ANALYSIS OF CSF SAMPLES FROM A PHASE 2 CLINICAL TRIAL IN ALZHEIMER'S PATIENTS SHOW THAT CT1812 CAN MODULATE α-SYNUCLEIN

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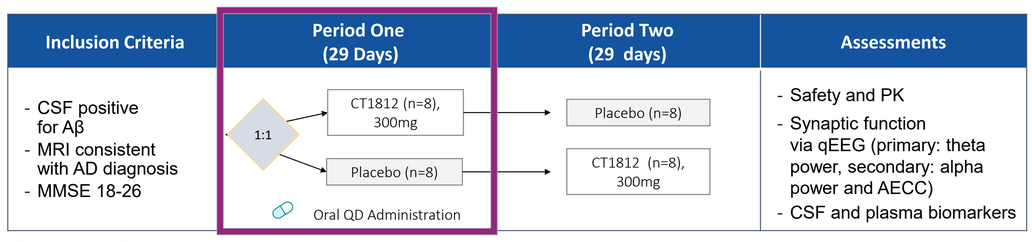
INTRODUCTION

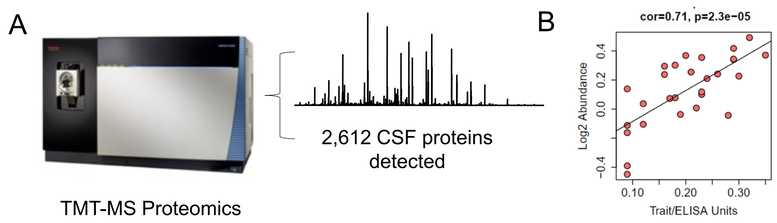
The presynaptic protein α-synuclein (αSyn), mainly associated with synucleinopathies like Parkinson and dementia with Lewy bodies (DLB), is also involved in the pathophysiology of Alzheimer's disease (AD) and higher levels of αSyn in the CSF of patients with AD have been linked to cognitive decline. CT1812 is a first-in-class investigational therapeutic in development for AD and DLB[1]. Preclinical evidence indicates that CT1812 can displace toxic amyloid-β oligomers (AβO) and αSyn oligomers from binding to neuronal synapses[2,3]. To understand if our clinical leading candidate, CT1812 can modulate αSyn, we assessed total αSyn levels in CSF samples from a Phase 2, single site, double-blind, placebo controlled, cross-over study design trial in patients with mild to moderate AD (SEQUEL-NCT04735536) (Schema 1). To investigate further the biology surrounding αSyn, Pearson correlation analysis between αSyn concentrations and the CSF proteome from CT1812-treated only patients was performed to identify proteins highly correlated with the change in αSyn.



Schema 1: SEQUEL study design.

METHODS

Participants (16) were randomized to receive 29 days of either CT1812 (300 mg, PO, qD) or placebo during the first treatment period. Following a two-week washout, participants then switched treatment for another 29 days period. Total αSyn in CSF samples was measured by ELISA (Euroimmun) at baseline and after 29 days of treatment and change from baseline calculated. Tandem-mass tag mass spectrometry (TMT-MS) proteomics was performed on CSF collected at the same time points (Schema 2). To identify correlate to αSyn, Pearson correlation analysis was performed between CSF αSyn levels and each protein in the CSF proteome ($p \leq 0.05$ and $p \leq 0.01$) from CT1812-treated only patients. Pathway analyses were performed using STRING (v12.0) and Metacore (v23.4.71500) using two different p-value criteria ($p \leq 0.05$ and $p \leq 0.01$). Data show here are only for day 29 (period 1).



Schema 2. Following CSF sample analysis via TMT-proteomics (A), Pearson correlation analysis was performed between αSyn and each protein in the CSF proteome (B).

