Childhood Eye Cancer Trust (CHECT) Research Grant Final Report (end of grant report)

Please complete all sections of this report and return to petra.maxwell@chect.org.uk.

Project title:	Assessing the feasibility of pluripotent stem cell derived retinal organoids as a model system to test the safety and efficacy of chemotherapeutic agents in Retinoblastoma		
Project reference:	(To be completed by CHECT)		
Total award:	£50,000		
Details of any additional funding:			
Lead investigator:	Prof. Majlinda Lako	Administering institution:	Newcastle University
Start date of award:	1.10.2021	End date of award:	30.09.2022

1. **Final Report**
   Please structure your report as follows:

   a. Summary of findings/results/outcomes of this project (with reference to the aims and objectives stated in your original application).
   b. If your aims and objectives changed during the course of the project, please explain why and in what way.
   c. Any problems or challenges impacting on the findings / results / outcomes of this project.
   d. How will these findings or outcomes impact patients or the public, and in what timescale? Do you foresee any obstacles / barriers to patients benefitting from the research findings?

a. Summary of findings: This project aimed to achieve the following objectives:

Objective 1: To validate the utility of RB pluripotent stem cell derived organoid model for *in vitro* evaluation of chemotherapeutic drugs.
Objective 2: To investigate the impact of RB chemotherapeutic drugs on the adjacent retinal cells.
Objective 3: To investigate the impact of RB chemotherapeutic drugs on the RPE cells.

Objectives 1 and 2 have been fully achieved. To assess the application of retinal organoids for testing therapeutic agents, we incubated control and pRB-depleted organoids with varying doses of three drugs used in current treatments of Rb tumours: Melphalan, Topotecan and TW-37 (**Figs. 1, 2**). With the kind support of Fight for Sight we have derived control and pRB-depleted organoids from a human embryonic stem cell line (hESC) and a patient-specific induced pluripotent stem cells (hiPSCs, [1]. Melphalan is an alkylating agent that is highly effective against Rb, but high

concentrations [2] are needed to reach its metronomic IC50 (50% inhibitory concentration) *in vitro*, attainable only after intraarterial or intravitreal chemotherapy [3]. Topotecan is a topoisomerase inhibitor, which prevents topoisomerase-I from re-ligating the nicked DNA strand, resulting in DNA damage and cell death [4]. It is effective against Rb in combination with Melphalan. Bcl-2 inhibitors such as TW-37 act by competing with pro-apoptotic proteins (such as Bid, Bim and Bad) and induce apoptosis [5]. Day 150 control and pRB-depleted hESC and hiPSC organoids were incubated with each of these three drugs for 72 hours.

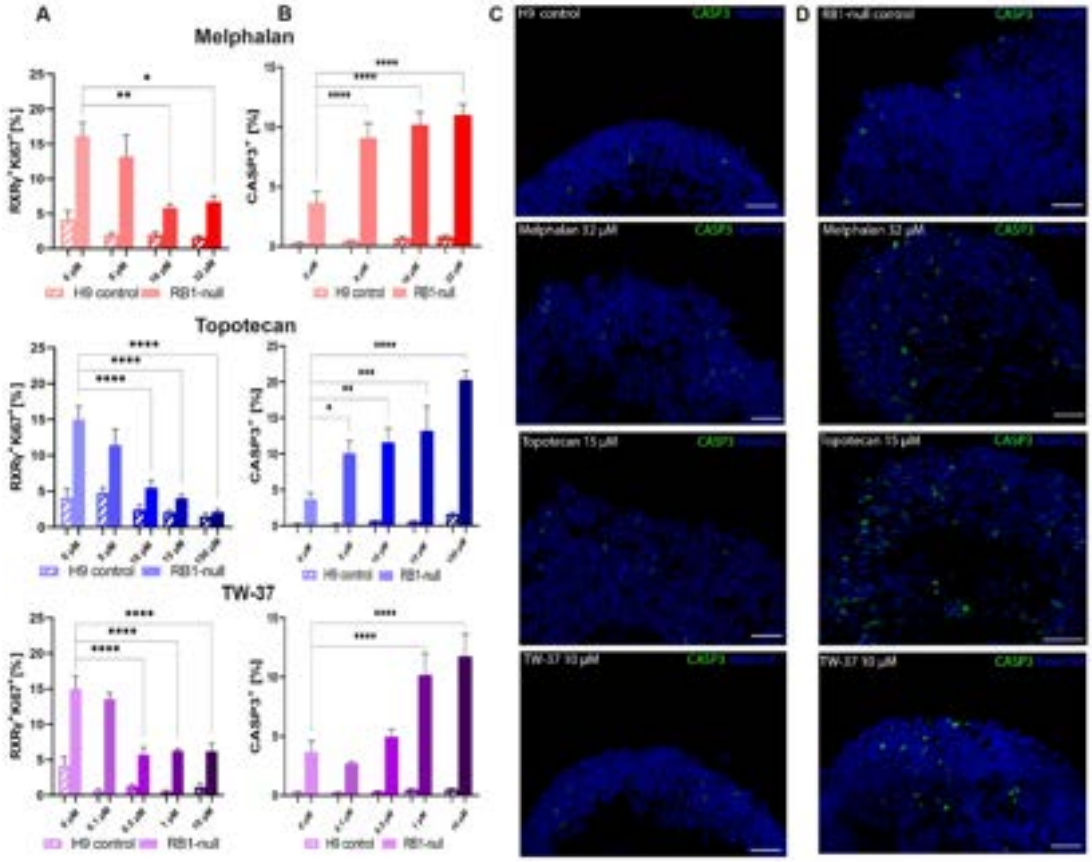

Fig. 1: **Assessment of clinically used chemotherapeutic agents for Rb treatment in control and RB1-null hESC organoids. A.** Bar graphs showing the percentage of proliferating cone precursors ($RXR\gamma^{+}Ki67^{+}$) in immunostained sections of treated organoids agent (Melphalan; 8, 16, 32 µM, Topotecan; 5, 10, 15, 150 µM, TW-37; 0.1, 0.5, 1, 10 µM alongside vehicle only sample; 0.1% DMSO). **B.** Apoptotic response (cleaved-caspase-3; CASP3) after application of chemotherapeutic agents. Data presented as mean ± SEM (n = 5 sections from each biological replicate). **C-D.** Representative immunostaining of CASP3 counterstained with Hoechst for control (C) and RB1-null (D) hESC organoids; vehicle only, Melphalan 32 µM, Topotecan 15 µM, and TW-37 10 µM. Scale bars; 50 µm.

