

# Retinoblastoma

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## Abstract

Retinoblastoma is a rare cancer of the infant retina, which forms when both *RB1* alleles mutate in a susceptible retinal cell, likely a cone photoreceptor precursor. Loss of the tumour suppressor functions of the retinoblastoma protein, pRB, leads to uncontrolled cell division and recurrent genomic changes during tumour progression. Although pRB is expressed in virtually all tissues, cone precursors have biochemical and molecular features that may sensitize to *RB1* loss to enable tumourigenesis. Retinoblastoma is diagnosed in ~8,000 children each year worldwide. Patient survival is >95% in high-income countries, but <30% globally. However, outcomes are improving through increasing awareness for earlier diagnosis, new guidelines and sharing of expertise. Intra-arterial and intravitreal chemotherapy have emerged as promising methods to salvage eyes. Ongoing international collaborations will replace the multiple different classifications of eye involvement with standardized definitions to consistently assess eligibility, efficacy and safety of treatment options. Life-long follow-up is warranted since survivors of heritable retinoblastoma are at risk for developing second cancers. Defining the molecular consequences of *RB1* loss in diverse tissues may open new avenues for treatment and prevention of retinoblastoma as well as second cancers in patients with germline *RB1* mutations.

## [H1] Introduction

Retinoblastoma is a rare cancer usually initiated by biallelic mutation of the retinoblastoma gene (*RBI*) in a single susceptible developing retinal cell. Inheritance of one mutant *RBI* allele strongly predisposes to retinoblastoma tumours that form after the second *RBI* allele is mutated.<sup>1</sup> The principles discovered by studying retinoblastomas have led to the recognition that inactivation of tumor suppressor genes broadly contributes to human tumorigenesis.

Retinoblastoma initiates after an *RBI*<sup>-/-</sup> retinal cell undergoes limited proliferation to form a non-malignant retinoma (Figure 1)<sup>2</sup> An intraretinal tumour develops after genetic or epigenetic changes lead to uncontrolled proliferation. Most commonly, this is first detected when the white tumour is visible through the pupil (leukocoria) or blocks vision.<sup>3</sup> When noticed early, prompt treatment can cure the cancer and save the eye(s). However, delayed diagnosis can lead to incurable invasion of the optic nerve and brain, or to metastases elsewhere in the body that are sometimes cured by extensive intervention.

Several questions related to our understanding of retinoblastoma and its optimal clinical management remain. The cell of origin of retinoblastoma has been difficult to pinpoint; new research we review in this Primer has potential to improve our understanding of why the infant retina is uniquely predisposed to malignancy upon *RBI* loss. The evidence base for clinical management is weak. Rigorous randomized clinical trials (RCTs) in retinoblastoma are missing for multiple reasons: too few patients in countries with capacity to conduct clinical trials; often complex presentation (two eyes with differing severity); too few patients to interest the pharmaceutical industry; often-opposing schools of thought and difficulties in transdisciplinary collaborations; and high societal value on eyes and vision imposes considerations beyond curing the cancer. New technologies showing dramatic tumour responses have been quickly embraced, without rigorous clinical science. Differences in resource availability, medical expertise, and societal and health system structure vary between and within nations, making recommendations for optimal care complex and context-dependent.

To address these challenges, this Primer on retinoblastoma introduces unprecedented new science, ideas, therapies and global collaboration. The Internet has opened many avenues: parents diagnose retinoblastoma themselves; colleagues share patients around the globe; centres of excellence are mapped; and a common database for all children — no matter where they live — is within reach. These developments are set to empower a ‘learning health system’ that will build the evidence base to optimise care.

