Unbiased transcriptomic analysis of the Sigma-2 receptor modulator CT1812 in cell-based models of dry age-related macular degeneration

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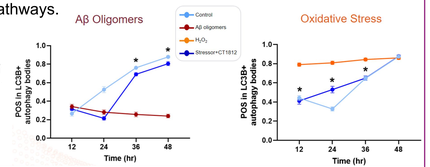
INTRODUCTION

Age-related macular degeneration (AMD) is one of the leading causes of blindness, with dry AMD accounting for the majority of AMD cases. There are several hallmarks of dry AMD including inflammation, oxidative stress, and the presence of amyloid-beta oligomers (AβO), which all can disrupt key homeostatic functions of retinal pigmented epithelium cells (RPE)[1-4]. The sigma-2 receptor (S2R) regulates pathways involved in age-related diseases, including oxidative stress and AβO toxicity[5]. A single nucleotide polymorphism (SNP) in the gene encoding sigma-2 receptor (*TMEM97*) has been identified that confers a decreased risk of AMD in genome-wide association (GWA) studies[6-8]. Furthermore, knock-down or knockout of *TMEM97* has been shown to protect RPEs in cellular stress models[9-10]. Proteomic analyses from aged patients suggest that S2R modulators affect pathways related to AMD[11-12]. Taken together, small-molecule targeting of S2R may fill an important unmet need as a non-invasive, more convenient treatment option for patients with dry AMD.

The S2R modulator CT1812 is currently in Phase 2 clinical trials for Alzheimer's disease (NCT03507790, NCT04735536) and dementia with Lewy bodies (NCT05225415), with plans for Phase 2 clinical trials for geographic atrophy secondary to dry AMD. Given the overlap in S2R-regulated pathways and dry AMD pathology, the ability to rescue key functional deficits in RPE cells, and the genetic link of S2R to dry AMD, it was hypothesized that the S2R modulator CT1812 will alter dry AMD-relevant transcripts and pathways.

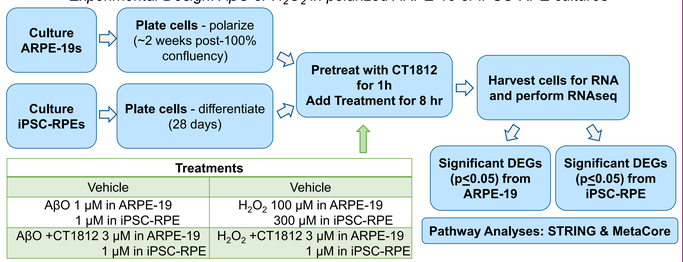
Schema: CT1812 rescues crucial function of RPE to traffic & degrade photoreceptor outer segments (POS) cargos following toxic insults[13]. Line graphs show colocalization of POS with autophagosome marker LC3 over time after introduction of the toxic stressor.



METHODS

Goal 1) Characterize human cell-based models for dry AMD via transcriptomic analysis

Experimental Design: AβO or H_2O_2 in polarized ARPE-19 or iPSC-RPE cultures

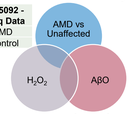


Treatments	
Vehicle	Vehicle
AβO 1 μM in ARPE-19 1 μM in iPSC-RPE	H_2O_2 100 μM in ARPE-19 300 μM in iPSC-RPE
AβO +CT1812 3 μM in ARPE-19 1 μM in iPSC-RPE	H_2O_2 +CT1812 3 μM in ARPE-19 1 μM in iPSC-RPE

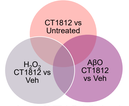
Goal 2) Compare transcripts from cell-based models and determine effects of CT1812 treatment

Compare gene expression changes to patient RPE/choroid meta-analysis[14]

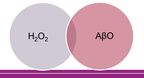
GSE29801 - Microarray Data	GSE135092 - RNAseq Data
41 AMD	26 AMD
50 control	105 control



Determine CT1812-induced gene expression changes within RPE models



Compare gene expression changes across in vitro stressors



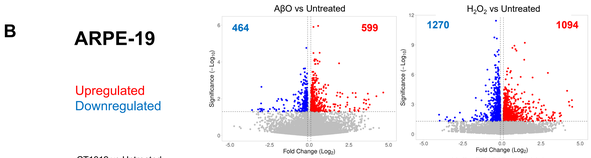
Determine CT1812-induced gene expression changes across RPE models

