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:	Investigation of the mutational landscape of retinoblastoma		
Project reference:	(To be completed by CHECT)		
Total award:	£29,220 (CHECT & Fight for Sight) + £6500 extension (CHECT)		
Details of any additional funding:			
Lead investigator:	Carmel McConville	Administering institution:	University of Birmingham
Start date of award:	1.5.14 / 1.11.18	End date of award:	31.5.21

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?

AIMS
The original aim of this project was to use next-generation sequencing (RNA-seq) of 8 retinoblastoma cell lines to survey the mutational landscape of retinoblastoma, as well as altered patterns of gene expression associated with identified mutations. This information is critical to understand exactly how these mutations co-operate with RB1 loss to disrupt normal growth control and to promote tumour progression.

Our initial results indicated that BCOR (BCL6 co-repressor) mutation was likely to be a significant event in retinoblastoma, and an extension to the study sought (i) to undertake further mutation studies of the BCOR gene in an additional 20 cell lines (ii) to confirm the *in vivo* origin of mutations and (iii) to carry out a pilot immunohistochemistry study to assess the relationship between BCOR mutation in cell lines and BCOR protein expression in matched tumour tissue. This should provide an essential foundation for further work to develop BCOR as a molecular marker in retinoblastoma, and also potentially for the development of targeted therapy based on BCOR or associated pathways.

SUMMARY OF RESULTS

I. RETINOBLASTOMA MOLECULAR PATHOLOGY

Mutational landscape of retinoblastoma.
We chose to use retinoblastoma cell lines recently established in this laboratory (Table 1) rather than tumour tissue, since tissue is often severely limited in amount and/or quality and contamination with normal retinal cells may limit sensitivity of the analysis. The availability of cell lines with known mutations will also facilitate future functional studies of mutated genes and testing of novel targeted therapeutics.

RNA-seq of 8 cell lines (in duplicate) allowed the identification of a total of 93 potentially pathogenic missense mutations (as predicted by SIFT/Polyphen/Mutation Taster) as well as 8 frameshift/nonsense/indel mutations. The most significant mutated genes were BCOR (BCL6 co-repressor, p.Val1237fs, p.Leu775*), BCL9 (BCL9 transcriptional co-activator, p.Arg485del), RECQL4 (RecQ like helicase 4, p.Leu720fs) and PHF23 (PHD finger protein 23, p.Lys132del). However, with the exception of BCOR, no genes were recurrently mutated. In view of additional independent reports of BCOR mutation in retinoblastoma[1-3], highlighting the significance of alterations in this gene, further analysis focused on a more in depth investigation of the pattern and frequency of BCOR mutation in a larger panel of cell lines, as well as the consequences for downstream BCOR-regulated pathways.

BCOR loss of function mutations are frequent in retinoblastoma cell lines
RNA-seq data from the initial 8 retinoblastoma cell lines was supplemented by Sanger sequencing of cDNA in a further 20 cell lines. Overall, transcript alterations were identified in 16 lines (16/28, 57%) and included deletions, as well as nonsense and frameshift mutations, predicted to cause loss of protein function. No missense mutations were identified. The observed alterations included only 5 single nucleotide variants (SNV) or small indels, while the remaining 11 cases showed either complete transcript loss (n=4), aberrant splicing resulting in deletion of 2 or more exons (n=4), intra-exonic deletion (n=2) or gene rearrangement (n=1) (Figure 1).

Additional Sanger sequencing of genomic DNA (gDNA) from the retinoblastoma cell lines and matched lymphoblastoid cell lines or ocular stromal cells from the same patients confirmed that all mutations were somatic in origin. In addition it was observed that in female patients, the mutated allele was always the expressed allele. gDNA sequencing also provided additional information about the genomic sequence variants responsible for aberrant/missing transcripts. Aberrant splicing of exon 12 $\rightarrow$ exon 15 (RB31), exon 8 $\rightarrow$ exon 11 (RB36), exon 1A$\rightarrow$ exon 4 (RB47) and exon 1A $\rightarrow$ exon 11 (RB57) was not a consequence of splice site mutations, but was caused by deletion of the intervening introns/exons (Figure 1). Complete transcript loss in RB44 was associated with deletion of exons 2 (first coding exon), 3, 4 and 5, while RB34, RB43 and RB56 showed deletion of the entire gene. One cell line (RB46) appeared to be

genetically heterogeneous, showing a 22bp deletion within exon 7 in approximately 37% of RNA-seq reads, with a similar finding in gDNA, although at a lower level due to the contribution of normal sequence from the second chromosome X.