## **[H1] Epidemiology**

### **[H2] Global incidence and outcomes**

The expected number of patients with retinoblastoma annually per country is most accurately calculated by multiplying the global retinoblastoma incidence (1 in 16,000–18,000 live births) by number of forecast surviving infants (birth rate minus infant mortality rate) (Table 1).<sup>4-6</sup> Using these formulas, approximately 8,000 new cases are predicted each year. The number of children predicted in each country are indicated in Supplementary Table 1. Calculations as in Table 1 accurately predict retinoblastoma in many countries.<sup>7-9</sup> There is no validated evidence of geographical or racial variations of retinoblastoma incidence.<sup>6,10,11</sup>

Of all affected children, 11% reside in high-income, 69% in middle-income and 20% in low-income countries.<sup>12</sup> Although prevalence is higher in middle- to low-income countries (Figure 2), most retinoblastoma treatment centres are in middle- and high-income countries, creating a gap in healthcare access (Table 2). Consistent with income being a surrogate for non-economic measures of standard of living, retinoblastoma in low-income countries is associated with low patient survival (~30%<sup>13,14</sup>) compared with high-income countries (>95%<sup>8</sup>), but comprehensive nation-wide data are lacking.

Poor outcome correlates with late diagnosis, difficulty accessing retinoblastoma-specific health care, and socio-economic issues leading to poor compliance, including family refusal to remove the affected eye (enucleation) and abandonment of therapy.<sup>11,15,16</sup> Without timely diagnosis and appropriate treatment, difficult-to-cure metastatic disease may develop. Fortunately, with early diagnosis,<sup>17</sup> many eyes can be safely treated and support a lifetime of good vision, pointing to key elements for global focus: awareness, collaboration, and affordable expert care.

### **[H2] Solutions for global retinoblastoma**

A number of initiatives address the inequality in retinoblastoma treatment between developing and developed countries. In 2009, the Canadian National Retinoblastoma Strategy (NRbS) published the first-ever retinoblastoma clinical practice guidelines,<sup>8</sup> adapted by the Kenyan National Retinoblastoma Strategy (KNRbS) and published in 2014 in partnership with the Kenyan Ministry of Health.<sup>18</sup> The KNRbS has supported the standardization of histopathologic examination of the enucleated eye to inform treatment decisions and discussion of prognosis with families. Adoption of upfront (first-line) enucleation with implants and immediate prosthetic eyes<sup>19</sup> (sourced from India), parent-to-parent interactions to allay uninformed fears, and standardization of information provided to parents has reduced the rate of non-compliance with treatment.<sup>18,20</sup> In 2013, the Paediatric Oncology in Developing Countries Committee of the SIOP (International Society of Paediatric Oncology) published a consensus guideline for the management of retinoblastoma in countries with limited resources<sup>21</sup> with clear ideas that can shape resource development. The Mexican National Strategy<sup>22</sup> and the Brazilian SOBOPE (Brazilian Society of Paediatric Oncology)<sup>23</sup> guidelines are also applicable at a national level with governmental support for treatment.

Peer-to-peer collaborations and twinning programmes have built a framework for knowledge and expertise exchange, filling gaps in specialized training, and donating equipment and resources with the ultimate aim to

ensure sustainable local capacity to deliver optimal health care.<sup>24</sup> Retinoblastoma-specific twinning programmes include partnerships between St. Jude's Children's Research Hospital (USA) and the Middle-East,<sup>25</sup> Central America<sup>26</sup> and Mexico,<sup>22</sup> and between the Institut Curie and a centre in Bamako, Mali.<sup>27</sup> The Central American Association of Paediatric Hematology Oncology (AHOPCA) created a cooperative group and implemented multicentre protocols for retinoblastoma treatment,<sup>26,28,29</sup> a major achievement, not yet paralleled in developed countries. The AHOPCA funding is now 90% local. Twinning programmes benefit from strong participation of both governmental and non-governmental organizations (NGO). However, where government may prove volatile and unpredictable, sustainable local capacity is a challenge. Less formal cooperation between developed and less developed countries can also result in highly efficient programmes.<sup>30,31</sup>