To assess if these drugs were acting on proliferating cone precursors in both RB1-null and patient-derived *RB1*$^{-/-}$ organoids, we performed quantitative immunofluorescence analysis revealing 16-32 µM Melphalan, 10-150 µM Topotecan and 0.5, 1 and 10 µM TW-37 to be the most effective doses in RB1-null organoids, as they significantly reduced the percentage of proliferating cone precursors

to similar levels found within the hESC-control organoids (**Fig. 1A**). In addition to the above concentrations, we found 8 µM Melphalan and 5 µM Topotecan to significantly reduce the percentage of RXRγ^{+} Ki67^{+} cells in hiPSC-organoids (**Fig. 2A**). We didn't detect significant changes in the proliferating cone precursors of hESC- or hiPSC-derived wild-type organoids (**Fig. 1A, Fig. 2A**).

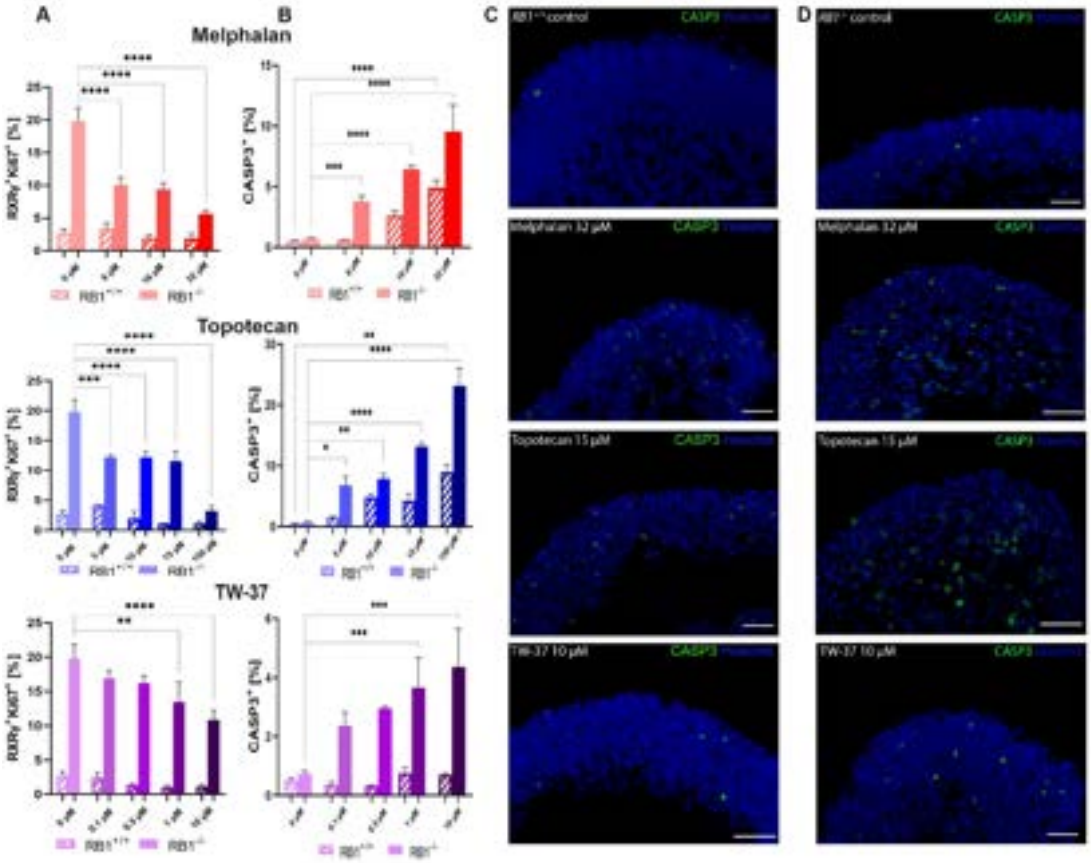

Fig. 2: **Assessment of clinically used chemotherapeutic agents for Rb treatment in hiPSC patient-derived *RB1*$^{-/-}$ and isogenic control *RB1*$^{+/+}$ organoids.** **A.** Bar graphs showing the percentage of proliferating cone precursors (RXRγ^{+}Ki67^{+}) in immunostained sections of treated organoids (Melphalan; 8, 16, 32 µM, Topotecan; 5, 10, 15, 150 µM, TW-37; 0.1, 0.5, 1,10 µM alongside vehicle only sample; 0.1% DMSO). **B.** Apoptotic response (cleaved-caspase-3; CASP3) after application of chemotherapeutic agents. Data presented as mean ± SEM (n = 5 sections from each biological replicate). **C-D.** Representative immunostaining of CASP3 counterstained with Hoechst for *RB1*$^{+/+}$ (C) and *RB1*$^{-/-}$ (D) organoids; vehicle only, Melphalan 32 µM, Topotecan 15 µM, and TW-37 10 µM. Scale bars; 50 µm.

