A transcriptomic analysis to investigate the role of sigma-2 receptor modulators CT1812 and CT2168 in a mouse model of Alzheimer's disease.

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INTRODUCTION

Amyloid-beta (Aβ) oligomers bind to receptors on neurons and cause synaptotoxicity and cognitive decline in Alzheimer's disease (AD)[1]. Sigma-2 receptor (S2R) modulators, such as our investigational therapeutic CT1812[2], can displace Aβ oligomers from binding to neuronal synapses and clear the oligomers to cerebrospinal fluid. We have previously demonstrated (Figure 1)[1] that decreasing binding of Aβ oligomers to neuronal synapses can restore cognitive activity in hAPPsI transgenic mouse model of AD[1, 3]. To further investigate the biological processes of S2R modulators and their molecular mechanism of action, we performed RNA sequencing analysis in an *in vivo* AD mouse model, hAPPsI, treated with our investigational therapeutic, CT1812, and a chemically distinct S2R modulator, CT2168.

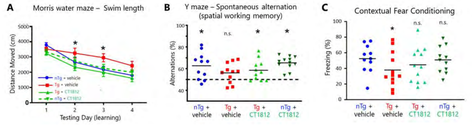

Figure 1. Treatment with CT1812 improves learning and memory deficits in transgenic AD mouse model. **A**), hAPPsI transgenic mice (Tg) treated with CT1812 (Tg + CT1812) learn the Morris water maze task significantly better than Tg vehicle-treated mice (Tg + vehicle; P = 0.016, two-way repeated measures analysis of variance, Bonferroni post hoc: *P < 0.5; mean ± SEM). CT1812 treatment does not affect non-transgenic animal (nTg) performance (nTg + CT1812). **B**), Tg mice treated with CT1812 remember previous arms entered in the Y maze task significantly better (P = 0.013, Student's t test) than chance (dashed line), but Tg vehicle-treated animals do not (nTg + vehicle, 62.7 ± SD 12.2%; Tg + vehicle, 56.1 ± SD 9.2%; Tg + CT1812, 58.5 ± SD 9.4%; nTg + CT1812, 65.3 ± SD 6.0%). **C**), Tg mice show deficits in the Contextual Fear Conditioning test (P = 0.037, Student's t test), while Tg and nTg mice treated with CT1812 do not (nTg + vehicle, 52.5 ± SD 5.4%; Tg + vehicle, 37.9 ± SD 6.4%; Tg + CT1812, 44.6 ± SD 6.5%; nTg + CT1812, 50.9 ± SD 5.1%). Each data point in (B) and (C) represents an individual mouse with the mean of all the points represented by the horizontal lines.[1]

METHODS

Five-month-old, male, non-transgenic mice (nTg) were dosed with vehicle and age-matched male transgenic mice (Tg) with the human APP London (717) and Swedish (670/671) mutation hAPPsI were dosed with vehicle or either CT1812 (10 mg/kg) or CT2168 (5 mg/kg), given orally, once daily for 7 days. Unbiased RNA sequencing analysis (N=10 per group) was conducted to evaluate differentially expressed genes (DEGs) between nTg and Tg mice, and to assess the effect of CT1812 and CT2168 compared to vehicle. STRING and MetaCore pathway analyses were performed using gene lists of $p \leq 0.05$. The animal study was performed by QPS Custom-build research in Austria.

Group	Genotype	Compound	Dose (mg/kg)	Schedule
A	nTg	vehicle	N/A	Once a day for 7 days
B	hAPPsI (Tg)	vehicle	N/A	Once a day for 7 days
C	hAPPsI (Tg)	CT1812	10	Once a day for 7 days
D	hAPPsI (Tg)	CT2168	5	Once a day for 7 days

Table 1. Summary table of treatment groups

Figure 2. Study design. Animals were sacrificed 24 hours after last dose, and brain was collected. Of the brain samples, hippocampus samples were used for transcriptomic analyses.

REFERENCES

1. Izzo NJ et al., Alzheimer's Dement. 2021 Aug;17(8):1365-1382;
2. Clinical trials NCT03507790, NCT05531656
3. Izzo NJ et al., PLoS One. 2014 Nov 12;9(11):e111898
4. Clinical trials NCT04735536, NCT03507790

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RESULTS

Total differentially expressed genes in transcriptomic analyses

Condition	# of DEGs (p≤0.05)	# of downregulated genes	# of upregulated genes
Tg vs nTg	909	541	368
Tg: CT1812 vs vehicle	2031	930	1101
Tg: CT2168 vs vehicle	365	188	177

Table 2. Summary table of up- or downregulated differentially expressed genes (DEGs) for each treatment group.

