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

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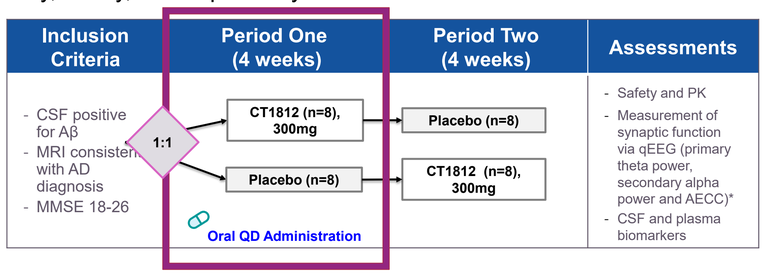
INTRODUCTION

CT1812 is an oral small molecule sigma-2 receptor (S2R) modulator in development for Alzheimer's disease (AD) and dementia with Lewy bodies (DLB)[1]. Preclinical and clinical evidence indicate that CT1812 can displace toxic amyloid-β oligomers (AβO) from binding to neuronal synapses leading to a displacement of AβO into the CSF[2]. A phase 2, single site, double-blind, placebo controlled, crossover design study (SEQUEL; schema 1) was conducted in 16 participants with mild to moderate AD (EUDRACT NUMBER 2019-003552-36; NCT04735536) to evaluate the effect of the S2R modulator CT1812 on quantitative electroencephalography (EEG) activity, safety, and exploratory biomarkers.

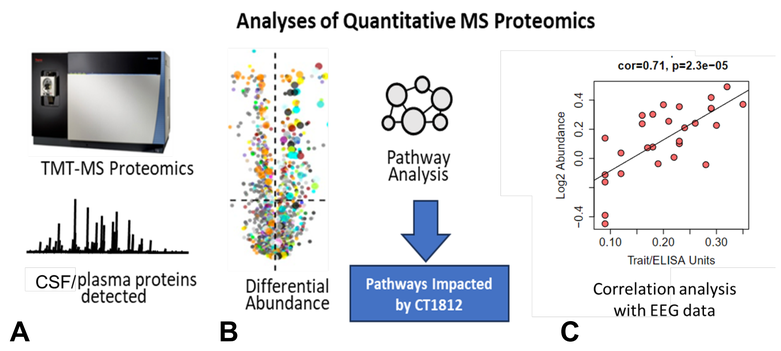


Schema 1: * *data for qEEG analysis can be seen at poster#. LP024*

An unbiased assessment of CSF and plasma proteomes for period one (day 29) from the 16 patients of SEQUEL was performed to identify pharmacodynamic biomarkers of CT1812, and to identify correlation between EEG parameters and each protein in the CSF proteome.

METHODS

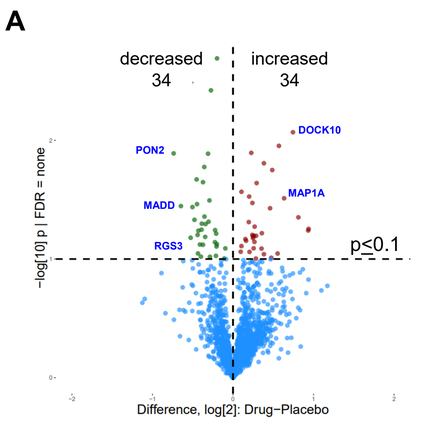
Participants were randomized to receive four weeks of either CT1812 (300 mg, PO, qD) or placebo during the first treatment period. Following a two-week washout, participants then switched treatment for another four-week period. Tandem-mass tag mass spectrometry (TMT-MS) proteomics was performed on CSF and plasma (schema 2) collected at baseline, day 29 (immediately after the first treatment period) and day 72 (immediately following the second treatment period) for longitudinal assessments. Treatment effects were assessed through differential abundance analyses using two statistical levels (p<0.1, p<0.05) followed by pathway analyses (MetaCore, STRING). To identify correlates to EEG, Pearson correlation analyses were performed across several EEG parameters and each protein in the CSF proteome (p<0.05). Data from global theta power correlations are shown, to assess local changes in neuronal activity; and data from global alpha AECc, as a measure of functional connectivity, correlations are shown.