To assess the drug specificity, we also assessed cell killing in control and pRB-depleted organoids (**Fig. 1B-D, Fig 2B-D**). Melphalan and Topotecan did cause an increase in percentage of Caspase-3^{+} apoptotic cells in all concentrations tested, while TW-37 did so only in the two highest concentrations in both RB1-null and patient-derived RB1$^{-/-}$ organoids (1 and 10 µM; **Fig. 1B, D, Fig. 2B, D**). The level of Caspase-3^{+} apoptotic cells in hESC-derived wild-type organoids remained very similar to untreated control (**Fig. 1B, C**), whereas in hiPSC-derived wild-type the highest

concentration of Melphalan (32 µM) and Topotecan (150 µM) significantly increased Caspase-3^{+} apoptotic cells (**Fig. 2B, C**). ***These assays in combination, point to 16 µM Melphalan, 10 µM Topotecan and 1 µM TW-37 as most effective in lowering the level of proliferating cone precursors in both hESC and hiPSC models, while retaining the healthy tissue unaffected***. Together these data suggest that RB1-null and patient-derived *RB1*$^{-/-}$organoids provide a useful platform for testing current and new Rb treatments.

Objective 3: We have tested the same three drugs as above (Melphalan, Topotecan and TW-37) on the retinal pigment epithelium (RPE) cells. We incubated hESC control- and patient *RB1*$^{+/+}$ hiPSC-derived RPE cells with varying doses of the chemotherapeutic drugs for 72 hours (**Figs. 3-6**). Evidence suggests that the RPE, which is exceptionally vital for the health and function of the neural retina, is affected during Rb treatment with chemotherapy with cytotoxicity, RPE hyperplasia, gliosis, mottling, and other alterations manifesting after chemotherapy in Rb patients [6-8].

To assess if these drugs were acting on the cell cycle in both hESC- and patient *RB1*$^{+/+}$ hiPSC-derived RPE cell lines, we performed cell-cycle phase distribution analysis revealing no significant changes the percentage of cells in any of the phases of the cell cycle in response to all three drugs (**Fig. 3a-f**). At tested concentrations of each drug, we did not detect any cell accumulation in the S-phase nor the prevention of the cycle's progression to the G2/M-phase, indicating no alteration to the cell cycle with the drug treatments compared to untreated controls.

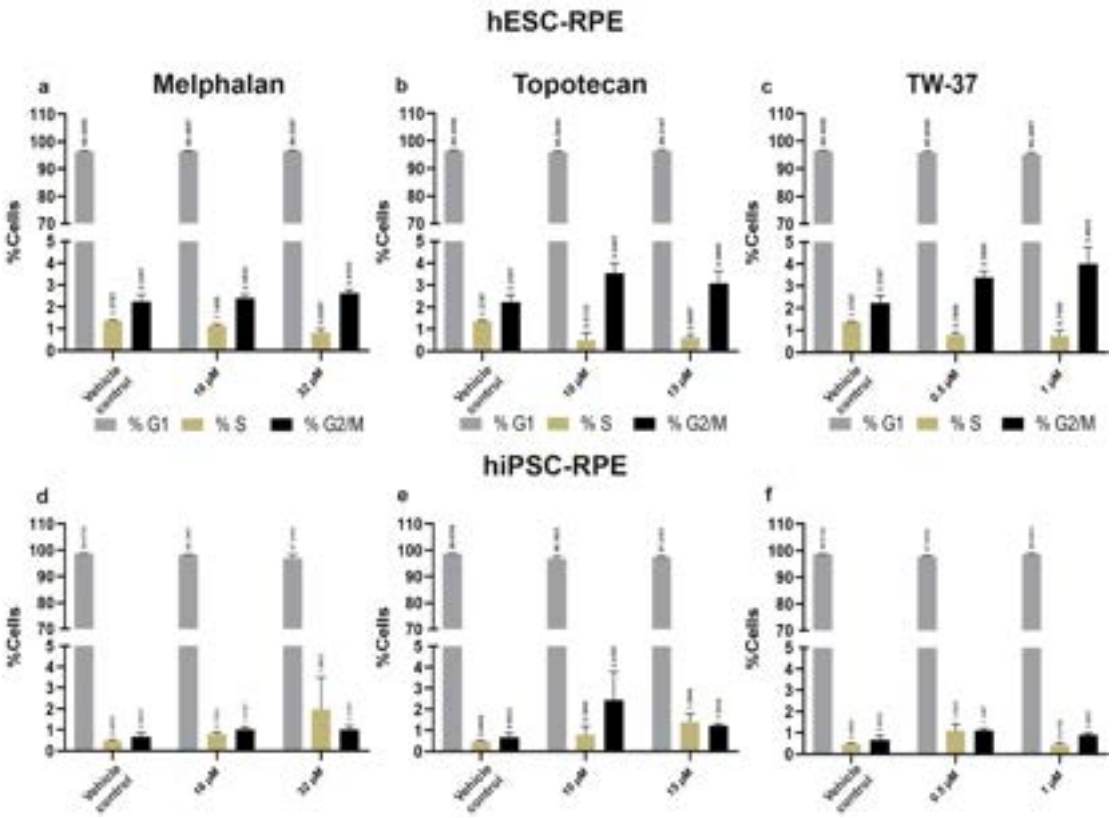

Fig. 3: Cell cycle phase distribution analysis of clinically used chemotherapeutic agents for Rb treatment in H9 hESC- and patient isogenic control *RB1*$^{+/+}$ hiPSC-derived RPE cells. Bar graphs showing percentage of cells (%) of G1-, S-, and G2/M-phase in drug-treated hESC- and patient *RB1*$^{+/+}$ hiPSC-derived RPE cells (Melphalan, **Fig. 3A**, **D**; 16, 32 µM, Topotecan, **Fig. 3B, E**; 10, 15 µM, TW-37, **Fig. 3C, F**; 0.5, 1 µM alongside vehicle control: 0.1% DMSO). Data presented as mean ± SEM (n = 3 biological replicates).

