# Sigma-2 Receptor Modulation Promotes Retinal Pigment Epithelial Cell Survival Following Chronic 7-Ketocholesterol Exposure

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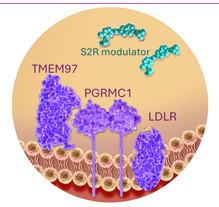
## INTRODUCTION

A hallmark of dry age-related macular degeneration (AMD) is the formation of drusen, which consists of proteins and oxidized lipid products under the retinal pigment epithelium (RPE). The oxysterol 7-Ketocholesterol (7KC) is the most abundant form of oxidized lipid present in drusen and is thought to play a role in AMD pathogenesis leading to RPE cell demise, the defining feature of geographic atrophy (GA). There are currently no FDA-approved therapies combating the accumulation of oxidized lipids in the retina that occurs in dry AMD.



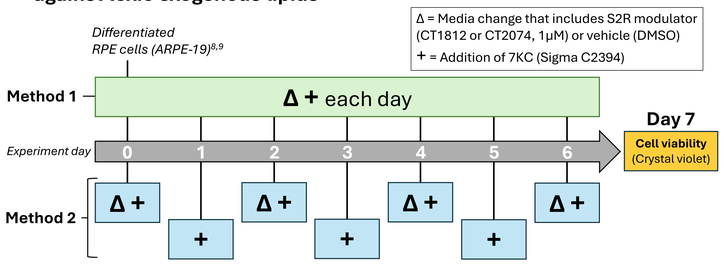

**Schema 1.** The sigma-2 receptor complex is comprised of TMEM97 and PGRMC1 with protein-protein interactions that include the LDL receptor (LDLR) (*purple*). We have demonstrated that S2R modulators (*cyan*) CT1812 (zervimesine) and CT2074 bind TMEM97[1].

The sigma-2 receptor (S2R, TMEM97) has been linked to dry AMD in genome-wide association studies[2]. Moreover, the S2R plays multiple roles in cellular homeostasis pathways, including regulation of lipids and vesicular trafficking[3-5]. We previously demonstrated *in vitro* that small molecule modulators of S2R, including investigational therapeutic CT1812 (zervimesine), can rescue RPE functional deficits induced by oxidative stress or toxic amyloid-β oligomers[6]. Here, we interrogate whether S2R modulators may also improve RPE viability in an *in vitro* model of oxidized lipid accumulation.

**GOAL: Provide proof-of-concept that S2R modulators are protective against RPE cell death induced by oxysterol 7KC**

## METHODS

- **Aim 1: Develop an *in vitro* assay assessing RPE cellular demise induced by chronic exposure to oxysterol 7KC[7]**
- **Aim 2: Demonstrate proof-of-concept of S2R modulator RPE protection against toxic exogenous lipids**

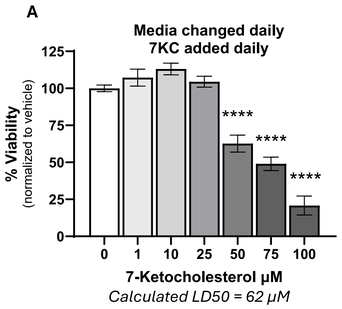



*See **Poster #A0491** for additional studies conducted using CogRx S2R modulators*

*"Delineating Mechanisms of Sigma-2 Receptor Modulators in Regulating Retinal Pigment Epithelial Lipid Uptake"*
*Wednesday May 7, 10:15AM – 12PM*

## RESULTS

### Chronic exposure to 7KC induces a concentration-dependent decrease in cell viability



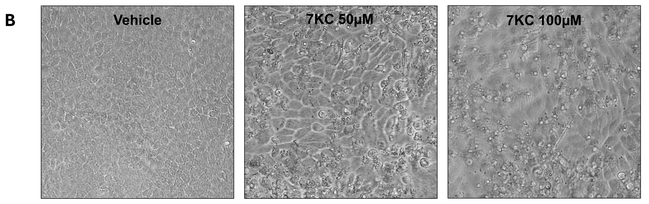
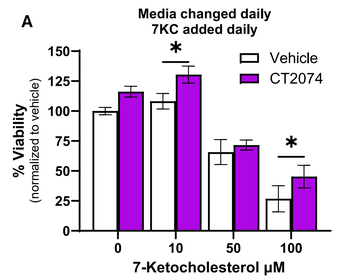




**Figure 1**. A. Differentiated RPE cells (ARPE-19) were treated once daily for seven days with increasing concentrations of 7KC (1-100 μM) or vehicle (EtOh-PBS), administered on top of a full media change. Cell viability was quantified by crystal violet assay seven days after experiment initiation. Absorbance values were normalized to vehicle (N=4 experiments, mean +/-SEM; one-way ANOVA; ****p<0.0001. B. Representative brightfield images of cultures treated with vehicle , 50μM 7KC , or 100μM 7KC.