RESULTS

CT1812 Decrease Levels of αSyn in CSF After 29 Days of Treatment

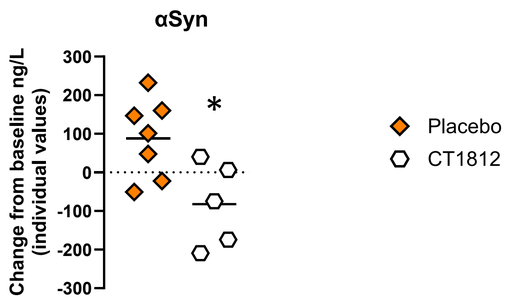


Figure 1: After 29 days (period 1), a statistically significant change from placebo was seen for αSyn in CSF samples of CT1812-treated patients (placebo mean 87.91 ng/L; CT1812 mean -82.32 ng/L; p<0.05 Student's t-test).

Sets of Proteins Associated with αSyn CSF levels

A

p-value	Correlates #	Positively Correlated	Inversely Correlated
p≤0.05	**476**	**187**	**289**
p≤0.01	**188**	**71**	**117**

B

Protein	Protein ID	p-value	αSYN cor.
PCDHGB5	Q9Y5G0	4.09E-07	1.00
GFER	P55789	6.99E-04	1.00
HSPA8	P11142	6.11E-06	0.99
SYN2	Q92777	6.51E-04	0.99
PLXDC1	Q8IUK5	1.25E-05	0.99
LINGO1	Q96FE5	1.44E-05	0.99
CDH13	P55290	4.36E-05	0.99
DCBLD1	Q8N8Z6	6.85E-05	0.98
TUBA4A	P68366	2.89E-03	0.98
GLOD4	Q9HC38	1.45E-04	0.98
RAP1B	P61224	1.58E-04	0.98
FREM2	Q5SZK8	1.90E-04	0.97
CD48	P09326	2.12E-04	0.97
GSS	P48637	2.24E-04	0.97
SEC14L2	O76054	5.53E-03	0.97

Protein	Protein ID	p-value	αSYN cor.
IGKV1D-43	A0A0B4J1Z2	4.46E-07	-1.00
TNFSF8	P32971	2.55E-05	-1.00
FAM177A1	Q8N128	2.27E-06	-1.00
SIRPB2	Q5JXA9	4.34E-05	-1.00
NA	ApoE2	1.04E-04	-1.00
IGKV6-21	A0A0C4DH24	1.13E-04	-1.00
B4GALT5	O43286	2.47E-03	-1.00
IGLV3-10	A0A075B6K4	1.90E-04	-1.00
ADAMTS16	Q8TE57	2.13E-04	-1.00
FLNB	O75369	4.22E-03	-1.00
SNX3	O60493	3.38E-04	-1.00
IGHV3-30-5	P0DP03	4.34E-03	-1.00
NDNF	Q8TB73	4.75E-03	-1.00
GAB4	Q2WGN9	4.91E-03	-1.00
ERMN	Q8TAM6	7.39E-03	-0.99

C

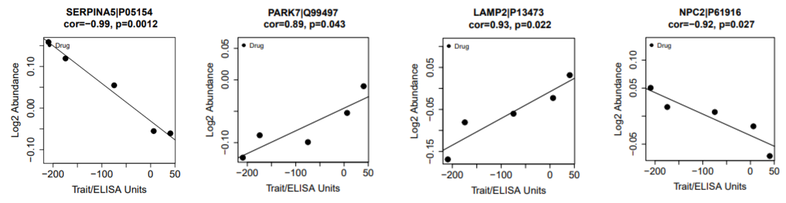


Figure 2: (**A)** CSF proteins were identified to be significantly correlated using Pearson correlation analyses with αSyn CSF levels ($p \leq 0.05$ and $p \leq 0.01$) in drug treated patients. (**B)** Topmost directly (red) and inversely (green) CSF proteins corelated to αSyn are listed ($p \leq 0.01$). (**C**) Other proteins of interest correlating with αSyn (x axis).