Pathways relevant to dry AMD pathology are represented by treatment with AβO or H_2O_2

A

Condition	Number of Differentially Expressed Genes			
	ARPE-19 p≤0.05	ARPE-19 p≤0.01	iPSC-RPE p≤0.05	iPSC-RPE p≤0.01
CT1812 vs vehicle	941	224	167	17
AβO vs untreated	1063	336	249	43
AβO +CT1812 vs vehicle	1839	710	371	59
H_2O_2 vs untreated	2364	1039	1022	431
H_2O_2 +CT1812 vs vehicle	634	132	389	63

B



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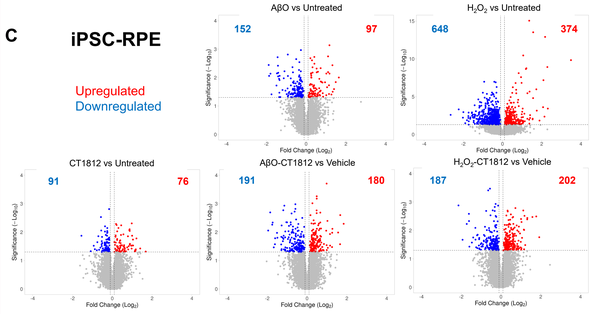


D

AβO vs untreated p≤0.05

ARPE-19 Pathway Map	P-value	iPSC-RPE Pathway Map	P-value
Development – VEGF signaling and activation	7.738E-05	Immune response - Alternative complement pathway	1.334E-08
Development – GDNF ligand family/RET receptor	3.318E-04	Immune response - IFN-alpha/beta signaling via JAK/STAT	4.777E-08
Development - Negative regulation of WNT/beta-catenin signaling	1.283E-04	IFN-gamma actions on extracellular matrix and differentiation	3.316E-07
Signal transduction – Non-canonical WNT5A signaling	3.318E-04	Immune response - Antiviral actions of Interferons	4.804E-06
Development – Ligand-dependent activation of ESR1/AP-1 pathway	3.327E-03	Immune response - Classical complement pathway	5.380E-06

E

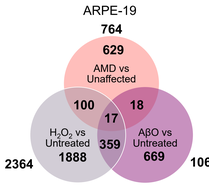
H_2O_2 vs untreated p≤0.01

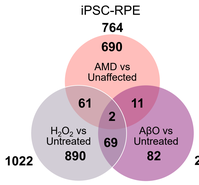
ARPE-19 Pathway Map	P-value	iPSC-RPE Pathway Map	P-value
Transcription - HIF-1 targets *	3.562E-05	Oxidative stress - ROS-induced cellular signaling	3.199E-09
Apoptosis and survival - NGF/ TrkA PI3K-mediated signaling	1.255E-04	Immune response - IFN-alpha/beta signaling via JAK/STAT	3.100E-08
Immune response - CD40 signaling	1.400E-04	DNA damage - p53 activation by DNA damage	8.277E-07
Signal transduction - mTORC2 downstream signaling *	2.353E-04	Transcription - HIF-1 targets *	1.800E-05
Apoptosis and survival - Caspase cascade	3.060E-04	TGF-beta-dependent induction of EMT via MAPK	3.527E-05

Fig. 1. A) Summary of differentially expressed genes (DEGs) in each treatment condition in ARPE-19s and iPSC-RPE cultures. B,C) Volcano plots illustrate significant changes (red, upregulated; blue, downregulated) in mRNA expression due to treatment (p<0.05). D, E) Metacore Pathway Analysis (Version 23.1.71200) conducted using p-value criterion designated in bold-face text. Pathways identified in non-relevant disease pathologies/organs were excluded from Top 5 pathways. Pathways within the Top 50 common to both ARPE-19 and iPSC-RPE are indicated with asterisks (*).

