Unbiased proteomic and transcriptomic analysis of Sigma-2 receptor modulation in an *in vivo* model of synucleinopathy

Britney N. Lizama[1], Aidan J. Reaver[1], Kiran Pandey[2], Duc M. Duong[3], Nicholas T. Seyfried[3], Anthony O. Caggiano[1], Mary E. Hamby[1]

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

PSTR402.10

NO – PHOTO / RECORD

INTRODUCTION

Synucleinopathies, which include Parkinson's disease and dementia with Lewy bodies (DLB), comprise the second most prevalent neurodegenerative disease worldwide. Alpha synuclein (αSyn) oligomers are the toxic form of αSyn protein contributing to neurodegeneration[1]. αSyn oligomers can bind to synapses and spread trans-synaptically to anatomically connected regions. Small molecule modulators of the sigma 2 receptor (S2R) block αSyn oligomers from binding to neurons and reverse oligomer-mediated trafficking deficits *in vitro*[2] (**Figure 1**; **Schema 1**).

To elucidate the mechanisms by which the S2R impacts aspects of synucleinopathies, S2R modulators CT1812 and CT2168 were tested in an *in vivo* preformed fibril (PFF) model of pathological αSyn spreading[3]. Using proteomics and transcriptomics, we assessed proteins, transcripts, and pathways in the PFF model that were altered by S2R modulators.

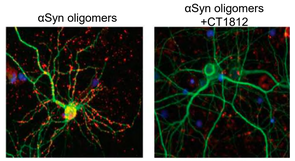
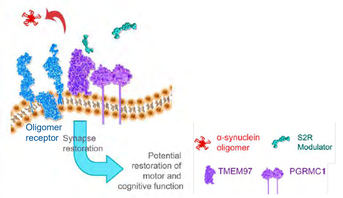


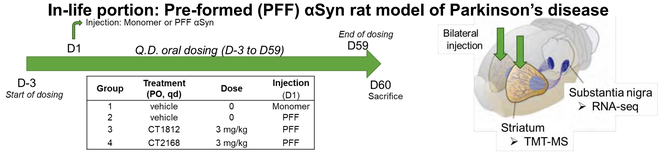
Figure 1: S2R modulators block the effects of αSyn oligomer binding to neurons. Representative microscopy images of primary neuronal cell cultures exposed to αSyn oligomer are shown with vehicle treatment (left) or after the addition of an S2R modulator (right). αSyn (red) binds to neuronal processes (MAP2, green). The addition of an S2R modulator decreases the levels of αSyn oligomers bound to neurons



Schema 1: Model of S2R protein interactions and hypothesized role of S2R small molecule modulators in restoring function in models of synucleinopathy

METHODS

In-life portion: Pre-formed (PFF) αSyn rat model of Parkinson's disease



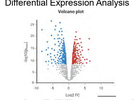
Group	Treatment (PO, qd)	Dose	Injection (D1)
1	vehicle	0	Monomer
2	vehicle	0	PFF
3	CT1812	3 mg/kg	PFF
4	CT2168	3 mg/kg	PFF

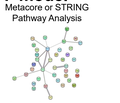
Treatment Group	Condition	TMT-MS (Final n)	RNAseq (Final n)
Group 1	Monomer αSyn +Vehicle	9	10
Group 2	PFF αSyn + Vehicle	10	7
Group 3	PFF αSyn + CT1812	9	10
Group 4	PFF αSyn + CT2168	9	10

Study Goals

Goal 1: Define the transcriptomic and proteomic profile of PFF model

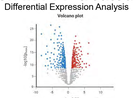
Differential Expression Contrasts	
Striatum proteome	PFF αSyn vs Monomer αSyn
SN transcriptome	PFF αSyn vs Monomer αSyn

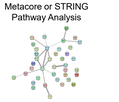




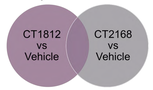
Goal 2: Identify effects of S2R modulation in PFF-treated animals