### Treatment with a S2R modulator improves RPE cell viability when co-administered with 7KC



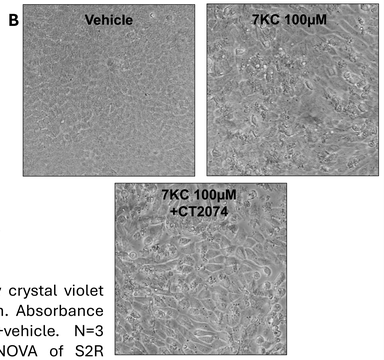
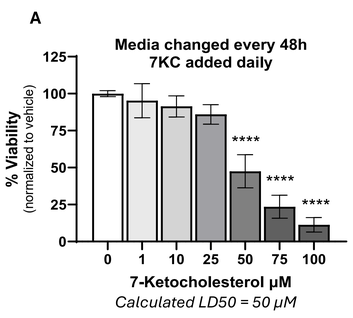




**Figure 2**. A. Cell viability was quantified by crystal violet assay seven days after experiment initiation. Absorbance values were normalized to 0μM 7KC +vehicle. N=3 experiments, mean +/- SEM; two-way ANOVA of S2R modulator CT2074 vs vehicle. *p<0.05. B. Representative brightfield images of cultures treated with vehicle, 100μM 7KC, or 100μM 7KC +CT2074.

### Assay optimized to increase effect size of 7KC-mediated cell death



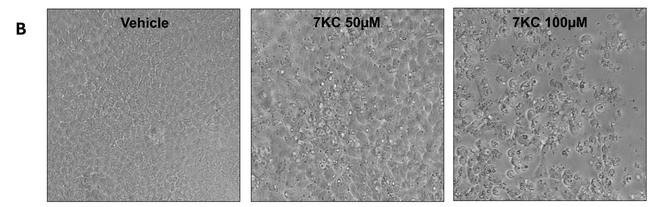
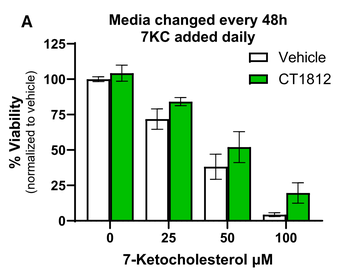




**Figure 3**. A. Differentiated RPE cells (ARPE-19) were treated for seven days with increasing concentrations of 7KC (1-100 μM) or vehicle (EtOh-PBS), administered on top of a full media change every 48 h. Between media changes, 7KC was re-administered on top of existing media. Cell viability was quantified by crystal violet assay seven days after experiment initiation. Absorbance values were normalized to vehicle (N=4 experiments, mean +/-SEM; one-way ANOVA; ****p<0.0001. B. Representative brightfield images of cultures treated with vehicle , 50μM 7KC , or 100μM 7KC.

### Preliminary results indicate that S2R modulator CT1812 may improve RPE cell viability during chronic 7KC treatment



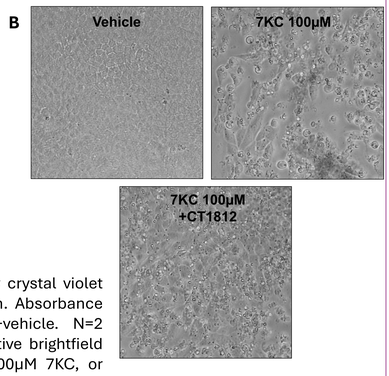




**Figure 4**. A. Cell viability was quantified by crystal violet assay seven days after experiment initiation. Absorbance values were normalized to 0μM 7KC +vehicle. N=2 experiments, mean +/- SEM. B. Representative brightfield images of cultures treated with vehicle, 100μM 7KC, or 100μM 7KC +CT1812.

## CONCLUSIONS

- 7-ketocholesterol (7KC) is a disease-relevant oxidized lipid species that induces concentration dependent cell death *in vitro*
- Preliminary data suggest that S2R modulators CT1812 and CT2074 can improve RPE cell viability under conditions of elevated 7KC
  - Further assay optimization is necessary to determine ideal assay conditions for rescue with S2R modulators

**These data support the therapeutic potential of S2R modulation for slowing RPE cell demise caused by oxysterol accumulation associated with dry AMD**

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