αSyn Correlated Proteins of Interest Connected to AD Phenotype

Protein Name	Link to AD
Heat shock protein A8 (HSPA8)	Key role in the homeostasis of tau, superoxide dismutase 1 and αSyn and in the balance between αSyn oligomeric and monomeric form
Synapsin II (SYN2)	αSyn oligomers can impair memory by selectively lowering synapsin expression
Niemann-Pick Disease Type C2 Protein (NCP2)	Regulates the transport of cholesterol through the late endosomal/lysosomal system, may play disease modifying role in AD
Leucine Rich Repeat And Ig Domain Containing 1 (LINGO1)	Decreases processing of AβPP in the amyloidogenic pathway by promoting lysosomal degradation of AβPP
Serpin Family A Member 5 (SERPINA5)	Associated with hippocampal vulnerability in AD; binds to tau and co-localizes within neurofibrillary tangles

Table 1: Correlates to αSyn CSF levels in CT1812-treated only patients (Pearson correlation, $p \leq 0.01$ $r>|0.7|$) associated to AD phenotype.

αSyn Correlates are Connected to S2R Complex Components

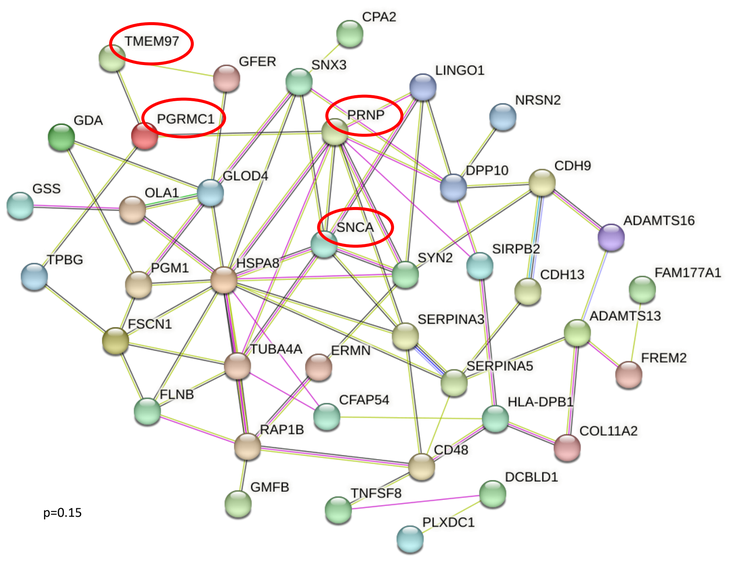


Figure 3: Protein-protein interaction map using STRING (v12.0) of the top 50 correlates to αSyn CSF levels in CT1812-treated only patients ($p \leq 0.01$ $r=|0.9|$) with the S2R complex components (TMEM97 (S2R)), added to this analysis to understand the relationship to CT1812's mechanism of action through S2R, PRNP and PGRMC1 and αSyn (SNCA).

Pathway Analyses Identify Immune Response, Protein Folding and Maturation Pathways Significantly Associated to αSyn Correlates

Top Metacore Pathway Maps, p≤0.05	p-value
Immune response-Alternative complement pathway	**4.10E-13**
Immune response Classical complement pathway	**1.14E-10**
Immune response-Lectin induced complement pathway	**7.94E-10**
Protein folding and maturation, posttranslational processing of neuroendocrine peptides	**1.10E-08**
Neurophysiological process Synaptic vesicle fusion and recycling in nerve terminals	**2.02E-07**

Top Metacore Pathway Maps, p≤0.01	p-value
Protein folding and maturation-Posttranslational processing of neuroendocrine peptides	**4.553E-05**
Neurophysiological process-Synaptic vesicle fusion and recycling in nerve terminals	**5.51E-05**
Immune response-Classical complement pathway	**6.05E-05**
Immune response-Alternative complement pathway	**6.05E-05**
Transport RAB3 regulation pathway	**5.33E-03**

Figure 4: αSyn-correlated proteins ($p \leq 0.05$; $p \leq 0.01$; $r>|0.7|$). were analyzed for pathway enrichment using Metacore (v23.4.71500). Top pathways are listed (non-relevant disease pathologies/organs excluded).