Differential Expression Contrasts	
Striatum proteome	CT1812 vs Vehicle
	CT2168 vs Vehicle
SN transcriptome	CT1812 vs Vehicle
	CT2168 vs Vehicle

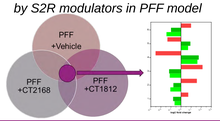




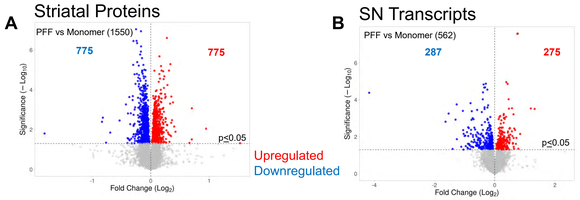
2A: Identify S2R-driven effects by comparing two chemically-distinct S2R modulators



2B: Identify proteins or transcripts normalized by S2R modulators in PFF model



PFF αSyn induces increased markers of immune response, and decreased dopaminergic and synaptic function markers



C

Gene	Protein ID	Log2FC	p-value
Nectin1	F1LNP8	-0.30	3.54E-07
Septin5	A0A338P769	-0.25	9.09E-08
Snca	P37377	-0.24	3.49E-07
C2cd4c	D3ZLU0	-0.23	4.54E-07
Syt17	A0A0G2K9H6	-0.23	4.06E-06
Sidt1	Q6Q3F5	-0.18	1.69E-06
RGD1306556	A0A8I6AS67	-0.17	1.21E-06
Stmn2	A0A8I5ZV30	-0.17	1.14E-07
Smap1	M0RC57	-0.17	6.59E-07
Slc39a10	D4A517	-0.15	2.57E-06
Tcirg1	G3V887	0.34	5.15E-06
Rnaset2	A0A8I6ALG0	0.31	1.08E-05
Abca1	F1LNL3	0.28	2.49E-07
S100b	A0A8I6A0X7	0.23	2.1E-05
Mfge8	Q1PBJ1	0.21	1.18E-05
Lgmn	Q5PPG2	0.18	1.08E-05
Ppt1	P45479	0.18	6.41E-06
Plxnb1	D3ZDX5	0.15	2.09E-06
Slc12a9	Q66HR0	0.15	1.62E-05
Polr2b	G3V8Y5	0.06	1.1E-05

D

Gene	Gene ID	Log2FC	p-value
Fam111a	012067	-4.17	4.15E-05
Tmem212	012608	-1.26	1.78E-04
Ccdc40	059401	-0.80	1.52E-04
Eno3	004078	-0.40	9.38E-05
Perp	011994	-0.39	3.98E-05
Slc6a3	017302	-0.37	1.48E-05
Bphl	017577	-0.35	2.18E-05
Th	020410	-0.32	2.12E-05
Snca	008656	-0.29	1.34E-05
Cpne7	015397	-0.24	2.82E-05
Cxcl13	024899	1.33	3.10E-04
Cxcl10	022256	1.20	2.93E-04
Rsad2	007539	0.76	2.61E-08
Nqo2	017820	0.75	2.70E-08
Nlrp1a	023143	0.57	2.87E-04
Ctgf	015036	0.47	3.01E-04
C3	046834	0.46	9.30E-05
Serpinb6a	017962	0.43	1.38E-05
Atr	010027	0.39	1.11E-05
Fam46a	010240	0.30	5.84E-04

All Gene IDs start with ENSRNOG00000

Fig. 2. Volcano plots illustrate significant changes (red, upregulated; blue, downregulated) in striatal protein levels **(A)** or nigral mRNA expression **(B)** due to treatment with PFF vs monomer (p≤0.05). Topmost significantly increased (red) and decreased (blue) proteins **(C)** and transcripts **(D)** are listed (p≤0.05), sorted by log-2 fold change in PFF-treated animals vs monomer. Proteins or transcripts of interest are labeled in bold, indicating those involved in immune response (yellow dots), dopaminergic signaling (purple dots), and synaptic function (green dots).