Schema 2. Following CSF or plasma sample analysis via TMT-proteomics **(A),** differentially expressed proteins were analyzed by Pathway analysis using Metacore (version 23.2.71300) and STRING (version 12.0). **C)** Pearson correlation analyses were performed between EEG parameters and each protein in the CSF proteome.

RESULTS

Differential Expression Analysis Identifies Plasma Biomarkers of CT1812 after 29 Days of Treatment



B

GENE	PROTEIN ID	Log2FC	p-value
PON2	Q15165	-0.74	1.29E-02
CTSG	P08311	-0.65	3.58E-02
RGS3	P49796	-0.53	6.62E-02
MADD	Q8WXG6	-0.50	3.65E-02
JAK3	P52333	-0.48	4.68E-02
LGALS1	P09382	-0.45	2.14E-02
MFF	Q9GZY8	-0.45	3.44E-02
ARMCX3	Q9UH62	-0.44	6.27E-02
NRDC	O43847	-0.43	8.98E-02
GLTP	Q9NZD2	-0.43	5.77E-02
PLOD1	Q02809	0.94	5.57E-02
MYH7B	A7E2Y1	0.93	5.75E-02
IGLV3-22	A0A075B6J6	0.81	4.45E-02
DOCK10	Q96BY6	0.74	8.55E-03
MAP1A	P78559	0.63	3.08E-02
STON2	Q8WXE9	0.57	1.11E-02
HAMP	P81172	0.55	8.99E-02
ARHGEF1	Q92888	0.49	1.78E-02
COX4I1	P13073	0.47	9.76E-02
PTGES2	Q9H7Z7	0.46	3.73E-02

Fig 1. Differential expression analysis of plasma samples, at 29 days, from AD patients given CT1812 vs placebo. **A)** Volcano plot illustrates differentially abundant proteins (CT1812 vs placebo) at p≤0.1. **B)** Topmost decreased (green) and increased (red) changes are listed (p≤0.1).

Pathway Analyses Identify Inflammatory, Amyloid-β, and Synaptic Pathways Significantly Altered in Plasma

A

Top Metacore Pathway Maps, p≤0.1	p-value
Signal transduction: Ephrin reverse signaling	5.57E-05
G-protein signaling: Rac1 activation	1.01E-03
Immune response: IL-3 signaling via JAK/STAT, p38, JNK and NF-kB	1.96E-03
Epigenetic regulation of neuronal hyperexcitability in neuropathic pain	2.55E-03
Immune response: HMGB1/TLR signaling pathway	4.18E-03

Top Metacore Pathway Maps, p≤0.05	p-value
Signal transduction: Ephrin reverse signaling	6.68E-04
Neurophysiological process: Synaptic vesicle fusion and recycling in nerve terminals	2.3E-03
Signal transduction: FGFR3 signaling	5.22E-03
Immune response: IL-3 signaling via JAK/STAT, p38, JNK and NF-kB	7.17E-03
Protein folding and maturation: Regulation of amyloid precursor protein processing	9.57E-03

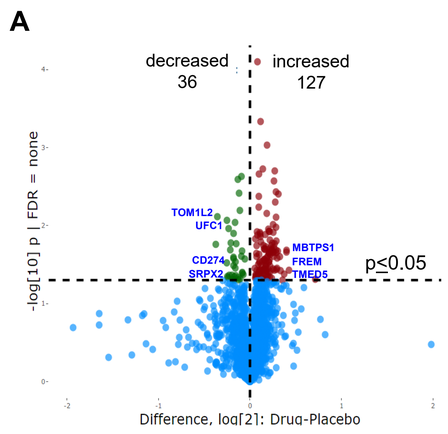
B

GO Term ID	Biological Process, p≤0.1	Strength	p-value
GO:0048156	Tau protein binding	**1.44**	3.2E-02
GO:0008092	Cytoskeletal protein binding	**0.64**	5.8E-03
GO:0005515	Protein binding	**0.25**	5.8E-03