To assess the drug specificity, we also assessed the cytotoxicity by lactate dehydrogenase (LDH) release into the supernatant and cell killing by apoptosis assay in hESC and patient *RB1*$^{+/+h}$ hiPSC-derived RPE cells (**Fig. 4**). Melphalan (320 µM; **Fig. 4a, d**) and TW-37 (10 µM; **Fig. 4c, f**) did cause a significant increase in the percentage of cytotoxicity tested at a 10-fold increase of the maximum dose used in clinical application in both hESC and patient hiPSC RPE cell lines, while Topotecan (150 µM; **Fig. 4e**) did so only in patient *RB1*$^{+/+}$ RPE cells. The percentage of apoptotic cells in hESC and patient *RB1*$^{+/+}$ hiPSC-derived RPE cells treated with two concentrations within the range used in clinical application remained very similar to untreated controls as no significant increase was detected (**Fig. 4a-f**). ***These assays, in combination, point to 8-32 µM Melphalan, 5-15 µM Topotecan and 0.5-1 µM TW-37 as the most effective doses in retaining the healthy RPE tissue unaffected in both hESC- and hiPSC-RPE models***.

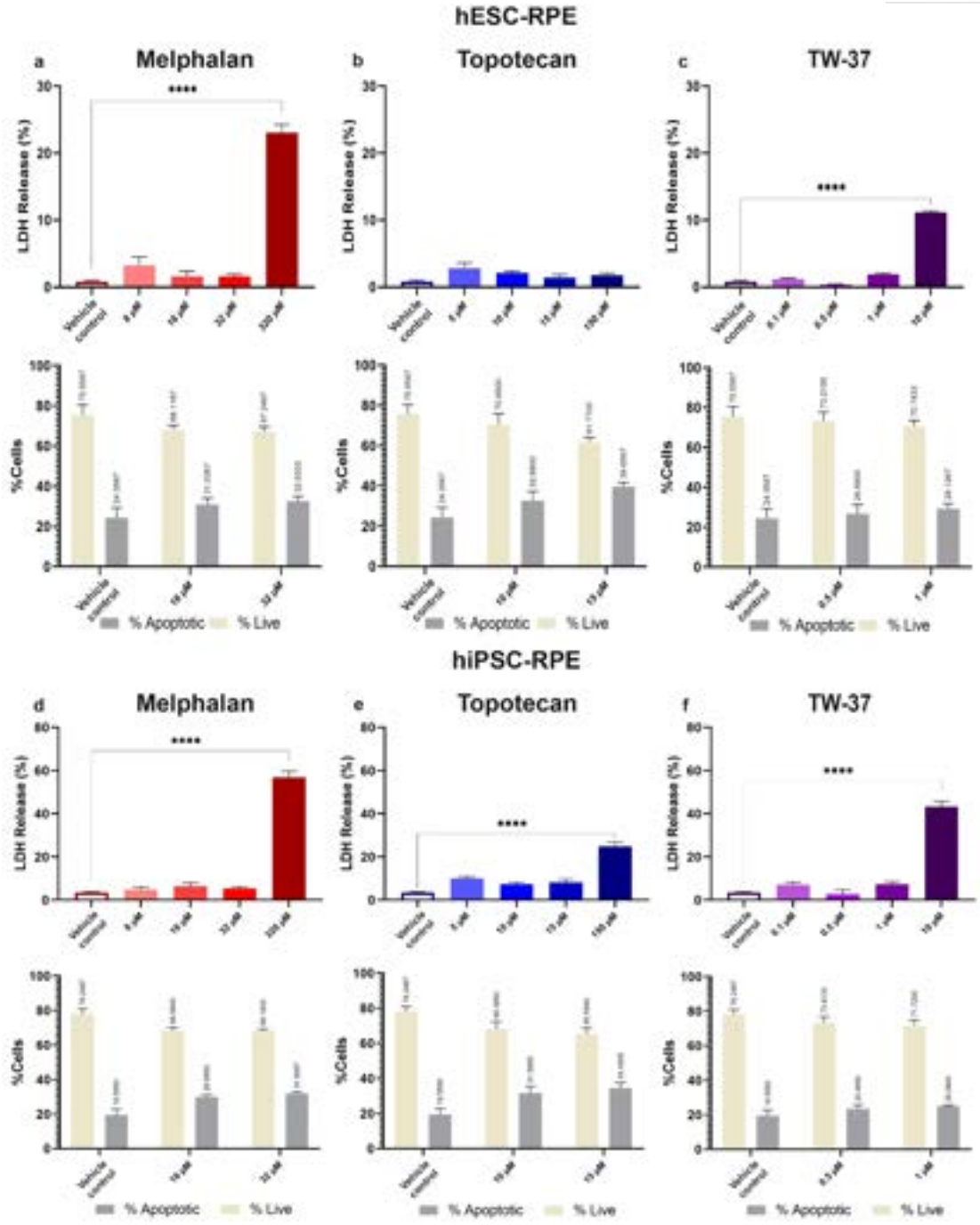

Fig. 4: Assessment of clinically used chemotherapeutic agents for Rb treatment in hESC- and patient isogenic control *RB1*$^{+/+}$ hiPSC-derived RPE cells. Bar graphs showing cytotoxicity of the specified agent measured by LDH release (%) into the supernatant, and the percentage of apoptotic and live cells (%) in drug-treated hESC- and patient *RB1*$^{+/+}$ hiPSC-derived RPE cells (Melphalan, **Fig. 4A, D**; 8, 16, 32 µM, Topotecan, **Fig. 4B, E**; 5, 10, 15, 150 µM, TW-37, **Fig. 4C, F**; 0.1, 0.5, 1,10 µM alongside vehicle control; 0.1% DMSO). Data presented as mean ± SEM (n = 3 biological replicates). Values of $p \leq 0.05$ were considered statistically significant (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$).