A successful national strategy has been developed in China. About 1,100 newly diagnosed cases are forecast annually, scattered over 32 provinces. Before 2005, enucleation was the only available treatment for most children. For better treatment options and follow-up, centres classified by expertise and resources were established in 28 hospitals covering 25 provinces (over 90% of the population), which profoundly reduced travel costs<sup>12</sup>. The improved efficiency and national collaboration from 2006 to 2014 has led to standardized classification and treatment of 2,097 newly diagnosed patients with retinoblastoma on common protocols, which has led to increased survival (unpublished data, J.Z.). In Argentina, a single centre with high expertise coordinates affiliated retinoblastoma clinics. This collaboration between governmental hospitals, local and international NGOs with prospective protocols has significantly improved survival in two decades, in addition to stimulating translational research and population-based incidence and survival studies.<sup>32 33,34</sup>

In a rapidly changing world, online strategies are facilitating global collaboration. The One Retinoblastoma World initiative ([www.1rbw.org](http://www.1rbw.org)) leads families to the nearest centre with known expertise.<sup>12</sup> The retinoblastoma-specific point-of-care database (eCancerCare<sup>RB</sup>, eCC<sup>RB</sup>) summarizes the medical record for retinoblastoma for each affected child. eCC<sup>RB</sup> is freely accessible online to every retinoblastoma centre with local ethics and privacy approvals. The Disease-specific, electronic Patient Illustrated Clinical Timeline (DePICT) is language-independent, is understandable by parents, and supports fully informed care choices.<sup>35</sup> With guardian consent, identifiable data is accessible to those in the circle of care of each child. Ultimately, the de-identified data will be fuel for powering a global learning health system for retinoblastoma.

## **[H1] Mechanisms/pathophysiology**

### **[H2] Genetic basis of retinoblastoma**

The search for the genetic basis of retinoblastoma provided the two-hit model of tumour suppressor gene inactivation. In 1971, Knudson discovered that the ages of retinoblastoma diagnosis are consistent with one rate-limiting event in bilaterally affected patients (who have heritable disease) (median age at diagnosis 12 months), and with two rate-limiting events in unilaterally affected patients with no family history (who usually have the non-heritable form) (median age at diagnosis 24 months).<sup>36</sup> This prompted the proposal that heritable retinoblastomas result from a germline mutation ('first hit', M1) and an acquired somatic mutation ('second hit', M2), whereas non-heritable retinoblastomas arise when two somatic mutations are present in the same transformation suppressor gene in a susceptible cell (Figure 3).<sup>37</sup> Chromosomal deletions in some patients

pointed to a chromosome (chr.) 13q14 locus. Loss of heterozygosity of chr. 13q14 polymorphic markers in 70% of retinoblastoma tumours<sup>38</sup> implied that the second hit involved the same locus. The breakthrough came when one retinoblastoma was found to be missing the sequence of a chr. 13q DNA clone,<sup>39</sup> that was shown to be a conserved exon sequence of *RBI*.<sup>39-42</sup> Although *RBI* loss consistently initiates cancer in the retina, *RBI* function can be lost with progression in almost all cancer types.<sup>43</sup>

*RBI* is a large (190 kb) gene with 27 exons, encoding a 4.7 kb mRNA that translates into a 928 amino acid protein, pRB. Many modifications impair pRB function, including point mutations, promoter methylation, and small and large deletions.<sup>44</sup> Even chromothripsis, a “shattering” of a genomic region resulting in widespread rearrangements, has been shown to underlie a minority of cases.<sup>45</sup> The A/B “pocket” region<sup>46</sup> harbours most missense mutations.