GO Terms Complement, Synaptic and Dopamine Metabolic Processes are Associated to αSyn Correlates

GO Term ID	Biological Process, p≤0.05	Strength	p-value
GO:0048842	Positive regulation of axon extension involved in axon guidance	**1.37**	3.37E-02
GO:0045964	Positive regulation of dopamine metabolic process	**1.31**	4.42E-02
GO:0006957	Complement activation, alternative pathway	**1.25**	4.90E-04
GO:0019835	Cytolysis	**1.16**	2.80E-04
GO:0006958	Complement activation, classical pathway	**1.11**	1.61E-06
GO:0006956	Complement activation	**1.1**	6.81E-09

GO Term ID	Biological Process, p≤0.01	Strength	p-value
GO:0061684	Chaperone-mediated autophagy	**1.64**	3.92E-02
GO:0006957	Complement activation, alternative pathway	**1.46**	1.38E-02
GO:0019835	Cytolysis	**1.31**	3.51E-02
GO:0043567	Regulation of insulin-like growth factor receptor signaling pathway	**1.31**	3.51E-02
GO:0006958	Complement activation, classical pathway	**1.24**	4.30E-03
GO:0006956	Complement activation	**1.19**	6.00E-04
GO:0051965	Positive regulation of synapse assembly	**1.18**	6.00E-04

Figure 5: STRING (v 12.0) pathway analysis of correlates to αSyn CSF levels in CT1812-treated only patients ($p \leq 0.05$; $p \leq 0.01$; $r>|0.7|$). GO terms sorted by strength.

CONCLUSIONS

- A significant decrease in CSF total αSyn levels was observed in AD patients after 29 days of treatment with CT1812
- Proteins highly correlated to CSF αSyn levels are associated with AD phenotype and pathways related to complement, inflammation, dopamine metabolism and synapse biology
- Protein-protein interaction mapping show a highly interconnected network with αSyn as a hub, and illustrate the connectivity with proteins comprising S2R

Findings highlight molecular mechanisms through which CT1812 may affect neurophysiology in Alzheimer's disease and will be validated in upcoming Phase 2 clinical trials with CT1812 in AD (NCT03507790) and DLB (NCT05531656)

Other Posters on CT1812 by Cognition Therapeutics

Abstract 2904: Plasma Proteomic Analysis from Alzheimer's Patients In SPARC Clinical Trial to Identify Pharmacodynamic Biomarkers of the S2R Modulator CT1812
B.N. Lizama, D. Duong, K. Pandey V. Di Caro, A. Mecca, R. O'Dell, C. van Dyck, M. Grundman, A.O. Caggiano, N. Seyfried, M.E. Hamby

Abstract 2964: Identification of New Pharmacodynamic Biomarkers of CT1812 That Correlate With Favorable Functional Connectivity of the Brain
V. Di Caro, K. Pandey, E. Cho, D. Duong, W. de Haan, M Grundman, N. Seyfried, A. Caggiano, E. Vijverberg, M. Hamby

ClinicalTrials.gov: NCT04735536
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Corresponding author: mhamby@cogrx.com

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1. Clinical trials NCT03507790, NCT05531656.
2 Izzo N et al. Preclinical and clinical biomarker studies of CT1812: A novel approach to Alzheimer's disease modification. Alz & Dementia 2021.
3 Limegrover CS et al. Sigma-2 receptor antagonists rescue neuronal dysfunction induced by Parkinson's patient brain-derived α-synuclein. J Neurosci Res 2021.

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