To evaluate the impact of drugs on RPE cells, we assessed the gene expression of several RPE markers by quantitative real-time PCR (qPCR) in drug-treated hESC- and hiPSC-derived RPE cells (**Fig. 5**). The selected markers are relevant genes in the RPE whose function of the encoded protein include apical polarity maintenance (Ezrin, *EZR*); tight junction maintenance (*ZO-1*); melanin conversion pathway (*TYR*); transport of retinol (transthyretin, *TTR*) and retinol conversion in the visual cycle (*RPE65*). There were no changes in the expression of markers evaluated in hESC-RPE treated with Melphalan. However, a significant downregulation of *EZR, TYR, TTR, and RPE65* was observed in hiPSC-RPE treated with 32 µM Melphalan. The difference in gene expression was also observed between hESC- and hiPSC-RPE treated with Topotecan. While both models displayed significant downregulation of *TYR, TTR* and *RPE65* expression, *EZR* expression was only downregulated in hiPSC-RPE cells. TW-37 also caused a different response in hESC- and hiPSC-RPE models. Ezrin gene expression was upregulated in hESC-RPE, *ZO-1, TYR* and *TTR* in hiPSC-RPE, whilst *TTR* was downregulated in hESC-RPE cells. Together these data demonstrate that amongst the three tested drugs, 10-15 µM Topotecan treatments modulated the expression of genes involved in melanin and retinol pathways in both PSC-RPE models. The other two drugs caused changes in gene expression in one of the PSC-RPE models, suggesting donor-specific effects that may be dependent on the genetic background.

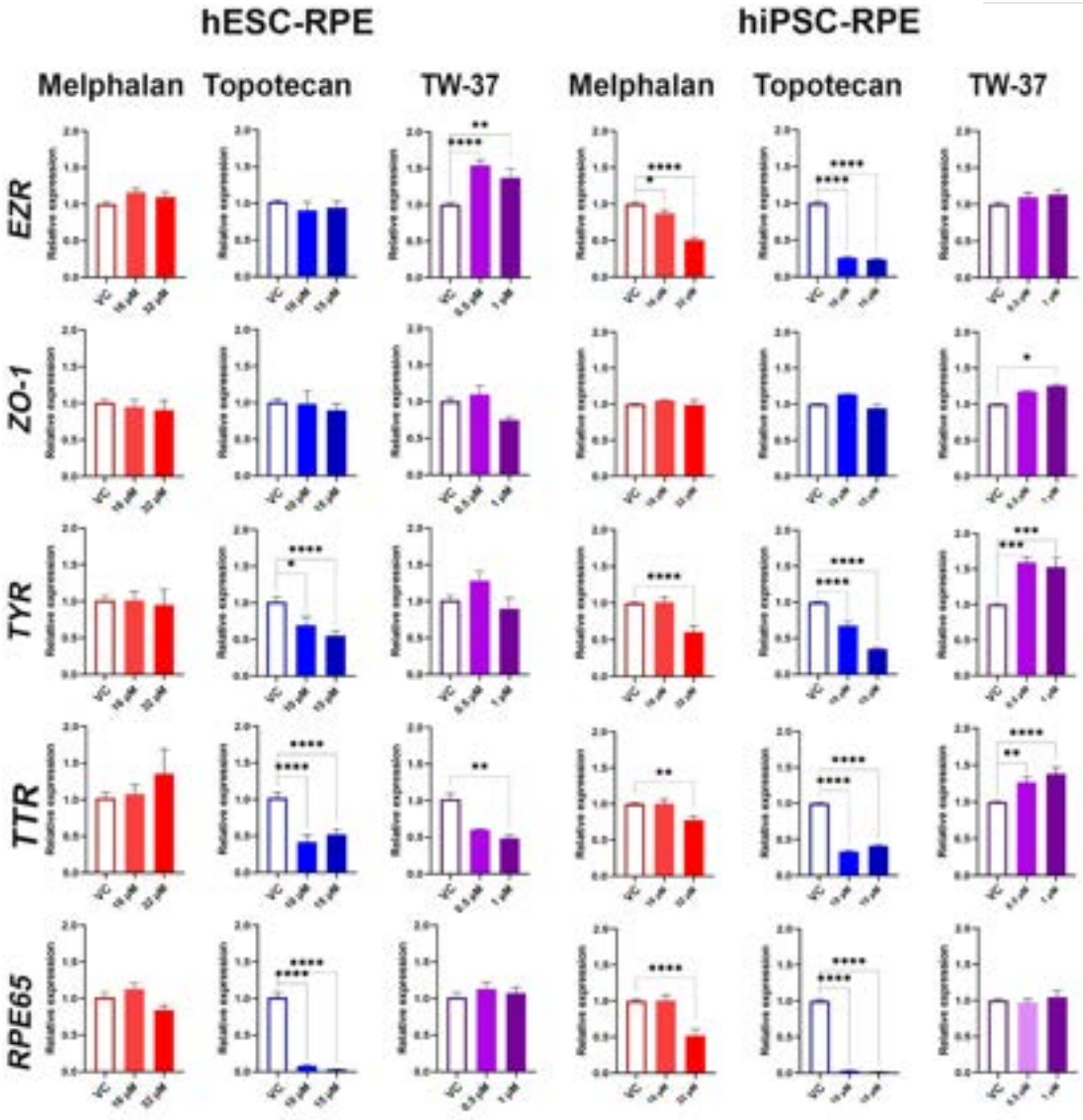

Fig. 3. Quantitative real-time PCR validation of selected genes of clinically used chemotherapeutic agents for Rb treatment in H9 hESC- and hiPSC-derived RPE cells. Gene expression levels of *EZR, ZO-1, TYR, TTR,* and *RPE65* normalized relative to the housekeeping gene *GAPDH* in drug-treated H9 hESC- and hiPSC-derived RPE cells (Melphalan; 16, 32 µM, Topotecan; 10, 15 µM, TW-37; 0.5, 1 µM, alongside vehicle control; 0.1% DMSO). Data presented as mean ± SEM (n = 3 biological replicates). Values of $p \leq 0.05$ were considered statistically significant (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$). VC; vehicle control.