Transcript changes and pathways identified in patient RPE are recapitulated in *in vitro* models

A





B

Meta-analysis Comparison with AβO p≤0.05

Pathway Map	P-value
De novo IMP biosynthesis *	4.761E-04
UMP biosynthesis *	1.600E-02
Mitochondrial ketone bodies biosynthesis and metabolism *	2.017E-02
Pentose phosphate pathway	2.847E-02
Nitrogen metabolism	3.012E-02

Meta-analysis Comparison with AβO p≤0.05

Pathway Map	P-value
SCAP/SREBP Transcriptional Control of Cholesterol and FA Biosynthesis	1.749E-04
Transport – Macropinocytosis	5.538E-03
Cell adhesion - Gap junctions	1.013E-02
Mitochondrial ketone bodies biosynthesis and metabolism *	1.105E-02
Endothelial cell contacts by junctional mechanisms	1.197E-02

C

Meta-analysis Comparison with H_2O_2 p≤0.05

Pathway Map	P-value
Unsaturated fatty acid biosynthesis *	2.267E-04
SCAP/SREBP Transcriptional Control of Cholesterol and FA Biosynthesis	1.162E-03
Development - NOTCH/ TGF-beta crosstalk in EMT *	1.162E-03
n-6 Polyunsaturated fatty acid biosynthesis	2.798E-03
n-3 Polyunsaturated fatty acid biosynthesis	3.067E-03

Meta-analysis Comparison with H_2O_2 p≤0.05

Pathway Map	P-value
ROBO2, ROBO3 and ROBO4 signaling pathways	1.707E-03
De novo IMP biosynthesis *	4.126E-03
SLIT-ROBO1 signaling	4.337E-03
TGF-beta-dependent induction of EMT via RhoA, PI3K and ILK	5.702E-03
TGF-beta-dependent induction of EMT via MAPK *	5.946E-03

Fig. 2: A) Venn diagrams of common DEGs (p≤0.05) identified *in vitro* models compared with DEGs identified in the macular samples from a meta-analysis study[14], irrespective of direction of effect. B) Metacore pathway analysis was conducted on the overlapping DEGs between the meta-analysis dataset[14] and *in vitro* models using p-value criterion p≤0.05. Pathways identified in non-relevant disease pathologies/organs were excluded from Top 5 pathways. Pathways within the Top 50 common to both ARPE-19 and iPSC-RPE are indicated with asterisks (*).

S2R modulator CT1812 alters pathways related to AMD pathology during stress conditions

A

AβO-CT1812 vs Vehicle p≤0.05

ARPE-19 Pathway Map	P-value	iPSC-RPE Pathway Map	P-value
Transcription - HIF-1 targets	1.663E-09	Immune response - BAFF-induced non-canonical NF-kB signaling	2.563E-04
Signal transduction - PDGF signaling via MAPK cascades	6.087E-09	Immune response - Role of PKR in stress-induced antiviral cell response *	3.180E-04
Neurogenesis - NGF/ TrkA MAPK-mediated signaling	6.623E-08	Apoptosis and survival - APRIL and BAFF signaling	7.151E-04
Cell adhesion – ECM remodeling	3.819E-06	Apoptosis and survival - NGF activation of NF-kB	7.881E-04
Development - Regulation of epithelial-to-mesenchymal transition	5.266E-06	Apoptosis and survival - Apoptotic TNF-family pathways *	9.497E-04

B

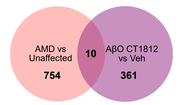
H_2O_2-CT1812 vs Vehicle p≤0.05

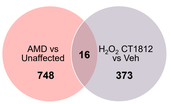
ARPE-19 Pathway Map	P-value	iPSC-RPE Pathway Map	P-value
Signal transduction - FGFR4 signaling	9.378E-05	Apoptosis and survival - APRIL and BAFF signaling	1.250E-03
Signal transduction - FGFR1 signaling	2.272E-04	Signal transduction - Role of MIF as an intracellular mediator	1.510E-03
Immune response - PGE2 signaling in immune response	1.106E-03	Cell cycle - Role of SCF complex in cell cycle regulation	5.134E-03
Transport - Clathrin-coated vesicle cycle	1.525E-03	Immune response - IFN-alpha/beta signaling via JAK/STAT	6.832E-03
Immune response - IL-5 signaling via JAK/STAT	2.958E-03	Cell cycle -DNA replication: elongation and termination	7.638E-03

Fig. 3. A, B) Metacore pathway analysis was conducted. Pathways identified in non-relevant disease pathologies/organs were excluded from Top 5 pathways. Pathways within the Top 50 common to both ARPE-19 and iPSC-RPE are indicated with asterisks (*).

