 5 most relevant GO terms were selected.

Figure 2. STRING pathway analysis (v12.0) of phosphoproteomics data with total 206 phosphopeptides (91 phosphoproteins). GO=gene ontology.

CT1812 Treatment Altered Proteins' Phosphorylation and Abundance

A.



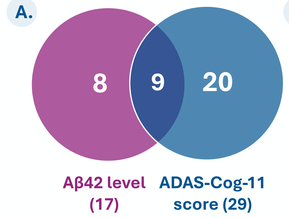
B.

Gene	UniProt ID	Phosphoproteome		Proteome	
		p-value	Log2FC	p-value	Log2FC
APOE	P02649	4.36E-02	0.22	8.50E-02	-0.14
MEPE	Q9NQ76	4.99E-02	-0.70	8.97E-02	-0.34

Figure 3. (A) Venn diagram to show overlapping proteins between SHINE-A phosphoproteins and differentially abundant proteins (p≤0.1). **(B)** p-value and fold change (Log2FC) for the 2 common proteins.

Correlated Phosphopeptides with CSF Aβ42 Levels and ADAS-Cog-11 Score were Identified

A.



B. Common 9 Phosphoproteins, p ≤ 0.05

Gene	Protein name	UniProt ID
AHSG	Alpha-2-HS-glycoprotein	P02765
APOL1	Apolipoprotein L1	O14791
CHGB	Secretogranin-1	P05060
ITIH2	Inter-alpha-trypsin inhibitor heavy chain H2	P19823
PLXDC2	Plexin domain-containing protein 2	Q6UX71
SERPINA10	Protein Z-dependent protease inhibitor	Q9UK55
SPARCL1	SPARC-like protein 1	Q14515
SPP1	Osteopontin	P10451
TALDO1	Transaldolase	P37837

C.

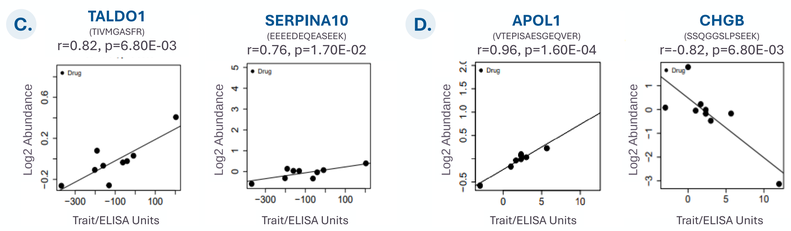
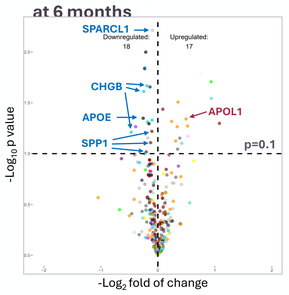


Figure 4. Comparative analysis between phosphopeptides correlates with CSF Aβ42 levels and ADAS-Cog-11 score in CT1812-treated group only by Pearson's correlation analysis. **(A)** Venn diagram to show common correlates (p≤0.05). **(B)** List of correlated phosphoproteins. **(C)** and **(D)** Representative scatter plots of correlated phosphopeptides with Aβ42 level and ADAS-Cog-11 score, respectively (p≤0.05, r=±|0.75|).

Major AD-Related Pathways and Four Phosphoproteins Replicated in SPARC, an Independent Cohort

A. Top Differentially Abundant Peptides: Drug-Placebo at 6 months



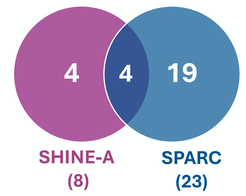
B.

GO Terms	Term description	Strength	p-value
1902998	Positive regulation of **neurofibrillary tangle assembly**	**2.15**	2.39E-02
1905908	Positive regulation of **amyloid fibril formation**	**2.02**	2.30E-03
0032805	Positive regulation of **LDL particle receptor** catabolic process	**1.97**	3.40E-02
1902949	Positive regulation of **tau-protein kinase activity**	**1.78**	5.40E-03
0034372	Very-low-density lipoprotein particle remodeling	**1.67**	8.20E-03

N.B. Top 5 most relevant GO terms were selected.

- 18 peptides (13 phosphoproteins) met the significance threshold of p ≤ 0.05 for differential abundance.
- **35 peptides (23 phosphoproteins)** met the significance threshold of p ≤ 0.1 for differential abundance.

C.



Gene	Phospho-residue	Protein name	UniProt ID
APOE	T212	Apolipoprotein E	P02649
CHGB	S259, S263, S130	Secretogranin-1	P05060
SPARCL1	S90	SPARC-like protein 1	Q14515
SPP1	T185, S280, S303	Osteopontin	P10451

D. Baseline Characteristics and Demographics

		SHINE-A	SPARC
Age (years)	mean, SD	71.7 (7.1)	70.0 (8.8)
Gender	% female	62.5	48
Race	White (%)	95.8	96
Weight (kg)	mean, SD	70.25 (14.8)	79.0 (12.1)
BMI (kg/m^2)	mean, SD	26.21 (5.0)	27.1 (4.5)
ApoE4 positive (+/+, +/-)	%	58.3	78
MMSE at baseline	mean, SD	21.1 (3.57)*	22.6 (1.92)
Anti-dementia drugs at baseline (AChE inh / memantine)	%	54.2	87

*Weighted average calculation from the data was used to compute this number.
SHINE-A MMSE at baseline was not calculated for total subjects, only all CT1812 vs placebo.

Figure 5. (A) Volcano plots to visualize the global phosphoproteomics change between CT1812-treated and placebo samples in the SPARC trial. Each data point in the scatter plot represents a protein, p≤0.1. **(B)** STRING pathway analysis (v12.0) of phosphoproteomics data with total 303 phosphopeptides (140 phosphoproteins). GO=gene ontology. **(C)** Venn diagram to show overlapping phosphorylated proteins by CT1812 between SHINE-A and SPARC trials, and the list of common phosphoproteins (p≤0.1). AD-related proteins highlighted in blue. **(D)** Baseline Characteristics and Demographics to show comparability between cohorts.

Conclusions

- The S2R modulator CT1812 mediates AD-related proteins' phosphorylation in CSF samples from Phase 2 clinical trials for Alzheimer's disease.
- Pathways enriched by CT1812 in SHINE-A are related to vesicle trafficking, neurofibrillary tangle assembly, amyloid fibril formation, low-density lipoprotein particle receptor catabolic processes, and tau-protein kinase activity.
- Identified phosphopeptides were correlated with Aβ42 levels and ADAS-Cog-11 score in participants treated with CT1812 in SHINE-A.
- Altered phosphoproteins by S2R modulator CT1812 in SHINE-A replicated in an independent cohort, SPARC.

This work was supported by funding from the National Institute on Aging (RF1AG057553 and R01AG058660)

CT1812 is an investigational therapeutic that has not been approved for any use by the US Food and Drug Administration

References: 1. Clinical trials NCT03493282, NCT03507790., 2. Izzo NJ et al., Alzheimer's Dement. 2021 Aug 17(8):1365-1382., 3. Izzo NJ et al., PLoS One. 2014 Nov 12;9(11):e111898.

CONTACT: echo@cogrx.com