Phagocytosis of shed rod photoreceptor outer segments (POSs) is one of the crucial functions of the RPE. To assess this function, untreated and drug-treated hESC- and hiPSC-derived RPE cells were incubated with FITC-labelled bovine rod POSs. Single-cell RPE suspension samples were analysed by flow cytometry for the percentage of cells able to ingest POSs (**Fig. 6a**) and the number of POSs ingested by each cell assessed through Median Fluorescence Intensity (MFI) (**Fig. 6b**). Drug-treated hESC- and hiPSC-derived RPE cells displayed no significant difference in the fraction of cells able to ingest POSs compared to vehicle-treated control (0.1% DMSO). However, 16-32 µM Melphalan

and 10-15 μM Topotecan treatments caused a significant decrease in amounts of FITC^{+} POSs internalized by individual cells in hESC-RPE cells (**Fig. 6b**). In contrast, only 32 μM Melphalan and 15 μM Topotecan treatments caused a significant decrease in POSs ingested in the hiPSC-derived RPE cell line. These results point to 16-32 μM Melphalan and 10-15 μM Topotecan treatments affecting the phagocytic activity of hESC-RPE cells, while only the highest concentrations within the clinical range, 32 μM Melphalan and 15 μM Topotecan, did so in the hiPSC-derived RPE cell line. TW-37 treatments for RPE cells caused no significant defects in POSs phagocytosis.

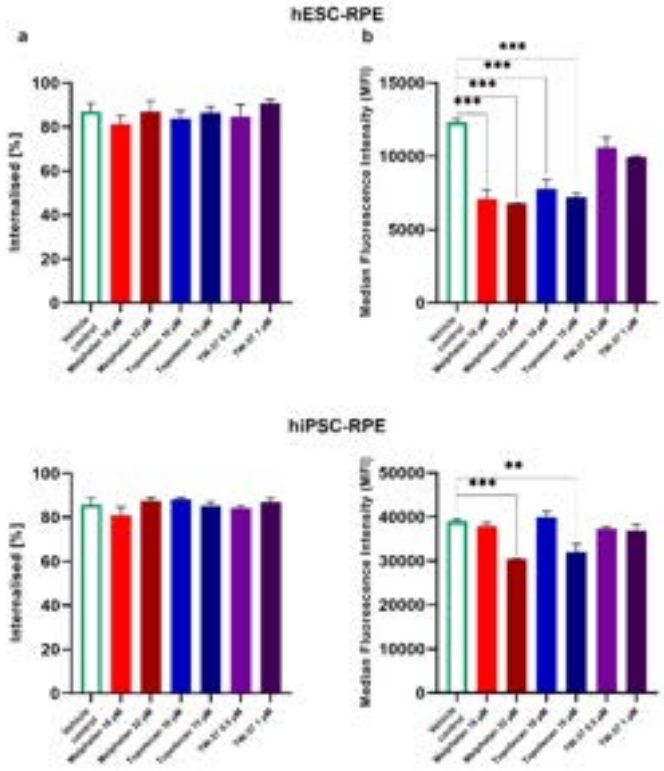

Fig. 6. Phagocytic activity assessment of clinically used chemotherapeutic agents for Rb treatment in H9 hESC- and hiPSC-derived RPE cells. Bar graphs showing the fraction of the total cell population that has internalized POSs (**a**) and the median fluorescence intensity (MFI) values indicating the number of FITC-POSs internalized by individual cells (**b**) in drug-treated H9 hESC- and hiPSC-derived RPE cells (Melphalan; 16, 32 μM, Topotecan; 10, 15 μM, TW-37; 0.5, 1 μM, alongside vehicle-treated sample; 0.1% DMSO). MFI indicates cell-surface receptor density involved in phagocytosis. Data presented as mean ± SEM (n = 3 biological replicates). Values of $p \leq 0.05$ were considered statistically significant (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$).

Together, our results demonstrate that although the most used Rb chemotherapeutic drugs do not cause cytotoxicity in RPE, their application *in vitro* leads to compromised phagocytosis and strength of the barrier function, in addition to changes in gene expression that could alter the visual cycle *in vivo*.

References

1. Rozanska, A., et al., *pRB-Depleted Pluripotent Stem Cell Retinal Organoids Recapitulate Cell State Transitions of Retinoblastoma Development and Suggest an Important Role for pRB in Retinal Cell Differentiation.* Stem Cells Translational Medicine, 2022: p. szac008.
2. Winter, U., et al., *Schedule-Dependent Antiangiogenic and Cytotoxic Effects of Chemotherapy on Vascular Endothelial and Retinoblastoma Cells.* PLOS ONE, 2016. **11**(7): p. e0160094.
3. Dalvin, L.A., et al., *Primary Intra-Arterial Chemotherapy for Retinoblastoma in the Intravitreal Chemotherapy Era: Five Years of Experience.* Ocular Oncology and Pathology, 2019. **5**(2): p. 139-146.