In the case of RB35 a BCOR rearrangment was evident from RNA-seq data. Manual inspection of soft-clipped sequence reads using IGV identified juxtaposition of an intergenic segment of chromosome 7q35 with BCOR exon 6 and exon 4. Increased read depth for the distal segment of exon 4, as well as exons 5 and 6 suggested a duplication of this region. The overall consequence of this rearrangement was the generation of a stop codon at the exon 6 - chromosome 7 junction (Table 2).

The observation of frequent structural variants among BCOR mutations (11 deletions vs only 5 SNV/single base Indels) prompted further investigation of the sequence context, in order to identify possible mutational mechanisms. Deletion breakpoints occurred within the BCOR gene in 9 cell lines, facilitating analysis of flanking sequences by Sanger sequencing. Analysis of 100bp of sequence flanking all breakpoints using RepeatMasker[4] failed to show any evidence of repetitive sequence elements (e.g. LINE, SINE) that might promote joining of non-contiguous sequences. However the majority of cell lines showed short sequences (3-6bp) present as direct or inverted repeats in the vicinity of deletion breakpoints. This suggests a possible role for non-B DNA structures as a source of genetic instability leading to DNA double strand breaks[5]. In the case of RB46 for example, an inverted repeat, 'ACAGCC[**CCACCC**]TCC[**GGGTGG**]ACAG', with the potential to form a cruciform structure, flanked by direct ACAG repeats, allowing slippage during replication is likely to have played a role in the observed 22bp deletion in this cell line (Figure 2). Guanine rich sequences, as observed in RB36, are also known to have a high propensity to form non-canonical G-quadruplex structures that may be mutagenic (Figure 2).

In vivo occurrence of BCOR mutations

In view of the unexpected high frequency of BCOR mutations in retinoblastoma cell lines (57%), it was important to establish that these originated *in vivo* and were not artifacts arising during library preparation for RNA-Seq or during cell culture. Two approaches were taken: (i) sequencing of the mutation in gDNA isolated from the original tumor (where available), or (ii) sequencing of the mutation in gDNA from 2 independent cell lines established in parallel from the original tumour sample. In the latter approach it was expected that mutations of *in vivo* origin would be present in both cell lines, but those arising during *in vitro* culture would be present in only one. Overall it was possible to verify the *in vivo* origin of mutations in 14 of 15 cell lines for which material was available (Table 2). In 3 cases (RB32, RB36, RB37), both mutated and wild-type sequences were identified in tumour tissue from male patients, suggesting either the presence of normal cells within the tumour sample or genetic heterogeneity of the tumour. Heterogeneity was confirmed in RB37 since sequencing of the RB1 gene showed a pure tumour sample with only the RB1 c.184C>T mutation and no normal sequence at this position (the clinically reported RB1 genotype was c.184C>T/LOH).

Two mutations were not found in matched tumour tissue. One of these (RB46) was found to be present in only 37% of RNA-seq reads and may represent a small subclone in the original tumour that was not represented in the tissue tested. The second mutation was identified in a slow-growing cell line (RB38) that showed a high level of differentiation during early culture (rosette structures with a central lumen clearly visible by phase contrast microscopy). The identical mutation was also present in a second cell line derived from the same tumour sample. Again the mutation may have been present in a small undifferentiated subclone that proliferated preferentially to become the major cell population in the cell lines. Subsequent immunohistochemistry studies confirmed that both of these tumours were well differentiated and largely BCOR positive, but with small areas that were negative for BCOR (see p.6 below).

These findings are consistent with previous reports that many retinoblastomas are genetically heterogeneous[6]. It is of interest that heterogeneity is also apparent in tumour histopathology, with many retinoblastomas showing regions of differentiated cells (Flexner Wintersteiner rosettes, fleurettes) as well

as undifferentiated regions. Further studies to investigate the relationship between tumour genetics (especially BCOR mutation) and histopathology will be important to explain the role of this heterogeneity in tumour biology and evolution.