CT1812 modifies genes and pathways in *in vitro* models also identified in patient RPE

A





B

Meta-analysis and AβO-CT1812 vs Vehicle

Pathway Map	P-value
N-Acylethanolamines, HSRL5-transacylation pathway	6.231E-03
Anandamide biosynthesis and metabolism	1.060E-02
Phospholipid metabolism p.2	1.083E-02

Meta-analysis and H_2O_2-CT1812 vs Vehicle

Pathway Map	P-value
Cell adhesion - Desmosomes	5.846E-03
Development - TGF-beta-induction of EMT via ROS	6.153E-03
Signal transduction - Negative regulation of BMP signaling	9.831E-03

Fig. 4 A) Venn diagrams of common DEGs (p≤0.05) identified in iPSC-RPE models treated with CT1812 compared with DEGs identified in the macular samples from the meta-analysis study.[14] B) Metacore pathway analysis was conducted on the overlapping DEGs between the meta-analysis dataset and *in vitro* models using p-value criterion p≤0.05. Non-relevant disease pathologies/organs were excluded from Top 3 pathways.

A subset of transcripts were found to be commonly altered across *in vitro* models

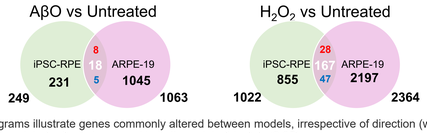
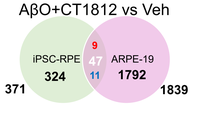


Fig. 5. Venn diagrams illustrate genes commonly altered between models, irrespective of direction (white), increased in both models (red), or decreased in both models (blue) (p≤0.05).

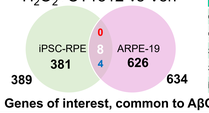
CT1812 modulates genes across *in vitro* models that are associated with RPE cellular biology and AMD

A



Pathway Map	P-value
Apoptosis and survival - Apoptotic TNF-family pathways	7.790E-05
Apoptosis and survival - Caspase cascade	1.846E-04
Immune response - IL-4-responsive genes in type 2 immunity	3.579E-04
Signal transduction - Thrombospondin 1 signaling	3.732E-04
Apoptosis and survival - p53 and p73-dependent apoptosis	6.114E-04

B



Pathway Map	P-value
Gamma-secretase regulation of angiogenesis	3.093E-05
Gamma-secretase proteolytic targets	2.235E-04
Signal transduction - Ephrin-B signaling	1.472E-02
Signal transduction - Ephrin-A signaling	1.685E-02
Neurophysiological process - Netrin-1 signaling	2.111E-02

C

Genes of interest, common to AβO- and H2O2-CT1812 vs Vehicle p≤0.05

Model	Gene	Change in Direction	Functional Description
iPSC-RPE	A	↑	Nucleotide exchange factor, involved in MAPK signaling
	B	↓	Plasma membrane component, elevated in AMD patient plasma
ARPE-19	C	↑	Plasma membrane component, involved in cell adhesion
	D	↑	Metallopeptidase, involved in regulating inflammation

Fig. 6. A,B) Venn diagrams illustrate genes commonly altered between models treated with CT1812, irrespective of direction of effect (white), increased in both models (red), or decreased in both models (blue) (p≤0.05). Tables list Top 5 pathways from Metacore analysis using overlapping genes. C) Table lists genes of interest relevant to dry AMD in the same direction of effect in stress models.

CONCLUSIONS

- *In vitro* oxidative stress and AβO toxicity models recapitulate some of the gene expression changes observed in AMD patient RPE.
- S2R modulator CT1812 modulates key pathways and genes related to dry AMD, including those related to the immune response, extracellular matrix, and cell survival.
- Comparative analysis with patient RPE shows that CT1812 modifies transcripts relevant to AMD biology and warrants further mechanistic study.

Preclinical evidence supports mechanistic rationale for CT1812 in modulating cellular processes active in dry AMD.

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