4. Rao, R., et al., *Eye salvage in diffuse anterior retinoblastoma using systemic chemotherapy with periocular and intravitreal topotecan.* Journal of American Association for Pediatric Ophthalmology and Strabismus, 2018. **22**(3): p. 235-237.e2.
5. Zeitlin, B.D., I.J. Zeitlin, and J.E. Nör, *Expanding circle of inhibition: small-molecule inhibitors of Bcl-2 as anticancer cell and antiangiogenic agents.* Journal of clinical oncology : official journal of the American Society of Clinical Oncology, 2008. **26**(25): p. 4180-4188.
6. Zolfaghari, E., et al., *Atypical Retinal Pigment Epithelial Hyperplasia and Glial Proliferation Masquerading as Progressive Recurrent Retinoblastoma: A Case Report Review and Clinicopathologic Correlation.* Ocular Oncology and Pathology, 2018. **4**(2): p. 116-121.
7. Süsskind, D., et al., *Toxic effects of melphalan, topotecan and carboplatin on retinal pigment epithelial cells.* Acta Ophthalmol, 2016. **94**(5): p. 471-8.
8. Xue, K., et al., *Ocular toxicity of intravitreal melphalan for retinoblastoma in Chinese patients.* BMC ophthalmology, 2019. **19**(1): p. 61-61.

b: If your aims and objectives changed during the course of the project, please explain why and in what way. No changes made during the course of the project.

c: Any problems or challenges impacting on the findings / results / outcomes of this project: Timely supply of reagents is an issue, but to overcome this, we make bulk orders a few months in advance to mitigate the impact on our research.

d: How will these findings or outcomes impact patients or the public, and in what timescale? Do you foresee any obstacles / barriers to patients benefitting from the research findings? Current Retinoblastoma treatments include surgery, radiation therapy, cryotherapy, laser therapy and chemotherapy. In the last ten years there has been a shift towards conservative treatments which aim to preserve both the globe and vision while minimising toxicity. Hence, intra-arterial and intra-vitreal chemotherapies, which avoid the complications of systemic chemotherapy (e.g., bone marrow suppression, alopecia and nutritional compromise in the short term and nephrotoxicity and ototoxicity in the longer term), have become more popular. Nonetheless, these also are associated with ocular and systemic morbidity such as vascular retinopathy, cystoid macular oedema, anterior segment toxicity and orbital fat atrophy, which limit their effectiveness as salvage options. One of the reasons for the observed ocular toxicity is that the current chemotherapy drugs are not designed specifically for the eye/retina. Furthermore, while currently available chemotherapy drugs kill the cancerous cells, they were not designed to target the pathways or molecular triggers that lead to uncontrolled cone proliferation in Retinoblastoma tumours. Our follow-up research built upon the results obtained by this project and funded by the Little Princess Trust will address both issues by identifying specific candidate drugs that act on the cell of origin that give rise to the Retinoblastoma tumours and testing those on the validate retinal organoid and retinal pigment epithelium models. We strongly believe that this approach will determine optimal chemotherapeutics that act specifically on the Retinoblastoma cell of origin, with minimal toxicity to the adjacent healthy retina. Through carefully planned pharmacokinetic studies, we will also assess drug absorption, stability and flux through the retina, which will determine the most appropriate mode of administration. In follow-up work, we will aim to perform preclinical drug toxicity testing *in vivo*, in collaboration with an experienced contract research organization (for example Charles River), for the selected drug candidates from this proposal. Importantly, we propose to study the drug candidates selected herein in a Phase 0 setting in patients who will have primary enucleation, which will allow us to identify evidence of drug delivery to the retina and any early evidence of anti-tumour effect. With this research vision we aim to be in the position of implementing new and much improved chemotherapy

treatments for the Retinoblastoma patients, in the next 5-10 years which is the main drive of our research and a key benefit to Retinoblastoma patients and their families.

2. Plain English summary (please refer to appendix 1).

Please provide a brief plain English summary of your final report above, including any findings or outcomes, and their potential impact on patients or the public. CHECT (and funding partners) will publish this summary in the public domain to demonstrate how we support research, therefore please do not include any confidential or commercially sensitive information.

- Aim(s) of the research
- Findings or outcomes
- Potential impact on Rb individuals and families

Aims of the research: Retinoblastoma is the commonest childhood cancer affecting the eye. Treatment of retinoblastoma aims to salvage the globe and visual function in addition to saving the patient's life. Current treatments include a combination of chemotherapy and focal treatments with an increasing shift towards local delivery. Several drugs are being used for chemotherapy; however, in addition to causing death of retinal cells, these also affect the adjacent retina and pigmented cell layer, both of which are essential for good vision. We have established several human models from patients with retinoblastoma and unaffected subjects, which can provide an efficient platform for drug testing upon successful validation. The goal of this project was to establish the feasibility of human models for assessing the efficacy of chemotherapy drugs in eradicating the retinoblastoma tumours and their impact on the adjacent retina and pigmented cell layer.

Findings: We have generated retinoblastoma organoids using a specific technique, which converts patient's blood cells into stem cells. These can be coaxed into cell aggregates (named organoids), which show the key features of tumors. We have selected three widely used chemotherapy drugs, which we have applied to the retinoblastoma organoids at different doses and time points. Our data shows an efficient and dose dependent response of these chemotherapeutic drugs in eradicating the starting cell type that results in development of retinoblastoma, validating the organoid model as a platform for new drug discovery. Using an additional model established in our lab namely the retinal pigment epithelium cells we have assessed the toxicity of the three selected chemotherapy drugs on the pigmented cell layer. Our data also demonstrate that function of the pigmented cell layer is compromised, indicating that great care has to be taken to deliver the chemotherapeutic drugs directly to the tumour without compromising the function of the retinal pigment epithelium, which is essential for the function and survival of retinal cells.

Potential impact on Rb individuals and families: This study was designed to test the safe and effective dose of current as well as novel drugs on the laboratory models (retinal organoids and retinal pigment epithelium). This valuable information will help shape treatment strategies e.g. new drug combinations and new dosage schedules to effectively treat retinoblastoma tumours while minimising damage to vision. The models validated during the course of this study model will be used to test new and existing chemotherapy drugs to increase the success of intravitreal injections of chemotherapy. This approach abolishes the need for recurrent access to patient samples and provides an efficient platform for testing the efficacy of new treatments for retinoblastoma in the lab, prior to human trials.