BCOR mutation is associated with an altered gene expression profile
Since BCOR is a transcriptional regulator we next sought to identify downstream changes in the cellular pattern of gene expression associated with BCOR mutation. Principal component analysis of expression data from the 8 cell lines that underwent RNA-seq showed good separation of lines with and without BCOR mutation, confirming a significant influence of BCOR mutation on the global gene expression profile. It was noted however that the genetically heterogeneous cell line RB46 (37% mutated reads) was located in an intermediate position in the PCA plot and thus this cell line was excluded from further analyses.

A total of 1091 genes showed significant differential expression in cell lines with (RB35, RB37, RB38, RB43) and without (RB33, RB40, RB45) BCOR mutation (≥2-fold difference; Benjamini-Hochberg adjusted p-value ≤0.05). Gene ontology (GO) analysis of genes expressed at higher levels in association with normal BCOR (n=599) identified significant enrichment of GO categories related to the detection/response to light stimulus and the phototransduction signaling cascade, characteristics of photoreceptor cells (rods and cones) which are one of the major classes of neuronal cell types in the retina (Table 3). In contrast, genes upregulated in BCOR mutated cell lines (n=492) were associated with GO terms including neurogenesis, axonogenesis and neuron development (Table 3). It was notable that these genes included several transcriptional regulators with roles in lineage specification of a range of different retinal neural cell types (Table 4), as well as NOTCH1, which is essential for the maintenance of a retinal progenitor cell population[7]. These observations suggest that in normal retina the co-repressor function of BCOR may be responsible for downregulation of genes which promote proliferation of retinal progenitor cells or lineage specific precursors, and that this leads directly or indirectly to differentiation of retinal photoreceptor cells.

In order to investigate the regulatory networks which are disrupted by BCOR mutation, the STRING database[8] was used to construct a protein-protein interactome (PPI) incorporating all differentially expressed genes (fold change ≥1.5; p≤0.05). Of 1082 genes with a corresponding protein annotation, 493 were connected by a total of 975 edges. This is significantly greater than the number of edges expected by chance from a random set of proteins (648, p=0.00), suggesting that proteins within the network are biologically linked. Topological analysis of the network using Cytoscape was undertaken to identify hub nodes that are highly connected and likely to function as key proteins within the network[9]. The 3 highest scoring hub nodes were NOTCH1, FYN and KIT, which together with directly interacting differentially expressed proteins formed an integrated network of 52 nodes and 104 edges (Figure 3).

NOTCH1 is of particular interest since it has been implicated in the maintenance of retinal progenitor cells and also in the suppression of photoreceptor differentiation[7,10]. NOTCH1 pathway components that showed significant upregulation and high levels of expression in the identified network included not only NOTCH1 itself (9-fold increase) but also TCF3/E2A, a NOTCH1 transcriptional regulator (2-fold increase)[11], CNTN1 (contactin N1) (8.5-fold increase), a neural cell adhesion protein that may also function as a NOTCH1 ligand[12] and SPEN/SHARP (SMRT/HDAC1 associated repressor protein) (1.7-fold increase). SPEN interacts directly with RBPJ, the main transcription factor that controls the Notch-dependent transcriptional response, mediating both positive and negative regulation of gene expression[13]. A key NOTCH1 effector TLE1, a co-repressor that interacts with multiple transcription factors to inhibit differentiation during neurogenesis[14], was also upregulated (3.4-fold increase).

KIT (stem cell factor receptor, CD117) and KITL (KIT ligand), which were upregulated 6-7-fold in BCOR mutated cell lines, may also play a role in maintaining a retinal progenitor cell phenotype in retinoblastoma. It is of interest that in mouse retina, activation of NOTCH signaling was found to result in an increased population of KIT positive progenitor cells[15].

FYN interacts with a variety of cell surface receptors that regulate intracellular tyrosine protein phosphorylation, and a potential target in retinoblastoma is the Ephrin receptor group[16]. Ephrins/Ephrin receptors mediate many developmental processes, particularly in the nervous system where they coordinate processes including cell migration and axon targeting, and also influence the proliferation and differentiation of neural progenitors[17,18]. EFNA3 and EFNB3 were upregulated more than 7-fold in BCOR mutated cell lines, while receptors EPHA4, EPHA5 and EPHB2 were upregulated 8-29-fold. This may have significance for the development of invasive phenotypes in retinoblastoma.