GO Term ID	GO Components p≤0.1	Strength	p-value
GO:0031307	Integral component of mitochondrial outer membrane	**1.57**	2.3E-02
GO:0015630	Microtubule cytoskeleton	**0.48**	3.1E-02
GO:0005856	Cytoskeleton	**0.39**	2.1E-02
GO:0005829	Cytosol	**0.36**	2.4E-06
GO:0031982	Vesicle	**0.35**	2.3E-03

Fig 2. A) Differentially abundant proteins (Fig 1; day29) at either p≤0.1 (*left*) or p≤0.05 (*right*) in plasma samples were analyzed for pathway enrichment using Metacore. Top pathways are listed (non-relevant disease pathologies/organs excluded). **B)** STRING pathway analysis was performed (p≤0.1), identifying top GO biological processes (*left*) and cellular components (*right*), sorted by strength.

Differential Expression Analysis Identifies CSF Biomarkers of CT1812 After 29 Days of Treatment



B

GENE	PROTEIN ID	Log2FC	p-value
FN3KRP	Q9HA64	-0.37	1.74E-02
TOM1L2	Q6ZVM7	-0.36	7.68E-03
CD274	Q9NZQ7	-0.25	3.00E-02
UFC1	Q9Y3C8	-0.25	8.56E-03
SRPX2	O60687	-0.25	4.49E-02
HSP90AA1	P07900	-0.24	4.66E-02
HLA-B	P30479	-0.23	1.09E-02
TPM1	P09493	-0.23	4.33E-02
ST6GALNAC2	Q9UJ37	-0.22	2.05E-02
TGFB1	P01137	-0.21	1.67E-02
ENPEP	Q07075	0.71	4.88E-02
FREM3	P0C091	0.43	3.73E-02
MBTPS1	Q14703	0.40	2.07E-02
PKLR	P30613	0.40	2.19E-02
FANCG	O15287	0.37	4.18E-02
TMED5	Q9Y3A6	0.36	5.00E-02
APOC2	P02655	0.33	3.78E-02
UMOD	P07911	0.33	2.50E-02
IGLV2-14	P01704	0.31	3.93E-03
APOM	O95445	0.30	2.12E-02

Fig 3. Differential expression analysis of CSF, at 29 days, from AD patients given CT1812 vs placebo. **A)** Volcano plot illustrates differentially abundant proteins (CT1812 vs placebo) at p≤0.05. **B)** Topmost decreased (green) and increased (red) changes (p≤0.05).

Pathway Analysis Identify Cholesterol, Lipoprotein Biology, and Wnt Signaling Pathways Significantly Altered in CSF

A

Top Metacore Pathway Maps, p≤0.1	p-value
Protein folding and maturation: Bradykinin / Kallidin maturation	1.22E-08
Cell adhesion: ECM remodeling	7.74E-08
Transcription: HIF-1 targets	1.1E-07
Cell adhesion: Cell-matrix glycoconjugates	9.78E-07
Signal transduction: Angiotensin II/ AGTR1 signaling via TGF-beta 1 and SMADs	9.54E-05

Top Metacore Pathway Maps, p≤0.05	p-value
Protein folding and maturation: Bradykinin / Kallidin maturation	5.92E-11
Cell adhesion: ECM remodeling	2.98E-06
Cell adhesion: Cell-matrix glycoconjugates	8.13E-06
Lipoprotein metabolism	1.04E-05
Development: ErbB3 signaling	9.85E-05

B

GO Term ID	Biological Process, p≤0.05	Strength	p-value
GO:0060228	Phosphatidylcholine -sterol O-acyltransferase activator activity	**1.95**	5.8E-04
GO:0031210	Phosphatidylcholine binding	**1.27**	2.7E-02
GO:0017147	Wnt-protein binding	**1.25**	2.8E-02
GO:0015485	Cholesterol binding	**1.11**	1.8E-02
GO:0005539	Glycosaminoglycan binding	**1.06**	3.3E-12