Proteins and transcripts altered by PFF αSyn enrich pathways related to neuronal processes and immune response

A Striatal Proteins: PFF vs Monomer, p≤0.05

Top Metacore Pathway Maps enriched by 1550 proteins	p-value
Neurophysiological process – Synaptic vesicle fusion and recycling in nerve terminals	1.22E-15
Transport – Clathrin-coated vesicle cycle	1.73E-06
Neurophysiological process – Receptor-mediated axon growth repulsion	4.00E-05
Development – Negative regulation of WNT/Beta-catenin signaling in the cytoplasm	4.43E-05
Signal transduction – Ephrin-B signaling	6.11E-05

GO Term ID	Biological Pathways	Strength	p-value
GO:0075525	Viral translational termination-reinitiation	1.1	3.40E-02
GO:0031632	**Positive regulation of synaptic vesicle fusion to presynaptic active zone membrane**	1.02	4.88E-02
GO:0051490	Negative regulation of filopodium assembly	1.02	4.88E-02
GO:1904428	Negative regulation of tubulin deacetylation	1.02	4.88E-02
GO:0048790	**Maintenance of presynaptic active zone structure**	1.0	2.20E-03

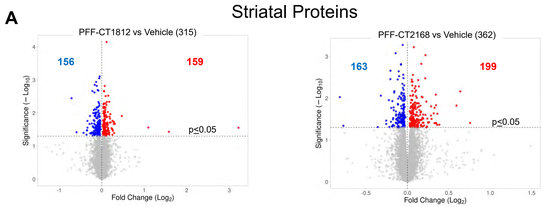
B SN Transcripts: PFF vs Monomer, p≤0.05

Top Metacore Pathway Maps enriched by 562 transcripts	p-value
Development – NOTCH signaling inhibition	1.06E-08
Regulation of immune cell differentiation by Notch signaling	5.36E-06
Development – Inhibition of ossification by Notch signaling	7.69E-06
Immune response – Classical complement pathway	1.15E-05
Transcription – HIF1 targets	2.50E-05

GO Term ID	Biological Pathways	Strength	p-value
GO:0045746	Negative regulation of Notch signaling pathway	0.92	6.60E-03
GO:0035082	Axoneme assembly	0.9	1.26E-06
GO:0006858	**Extracellular transport**	0.87	4.76E-02
GO:0035850	Epithelial cell differentiation involved in kidney development	0.87	4.76E-02
GO:0031670	Cellular response to nutrient	0.8	2.04E-02

Fig. 3. Metacore Pathway Analysis (Version 23.1.71200, green tables) and STRING pathway analysis (Version 16, purple tables) were conducted using significant (p≤0.05) 1550 striatal protein changes **(A)** or 562 nigral transcript changes **(B)** identified in PFF vs monomer. Pathways identified in non-relevant disease pathologies/organs were excluded from Top 5 pathways. Pathways of interest are labeled in bold.

S2R modulators induce changes in key pathways related to apoptosis, metabolism and transport

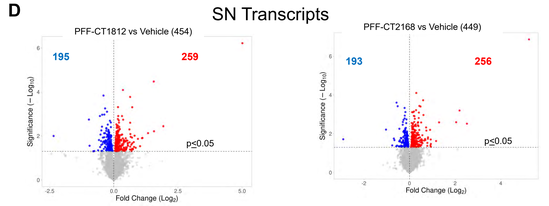


B PFF-CT1812 vs Vehicle p≤0.05

Top Metacore Pathway Maps enriched by 315 proteins	p-value
Aminoacyl-tRNA biosynthesis in mitochondrion	3.41E-06
Leucine, isoleucine and valine metabolism	1.95E-03
Development – NOTCH signaling in the nervous system	3.28E-03
Apoptosis and survival – Caspase cascade	3.59E-03
Development – YAP/TAZ-mediated co-regulation of transcription	3.59E-03