CT1812 and CT2168 treatments impact the transcriptomic profiles of treated animals

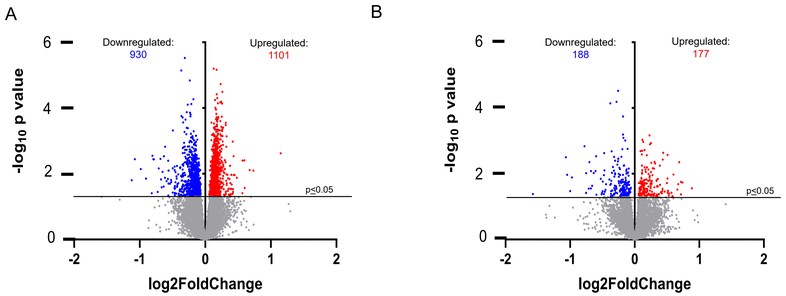

Figure 3. Volcano plots to visualize the global transcriptomic change after treatment with A) CT1812 and B) CT2168 (p≤0.05). Each dot is a gene; red indicates upregulated and blue indicates down-regulated genes.

Comparative analyses between treatment groups

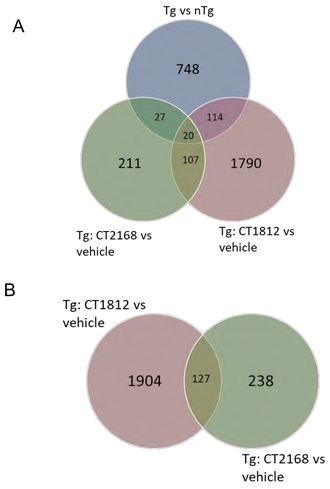

Figure 4. Distinct and Common DEGs (p≤0.05) between A) nTg and Tg treated animals with CT1812 and CT2168 and B) CT1812 and CT2168 treated animals.

Common DEGs after treatment with CT1812 and CT2168 with normalization towards nTg

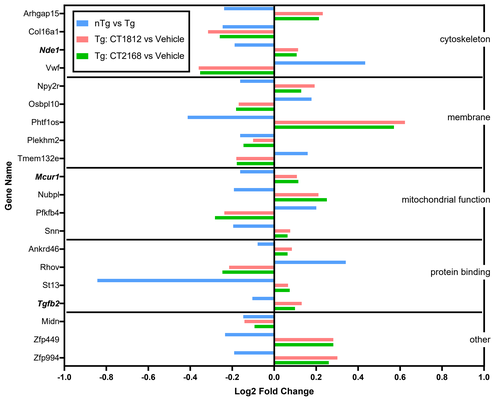

Figure 5. Forest plot showing directional fold changes of 20 common DEGs identified between nTg and Tg treated animals with CT1812 vs vehicle and CT2168 vs vehicle. Genes showing an aberrant expression in the Tg vs. nTg that are regulated in the opposing direction by CT1812 and CT2168 may underlie a possible regulatory mechanism of the analyzed S2R modulators. Genes in bold, such as ***Tgfβ2, Nde1***, and ***Mcur1*** *are* related to AD phenotype.

S2R modulators CT1812 and CT2168 alter pathways related to AD pathology

A

#	Top Transcriptomic Pathways (Tg: CT1812 vs vehicle; p≤0.05)	P-value
1	Transport Clathrin-coated vesicle cycle	1.42E-09
2	Proteolysis Ubiquitination pathway	3.68E-05
3	Transport RAB1A regulation pathway	4.22E-05
4	Development NOTCH signaling in the nervous system	1.31E-04
5	Immune response IL-15 signaling via JAK-STAT and PPAR cascades	2.79E-04
6	Neurogenesis NGF/ TrkA MAPK-mediated signaling	8.99E-04

B

#	Top Transcriptomic Pathways (Tg:CT2168 vs vehicle; p≤0.05)	P-value
1	Canonical WNT signaling pathway in colorectal cancer	8.85E-05
2	Signal transduction FGFR1 signaling	5.81E-04
3	Immune response Lysophosphatidic acid signaling via NF-kB	2.608E-03
4	Apoptosis and survival Caspase cascade	3.419E-03
5	WNT signaling in invasive-type melanoma cells	5.25E-03
6	Autophagy	6.162E-03

Table 3: Metacore pathway analysis (v. 23.1.71200) of transcriptomic data after treatment with A) CT1812 and B) CT2168. Pathways identified in non-relevant disease pathologies/organs were excluded from Top 6 pathways.

CONCLUSIONS

- For the first time, in a mouse model where cognitive performance can be improved by S2R modulators, a comprehensive transcriptomic analysis has been performed.
- Findings indicate that two chemically distinct S2R modulators, CT1812 and CT2168 can regulate key mRNA transcripts relevant to AD.
- Pathway analysis revealed that membrane trafficking, cytoskeleton remodeling, autophagy, inflammation, and WNT/β-catenin signaling pathways, pathways relevant to AD, can be impacted by CT1812 and CT2168.
- Interestingly, proteomic analysis of CSF and plasma from AD patients treated with CT1812 in recent clinical trials[4] have similar altered pathways as in this mouse model.
- Together, these findings highlight the mechanism of action of S2R modulators and how they influence pathways in AD models

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