GO Term ID	GO Components, p≤0.05	Strength	p-value
GO:0034366	Spherical high-density lipoprotein particle	**1.92**	2.2E-06
GO:0042627	Chylomicron	**1.79**	3.6E-07
GO:0034361	Very-low-density lipoprotein particle	**1.67**	9.4E-08
GO:0034364	High-density lipoprotein particle	**1.62**	1.2E-09
GO:0001527	Microfibril	**1.62**	4.1E-04

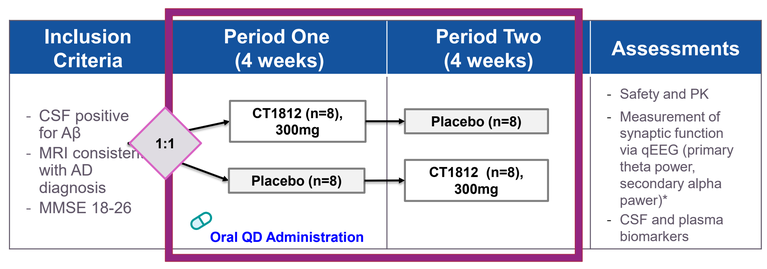
Fig 4. A) Differentially abundant proteins (Fig 3: day29) at either p≤0.1 (*left*) or p≤0.05 (*right*) in CSF samples were analyzed for pathway enrichment using Metacore. Top pathways are listed (non-relevant disease pathologies/organs excluded). **B)** STRING pathway analysis was performed (p≤0.05) to identify top biological processes (*left*) and top GO components (*right*), sorted by strength.

CONCLUSIONS - CT1812 TREATMENT

- **These results provide insight into potential pathway engagement biomarkers of CT1812 after four-week treatment**
- **Biological pathways, including cholesterol, lipid biology, inflammation, and WNT/β-catenin signaling are impacted by CT1812 treatment**

Findings highlight molecular mechanism through which S2R modulation may affect neurophysiology in Alzheimer's disease

Correlation Analysis of EEG and CSF Proteomic Data for all Patients at all Timepoints Irrespective of Treatment



Sets of Proteins and Biological Processes Associated with Global Theta Power

A

GENE	PROTEIN ID	THETA POWER GLOBAL.cor.	p-value
GABRD	O14764	0.71	1.71E-05
ATP1A2	P50993	0.70	5.06E-07
FAT1	Q14517	0.65	3.54E-06
CFHR3	Q02985	0.63	8.51E-06
B4GALT4	O60513	0.63	4.58E-04
KLK14	Q9P0G3	0.62	5.10E-04
LDLR	P01130	0.61	1.57E-05
PCDHGB5	Q9Y5G0	0.61	7.08E-04
FLNC	Q14315	0.60	2.27E-05
SPECC1	Q5M775	0.60	2.90E-05
CNPY3	Q9BT09	-0.70	3.07E-07
SCUBE3	Q8IX30	-0.64	1.92E-04
ADGRG1	Q9Y653	-0.60	2.09E-05
RAB4A	P20338	-0.60	3.39E-05
DPEP2	Q9H4A9	-0.59	4.47E-05
OMD	Q99983	-0.58	4.99E-05
PRSS23	O95084	-0.56	9.98E-05
GAL3ST4	Q96RP7	-0.55	1.51E-04
ITGA1	P56199	-0.55	2.48E-03
ANGPTL4	Q9BY76	-0.54	1.88E-04

B

GO Term ID	Biological Process, p≤0.1	Strength	p-value
GO:0051919	Positive regulation of fibrinolysis	**1.6**	1.50E-03
GO:0010873	Positive regulation of cholesterol esterification	**1.48**	2.08E-02
GO:0010916	Negative regulation of very-low-density lipoprotein particle clearance	**1.48**	2.08E-02
GO:0034384	High-density lipoprotein particle clearance	**1.46**	4.70E-04
GO:0034382	Chylomicron remnant clearance	**1.38**	3.00E-02