C PFF-CT2168 vs Vehicle p≤0.05

Top Metacore Pathway Maps enriched by 362 proteins	p-value
Transport – Rab-9 regulation pathway	2.15E-04
Development – Activation of ERK by Alpha-1 adrenergic receptors	2.33E-04
Translation – Translation regulation by Alpha-1 adrenergic receptors	5.05E-04
Signal transduction – ERK1/2 signaling pathway	6.42E-04
Proteolysis – Ubiquitination pathway	1.20E-03



E PFF-CT1812 vs Vehicle p≤0.05

Top Metacore Pathway Maps enriched by 454 transcripts	p-value
Apoptosis and survival – Granzyme B signaling	6.70E-09
Development – Self-renewal of adult neural stem cells	8.03E-08
Development – Epigenetic and transcriptional regulation of oligodendrocyte precursor cell differentiation and myelination	1.28E-07
Development – NOTCH signaling in the nervous system	1.60E-05
Autophagy	2.36E-05

F PFF-CT2168 vs Vehicle p≤0.05

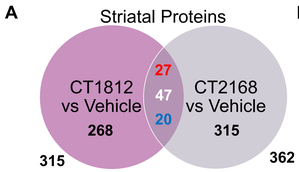
Top Metacore Pathway Maps enriched by 449 transcripts	p-value
Development – Self-renewal of adult neural stem cells	5.57E-06
Polyamine metabolism	1.65E-03
Apoptosis and survival – Ubiquitination and phosphorylation of TNF-alpha-induced NFkB signaling	1.78E-03
Development – YAP/TAZ-mediated co-regulation of transcription	6.68E-03
Development – NOTCH signaling in the nervous system	6.87E-03

Fig. 4. Volcano plots illustrate significant changes (p≤0.05; red, upregulated; blue, downregulated) in striatal protein levels **(A)** or nigral mRNA expression **(D)** due to treatment with CT1812 or CT2168 vs vehicle in PFF-injected animals. Metacore Pathway Analysis was conducted using significant (p≤0.05) striatal protein changes **(B-C)** or nigral transcript changes **(E-F)**. Top 5 pathways are listed, with pathways of interest indicated in bold. **Non-relevant disease pathologies/organs were excluded.**

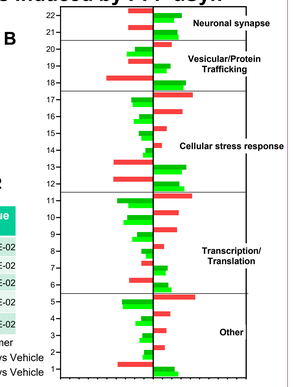
CONCLUSIONS

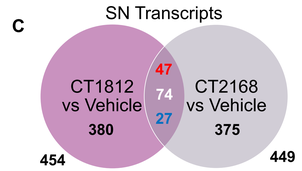
- PFF αSyn alters biological pathways related to vesicular trafficking in striatum and immune response in SN, congruent with the path of propagation of aberrant αSyn[4].
- S2R modulators CT1812 and CT2168 affect pathways associated with synucleinopathy, including apoptosis, metabolism, proteostasis, and protein/lipid transport.
- S2R modulators reverse a subset of protein and gene expression changes caused by PFF αSyn, including transcript level changes in neuroprotective cerebral dopamine neurotrophic factor (CDNF)[5].