The major conclusion drawn from these investigations is that normal BCOR function is likely to be important for retinal differentiation and that loss of function leads to activation of a regulatory network driving continued proliferation of progenitor cell types. This a significant finding since previous studies from this and other groups have shown that retinoblastoma disease progression is associated with loss of retinal differentiation[19-21]. BCOR mutation may potentially be a very useful biomarker therefore, signaling an increased risk of disease progression and metastasis.

II. RETINOBLASTOMA HISTOPATHOLOGY

In order to further assess the potential clinical utility of BCOR as a tumour biomarker, the next step was to determine the extent to which our cell line results parallel the *in vivo* situation. Consequently the aim of the final part of the project was to carry out a pilot immunohistochemistry study to investigate the relationship between BCOR mutation status in cell lines and BCOR protein expression in matched tumours. We particularly wished to investigate the pattern of BCOR expression in differentiated vs. undifferentiated tumour cells and in regions of tumour invasion into the choroid and the optic nerve.

Formalin-fixed paraffin-embedded (FFPE) tissue for immunohistochemistry was obtained from 14 retinoblastoma tumours (primary enucleations) from which cell lines had been established. This included 8 BCOR-mutated cell lines and 6 cell lines without identified mutations. Tumour sections were stained with a commercially available BCOR antibody, C10 (SC-514576 Santa Cruz), that has been successfully validated in several studies of paediatric sarcoma-like tumours [22,23]. Stained slides were digitally scanned using a Leica Aperio AT2 scanner, and images were imported into the QuPath Bioimage analysis software for futher analysis [24].

BCOR immunohistochemistry of BCOR mutation-negative retinoblastomas

In retinoblastoma tumours matching cell lines for which no BCOR mutations were identified (n=6), differentiated cell types were characterised by intense BCOR nuclear staining. RB40 and RB45 for example, were reported by the Clinical Pathology Service as well differentiated with abundant Flexner-Wintersteiner (FW) rosettes (which mimic photoreceptor differentiation) and showed strong BCOR positivity thoughout most/all of the tumour. In RB45 however, a small focus of choroid invasion was BCOR-negative (Figure 4). A third retinoblastoma, RB42, was also of interest: this MYCN-amplified tumour was reported as moderately differentiated with scattered FW rosettes, but also showed post-laminar optic nerve invasion and focal choroid invasion. It was observed that a large part of the anterior portion of the tumour contained frequent FW rosettes and showed strong BCOR positivity (Figure 5). In contrast, the posterior portion of the tumour, including a region of optic nerve invasion and adjacent choroid invasion, showed much reduced BCOR staining with some Homer Wright/neuronal (HW) rosettes (Figure 5). Since no BCOR mutation was observed in the RB42 matched cell line, and BCOR mRNA expression was relatively high, the low level BCOR staining in this region of the tumour may represent regulation of BCOR expression by translational control mechanisms e.g. small non-coding RNA, or alternatively the cell line may have been derived from the differentiated, BCOR-positive component of the tumour.

A further 3 tumours without detected BCOR mutations in the matched cell lines, were classified as poorly differentiated (RB33, RB49, RB53). The RB49 *cell line* showed a much reduced level of BCOR mRNA, suggesting either that a mutation was present but not detected by our methodology, or BCOR mRNA

expression was down-regulated by other means. In the tumour the bulk of the mass within the vitreous consisted of BCOR-negative cells. However some more differentiated BCOR-positive tumour cells, with FW rosettes were also present and located primarily in the sub-retinal region (Figure 6).

RB33 showed features similar to RB49, with the bulk of the tumour mass in the vitreous negative for BCOR, but with isolated regions of BCOR positive cells mostly located immediately adjacent to/within the retina and including FW rosettes and fleurettes. The histopathology of both of these retinoblastomas is consistent with an evolving pattern of BCOR expression, with tumour arising from the retina initially expressing BCOR, but with the development of substantial BCOR-negative tumour growth within the vitreous. The signficant BCOR-negative components of these two tumours also suggest that the frequency of BCOR down-regulation may be even greater than our mutation results indicate, and further investigation of mechanisms responsible will be important.