Fig 5. Correlation analysis across patients and timepoints (Day 1, 29 and 72) between EEG global theta power and proteomics. **A)** Topmost directly (red) and inversely (green) CSF proteins corelated to global theta power are listed (p≤0.05). **B)** STRING pathway analysis was performed for a list of 187 protein (p≤0.01) to identify top biological processes corelated to global theta power. GO terms sorted by strength.

Sets of Proteins and Biological Processes Associated with Global Alpha AECc

A

GENE	PROTEIN ID	ALPHA. AECc. GLOBAL.cor.	p-value
GANAB	Q14697	0.73	5.27E-08
LRFN2	Q9ULH4	0.71	1.22E-07
CHST1	O43916	0.71	1.68E-07
NPY	P01303	0.69	5.51E-07
EPHA5	P54756	0.68	8.13E-07
PTPRN	Q16849	0.66	1.65E-06
GATD3B	A0A0B4J2D5	0.66	1.65E-04
CD248	Q9HCU0	0.65	2.50E-06
PCDH20	Q8N6Y1	0.65	2.17E-04
PCSK2	P16519	0.65	2.95E-06
CALCOCO2	Q13137	-0.73	1.06E-05
ENPEP	Q07075	-0.72	1.00E-05
SERPINA6	P08185	-0.69	3.68E-07
FSIP2	Q5CZC0	-0.67	1.41E-04
F11	P03951	-0.66	1.64E-06
IMPG2	Q9BZV3	-0.65	2.61E-06
UMOD	P07911	-0.64	4.30E-06
MSTN	O14793	-0.64	4.68E-06
LILRB5	O75023	-0.64	3.47E-04
CRELD1	Q96HD1	-0.63	6.60E-06

B

GO Term ID	Biological Process, p≤0.1	Strength	p-value
GO:0019800	Peptide cross-linking via chondroitin 4-sulfate glycosaminoglycan	**1.73**	2.86E-02
GO:0030204	Chondroitin sulfate metabolic process	**1.24**	2.15E-02
GO:0008038	Neuron recognition	**1.05**	4.92E-02
GO:0006029	Proteoglycan metabolic process	**1.03**	3.50E-03
GO:0042246	Tissue regeneration	**1.02**	2.47E-02

Fig 6. Correlation analysis across patients and timepoints (Day 1, 29 and 72) between EEG global alpha AECc and proteomics. **A)** Topmost directly (red) and inversely (green) CSF proteins correlated to global alpha AECC are listed (p≤0.05). **B)** STRING pathway analysis was performed for a list of 522 proteins (p≤0.01) to identify top biological processes corelated to global alpha AECC. GO terms sorted by strength.

CONCLUSIONS – CORRELATION ANALYSIS

- **Sets of proteins were robustly and significantly correlated with regional and global theta power and global alpha AECC**
- **Several biological processes were identified via pathway analyses to be associated with global theta power and global alpha AECc**
- **Future analyses to pin-point drug related correlates will be warranted to identify proteins and pathways related treatment-related mechanisms that may underlie improvement in global theta power and alpha AECc**

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 P075: Proteomic Analysis of Plasma In a Phase 2 Clinical Trial In Alzheimer's Patients To Identify Pharmacodynamic Biomarkers of the S2R Modulator CT1812
B. Lizama, E. Cho, D. Duong, K. Pandey, C. Williams, A. Caggiano, N. Seyfried, V. Di Caro, 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

ClinicalTrials.gov: NCT04735536
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1. Clinical trials NCT03507790, NCT05531656.
2 Izzo et al. Preclinical and clinical biomarker studies of CT1812: A novel approach to Alzheimer's disease modification. Alz & Dementia 2021.
3. 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.

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