3. Publications

1. Queen R, Collin J, Zerti D, Dorgau B, Beh CS, Davey T, Coxhead J, Hussain R, Al-Aama J, Steel DH, Benvenisty N, Armstrong L, Parulekar M, Lako M. pRB-Depleted Pluripotent Stem Cell Retinal Organoids Recapitulate Cell State Transitions of Retinoblastoma Development and Suggest an Important Role for pRB in Retinal Cell Differentiation. Stem Cells Transl Med. 2022 Mar 23;szac008. doi: 10.1093/stcltm/szac008.
2. Cerna-Chavez R, Rozanska A, Poretti GL, Benvenisty N, Parulekar M and Lako M. Retinal pigment epithelium exhibits gene expression and phagocytic activity alterations when exposed to retinoblastoma chemotherapeutics. Exp. Eye Res. (under review).

4. Dissemination of results

Please list where and by whom any results/findings have been disseminated (e.g. conferences, workshops, public engagement events)

Presentations at conferences:

Conference	Author, title	Oral or poster
UK Stem cell meeting: 2019	Lako: making retinal organoids, a voyage of discovery	oral
Winter Annual Anatomy meeting: 2021	Lako: application of retinal organoids for disease modelling	oral
3D Hybrid Organotypic Systems: 2021	Lako: Optimizing the generation of retinal organoids and their application in cell replacement therapies	oral
3D Cell Culture Conference: 2021	Lako: applications of retinal organoids in drug discovery studies	oral
ERN-EYE 4th scientific workshop “Models for Rare Eye Diseases research”: 2021	Lako: Using primary tumour samples and patient-specific retinal organoids to understand the development of Retinoblastoma and design therapeutic strategies	oral
European Retinoblastoma symposium	Rozanska: A lab made retinoblastoma model	oral
ARVO 2022	Rozanska-Lako: pRB-Depleted Pluripotent Stem Cell Retinal Organoids Recapitulate Cell State Transitions of Retinoblastoma Development	oral
ISSCR Annual Meeting 2022	Cerna Chavez-Lako: pRB-depleted pluripotent stem cell retinal organoids as a 3D in vitro model for drug screening in Retinoblastoma	poster
Retinal organoid workshop, University of Leeds, July 2022	Lako: Pluripotent stem cell-derived retinal organoids to repair vision, understand and treat eye disease	oral

5. Intellectual property (IP)

Please list any IP arising from the research, and whether it is wholly owned by the researcher.

No IP arising so far, but we hope to protect IP on the organoid and RPE model use for drug discovery once the Little Princess Trust is under wat (see point 7 below).

6. Collaborations

Please list any collaborations which have arisen during or as a result of this research.

We have formed new and very fruitful collaborations with drug discovery (Prof. Steve Wedge and Dr. Ian Hardcastle), pharmacogenetics (Prof. Gareth Veal) experts at Newcastle University and Consultant Paediatric Oncologists at the Birmingham Children Hospital (Helen Jenkinson and Gerard Millen)

7. Future research and funding

Please provide details of any further research/ideas planned and where potential funding will be sourced from as a result of this project

We have been able to obtain a 2- year project grant from Little Princess Trust (£200,000) to use the retinal organoid and RPE models validated during the course of this project to test 37 candidate drugs for eradication of Rb tumours.

8. Any further comments:

We have enjoyed our interactions with CHECT and hope to work with you in the future.

Report completed by:	Prof. Majlinda Lako	Date of report:	24.10.2022

Appendix 1: Writing a plain English summary in your Childhood Eye Cancer Trust (CHECT) final report [i]

A plain English summary is a clear explanation of your research that should be accessible by an interested audience.

Your final report will be reviewed by experts on the CHECT Scientific Advisory Committee but also by lay members of the SAC and CHECT Board members who are not scientific experts. It will also be accessible through the CHECT website.

A good quality plain English summary providing an easy to read overview of your whole study will help:

- those carrying out the review (reviewers and Board and panel members) to have a better understanding of your research
- inform others about your research such as those affected by retinoblastoma (Rb), members of the public, health professionals, policy makers and the media
- research funders to publicise the research that they fund.

The summary is important. If it is felt that your plain English summary is not clear and of a good quality then you may be required to amend your summary prior to the final closure of the project.

What to include in your plain English summary

Your plain English summary should be 300 words or less. When writing the summary consider including the following information:

- Aim(s) of the research
- Findings or outcomes
- Potential impact on Rb individuals and families

How to write a plain English summary

The people who will read your summary will be an interested audience, but are not necessarily specialists. Therefore write your summary with this audience in mind, for example at the same level as an article in a newspaper.

There are a few simple rules for writing in plain English. In summary these are:

- Avoid wherever possible using jargon, abbreviations and technical terms. If you have to use them provide a clear explanation
- Avoid complicated English or uncommon words
- Use active not passive phrases: for example say ‘we will do it’ rather than ‘it will be done by us’
- Keep sentences short
- Think about the order and structure
- Break up the text. For example use bullet lists
- Ask patients / carers / colleagues to read a draft to find out if anything is unclear.

The plain English summary is not the same as a scientific abstract. Please do not cut and paste this or other sections of your final report to create the plain English summary. Further guidance on plain English summaries for research is available at:

- **www.amrc.org.uk/blog/how-to-write-a-lay-summary-of-a-research-project**
- **www.cancerresearchuk.org/funding-for-researchers/patient-involvement-toolkit-for-researchers/planning-your-patient-involvement/writing-for-a-lay-audience**

[i] Based on the NIHR guidance available at https://www.nihr.ac.uk/about-us/CCF/PPI/Plain_English_summaries_in_National_Institute_for_Health_Research_funded_research.pdf