Both S2R modulators reverse a subset of protein and gene expression changes induced by PFF αSyn



Top Metacore Pathway Maps enriched by 47 overlapping proteins	p-value
Pyruvate metabolism	1.27E-02
Aminoacyl-tRNA biosynthesis in mitochondrion	1.27E-02
LRRK2 in neuronal apoptosis in Parkinson's disease	2.59E-02
Development – Role of CNTF and LIF in regulation of oligodendrocyte development	4.23E-02
Apoptosis and survival – Cytoplasmic/mitochondrial transport of Bid, Bmf, and Bim	5.12E-02





Top Metacore Pathway Maps enriched by 74 overlapping transcripts	p-value
Proteolysis – Putative SUMO-1 pathway	2.02E-03
Development – Histone H3 demethylases in stem cells	2.46E-03
DNA damage – ATM/ATR regulation of G2/M checkpoint nuclear signaling	4.81E-03
Fructose metabolism	1.50E-02
Transport – Intracellular cholesterol transport	1.57E-02

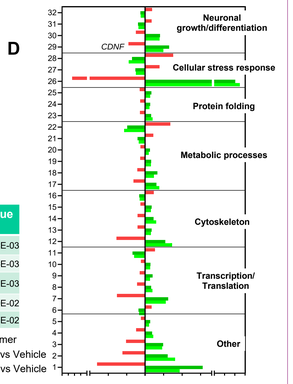


Fig. 5. Venn diagrams illustrate 47 striatal proteins **(A)** and 74 nigral transcripts **(C)** commonly altered by CT1812 and CT2168 treatment compared to vehicle irrespective of direction of effect (white), increased by both compounds (red), or decreased by both compounds (blue) (p≤0.05). Metacore Pathway Analysis was conducted using significant overlapping proteins or transcripts. Top 5 pathways are listed, with pathways of interest indicated in bold. **Non-relevant disease pathologies/organs were excluded. (B, D** 22 of the 47 proteins and 32 of the 74 transcripts were also significantly altered by PFF vs monomer. Forest plots illustrate changes in direction of the 22 striatal proteins **(B)** and 32 nigral transcripts **(D)** induced by compound treatments (green) compared to significantly changed proteins or genes induced by PFF vs monomer (red). **Cerebral dopamine neurotrophic factor (*CDNF*), for example in (D)**, is decreased by PFF vs monomer and normalized by CT1812 and CT2168.

Preclinical data illuminate effects of S2R modulators in cell survival and transport pathways, which are key factors in PFF-mediated synucleinopathy.

REFERENCES
1. Winner B, Jappelli R, Maji SK, et al. In vivo demonstration that alpha-synuclein oligomers are toxic. *Proc Natl Acad Sci* USA 2011; 108: 4194–9.
2. Limegrover CS, Yurko R, Izzo NJ, et al. Sigma-2 receptor antagonists rescue neuronal dysfunction induced by Parkinson's patient brain-derived α-synuclein. *J Neurosci Res* 2021: jnr.24782.
3. Patterson JR, Duffy MF, Kemp CJ, et al. Time course and magnitude of alpha-synuclein inclusion formation and nigrostriatal degeneration in the rat model of synucleinopathy triggered by intrastriatal α-synuclein preformed fibrils. Neurobiol Dis 2019; 130.
4. Duffy, M, Collier, TJ, Patterson JR, et al. Lewy body-like alpha-synuclein inclusions trigger reactive microgliosis prior to nigral degeneration. *J Neuroinflammation*. 2018, 15: 129.
5. Lindholm P and Saarma M. Cerebral dopamine neurotrophic factor protects and repairs dopamine neurons by novel mechanism. *Mol Psychiatry*. 2022; 27(3). 1310-1321.

ACKNOWLEDGEMENTS:
In-life portion of the study was conducted at Atuka, Inc. (Toronto, Canada) - All animal studies were conducted according to CCAC guidelines and under IACUC-approved Animal Use Protocols (AUPs)

This work was funded in part by the Michael J. Fox Foundation
CT1812 is in a Phase 2 clinical trial for DLB (NCT05225415)

Corresponding author: mhamby@cogrx.com

COGNITION™ Therapeutics