The pattern in RB53 in contrast, was almost entirely BCOR-positive, with intense staining in the endophytic component of the tumour. Infrequent FW rosettes were observed. It is possible that the mutation-negative cell line was established from tissue representing this region of the tumour. A separate exophytic region of the tumour showed less intensely stained tumour with larger, more anaplastic cells, which may represent an evolving more invasive phenotype. Micro-dissection of cells from this region of the tumour for analysis of BCOR mutation status would be of great interest to determine if a BCOR mutation had occurred.

All of these 3 poorly differentiated tumours were reported as having intralaminar optic nerve invasion, but invasion was not representated on the sections studied.

BCOR mutation-positive retinoblastomas

Among 8 retinoblastomas representing BCOR mutation-positive cell lines, all showed both BCOR-positive and BCOR-negative regions of tumour in varying proportions. Again there was a clear correlation between differentiation (FW rosettes) and strong BCOR staining. Thus, the two retinoblastomas reported as well differentiated, RB38 (OSA) and RB46 (MCL), showed a predominantly BCOR positive tumour, with only very small BCOR negative regions. It is of interest that the RB38 cell line was slow growing and showed features of differentiation (rosette-like structures) on initial culture, that were gradually lost with time as a more proliferative cell type emerged. In the case of the RB46 cell line, only 37% of sequence reads showed BCOR mutation, consistent with a heterogeneous cell population similar to the tumour. The moderately differentiated retinoblastoma RB47 (GLE) showed clearly demarcated areas of positive and negative BCOR staining with both FW and HW rosettes in the former, but only HW rosettes in the latter (Figure 7).

Poorly differentiated RB36 (MAH), RB37 (DAV) and RB43 (MCN), also showed clear blocks of intense positive and negative (or in the case of RB36(MAH) very low level) BCOR staining, with focal FW rosettes showing strong positivity (Figure 8). In RB37, cells invading the intra-laminar optic nerve were clearly BCOR negative (Figre 8). It is of interest that both RB36 and RB37 (from male patients) showed both normal and mutated gene sequence in tumour DNA (RB43 was not tested).

In the undifferentiated tumour RB35 (CLE), although largely BCOR-negative, an isolated area of BCOR-positive tumour cells, containing many apoptotic figures was observed comprising approximately 30% of the tumour as well as small areas of positive cells within or adjacent to the retina(Figure 8). RB50, reported as an atypical small round blue cell tumour, but with focal FW and HW rosettes, was almost entirely BCOR-negative and just one very small area of BCOR-positive tumour visible. No typical FW rosettes were observed in the section studied.

In summary, a preliminary analysis of BCOR immunohistochemistry in retinoblastoma tumours indicated that the pattern of staining is consistent with our conclusion from cell line studies, that BCOR expression is associated with tumour differentiation. Thus regions of retinal-type differentiation, observed as FW rosettes, invariably showed strong BCOR expression. It is of interest that FW rosettes were restricted to

BCOR-positive tumour tissue, but Homer Wright rosettes were observed in both BCOR-negative and BCOR-positive tumour tissue.

All poorly differentiated and undifferentiated retinoblastomas contained both BCOR positive and BCOR negative regions in varying proportions, with more differentiated BCOR-positive cells frequently located close to, or within the retina. This observation is consistent with the suggestion that early events following RB1 loss in retinal cells involve the formation of well differentiated lesions and that subsequent loss of differentiation is associated with tumour progression, allowing intra-vitreal growth and invasion into other ocular structures. Thus among the tumours studied, even those with an overall classification of 'poorly differentiated' or 'undifferentiated' showed remnants of BCOR-positive/differentiated tumour.

The recognized heterogeneity of retinoblastoma at the level of both histopathology and molecular pathology has led researchers to question how these different aspects of retinoblastoma biology might be linked. One proposal put forward is a tumor progression model in which early well differentiated lesions with a photoreceptor-like gene signature advance to undifferentiated lesions with loss of photoreceptor gene expression and higher proliferative capacity[21]. Similarly, analysis of tumour pathology in a further study showed that loss of differentiation and the development of severe anaplasia and pleomorphism correlated with decreased expression of photoreceptor genes[25]. However published studies do not provide information about specific gene mutation(s) which drive these events. Our CHECT-funded study is the first to link BCOR mutation with the regulation of differentiation and thus provides a novel marker, detectable at either the DNA or protein level, that could be used to categorize retinoblastomas with differing potential for progressive growth and invasion.

Clinical Significance
The development of BCOR as a tumour marker may have value in informing decisions regarding adjuvant chemotherapy post-enucleation. A very recent report from the European Retinoblastoma Group concluded that there was a lack of uniformity among treatment centres regarding indications for adjuvant treatment and that 'further biomarkers in addition to histopathological risk factors could improve treatment stratification'[26]. Additional research into the pattern of BCOR expression, particularly within the intermediate risk group (i.e. with massive choroidal invasion and/or post-laminar optic nerve invasion), currently treated with chemotherapy in most centres, may help to further stratify tumours within this group. It was noted for example that of 3 intermediate risk tumours in the present study (RB42, RB43, RB50), only one (RB50) showed BCOR loss throughout most of the tumour. If further studies confirm a relationship between BCOR loss and tumour progression this could be used to stratify patients to receive chemotherapy.

An additional area of clinical interest is the development of improved chemotherapeutic agents to minimize toxicity to the retina and associated vasculature, and thus improve visual outcome. The identication of BCOR and the pathways within which it functions, provides for the first time, novel target(s) that could be utilized for this purpose.

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.

The growth of a cancer is a consequence of the combined effects of mutations in several different genes. These mutations, or mistakes, cause the gene to function abnormally or not at all. In retinoblastoma, we know that the RB1 gene is almost always mutated, but this on its own is not enough to cause the cancer. Very little is known about other gene mutations and how they contribute to the growth and spread of the cancer.

In this CHECT-funded study our aim was to identify other mutated genes and to study their effects on retinoblastoma. We chose to do this by growing cancer cells from 28 different retinoblastomas in the laboratory and looking in detail at the DNA sequence of every active gene in each of the retinoblastomas, for evidence of mutations that might be important.

We found that there was only one gene, called BCOR, that was mutated frequently in this group of retinoblastomas. In 16 (57%) of the retinoblastomas, mutation in the BCOR gene meant that it could not function properly. We next compared the 16 retinoblastomas with BCOR mutation, with the remaining 12 retinoblastomas, that had a normal functional BCOR, to see what the difference was between them. We found that BCOR is important for the normal development of a class of retinal cells called photoreceptor cells, that function in the visual system in the eye. This happens through a process called 'differentiation', which means that cells develop very specific characteristics that are essential for their function e.g. the retinal photoreceptor cells become specialized to detect light.

When BCOR is mutated, the cells remain 'undifferentiated' and do not look or function like normal retinal photoreceptor cells. While almost all retinoblastomas have a RB1 mutation, our results suggest that those that also have a BCOR mutation have a higher probability of developing into a more aggressive cancer that can spread outside the eye, where it would be much more difficult to treat. We noticed for example that undifferentiated cells, without functional BCOR, were seen in parts of the cancer that were spreading away from the retina and into the optic nerve. Retinoblastoma cells in the optic nerve are known to signal a high risk of the retinoblastoma spreading outside the eye.

The significance of these observations is that BCOR could be used as a marker to differentiate between retinoblastomas with a high risk of spread (BCOR-mutated) or a lower risk (BCOR-normal). This information is important to help doctors decide on the best treatment for retinoblastoma patients. It is also possible that new drugs could be developed to reverse the effects of BCOR mutation by stimulating retinal photoreceptor cell differentiation.

Publications

Please list all published or accepted papers and abstracts from the work of this grant (journal style) (attach copies where available)

Molecular aspects of the study were submitted to the British Journal of Cancer ('The transcriptional co-repressor BCOR is mutated frequently in retinoblastoma cell lines, resulting in loss of differentiation control'. McConville C & Jenkinson H), but the manuscript was not accepted. A new manuscript incorporating both mutation information and histopathology will be prepared and submitted elsewhere.

3. Dissemination of results

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

Results were presented at a meeting of the European Retinoblastoma Group (EURBG) by Dr Jenkinson in 2019.

4. Intellectual property (IP)

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

5. Collaborations

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

6. 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

The retinoblastoma cell lines used in this study will be made available to other researchers.

7. Any further comments

Report completed by:	Dr Carmel McConville	Date of report:	3.9.21

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