pRB is best known as a cell cycle regulator that binds to E2F transcription factors to repress cell proliferation-related genes. Hyperphosphorylation of pRB by cyclin-dependent kinases (CDKs) in response to mitogenic signals normally relieves repression and promotes the G1 to S phase transition. pRB loss relieves this repression in the absence of mitogenic signals to enable cell cycle entry. It is tempting to surmise that pRB is primarily needed to suppress E2F transcription factors, and that loss of this function is the main cause of retinoblastoma formation.<sup>46</sup> However, several “low penetrance” *RBI* mutations encode proteins with minimal ability to bind E2F but unexpectedly these mutations predispose to far fewer tumours than *RBI* null alleles.<sup>47,48</sup> Thus, such defective E2F-binding alleles may function to substantially block retinoblastoma development through one or more of pRB’s E2F-independent functions.<sup>46</sup> For example, pRB also up-regulates p27, and this effect has been implicated in cell differentiation, apoptosis, and genomic integrity.<sup>46</sup> However, during cone precursor maturation increasing pRB is associated with decreasing p27 expression,<sup>49</sup> arguing that p27 would not mediate retinoblastoma suppression in these cells.<sup>49</sup> The loss of pRB N-terminus functions such as non-homologous end-joining may also be important but are not clearly implicated in retinoblastoma initiation.<sup>50,51</sup> Thus, the exact mechanism by which pRB normally suppresses retinoblastoma remains to be identified.

Biallelic *RBI* inactivation is necessary to initiate most retinoblastomas, but is not sufficient, since the benign retinal lesion, retinoma, has lost both *RBI*<sup>-/-</sup> alleles (Figures 1 and 3).<sup>2</sup> Further genetic or epigenetic changes are likely needed for malignant transformation.<sup>52</sup> Epigenetic alterations might drive retinoblastoma formation by inducing H3K4 trimethylation and H3K9 and H3K14 acetylation marks and expression of the *SYK* oncogene.<sup>53</sup> Comparative genomic hybridization studies identified DNA copy number gains encompassing the candidate retinoblastoma oncogenes mitotic kinesin *KIF14* and the p53 regulator *MDM4* (chr. 1q32), transcription factors *E2F3* and *DEK* (chr. 6p22), and the onco-miR clusters *miR-106b~25* (chr. 7q22.1) and *miR-17~92* (chr. 13q31), as well as losses encompassing the cadherin-11 (*CDH11*) (chr. 16q22) and pRB family member *RBL2* (chr. 16q12.2) tumour suppressor genes.<sup>52</sup> Whole-genome sequencing identified inactivating mutations in the transcriptional corepressor *BCOR* encoding the BCL-6 corepressor.<sup>53</sup> Interestingly, some tumors had no detected alterations,<sup>53</sup> consistent with acquisition of such changes during tumour progression.<sup>54</sup>

Many other genes and microRNAs also show altered expression in retinoblastoma compared to normal retina.<sup>52</sup> Gene expression profiles were initially proposed to reveal only a single relatively homogeneous class of retinoblastoma<sup>55</sup> and were subsequently proposed to segregate *RBI*<sup>-/-</sup> retinoblastomas into two subtypes.<sup>56</sup> However, analyses of a larger cohort recently revealed a spectrum of phenotypes reflecting progression from a

state with high photoreceptor differentiation and few cytogenetic changes to a state with lower differentiation, increased mRNA and ribosome biogenesis and mitosis gene expression signatures, and increased cytogenetic aberrations.<sup>54</sup> Thus, *RB1*<sup>-/-</sup> retinoblastomas appear to progress from self-limiting retinomas to differentiated retinoblastoma to less differentiated malignancies.

Whereas both *RB1* alleles are mutated in nearly all retinoblastomas, a subset of unilateral tumours (1.4%) show no evidence of *RB1* mutation but have high-level amplification of the oncogene *MYCN* (amplified *MYCN*, *MYCN*<sup>A</sup>).<sup>57</sup> These *RB1*<sup>+/+</sup>*MYCN*<sup>A</sup> tumours are always unilateral, are diagnosed in much younger children than unilateral *RB1*<sup>-/-</sup> tumours, and have a distinct morphology, reflecting a unique subtype. Although they also fall within the photoreceptor differentiation and mitosis/RNA biogenesis spectrum of *RB*<sup>-/-</sup> tumours, *RB1*<sup>+/+</sup>*MYCN*<sup>A</sup> tumors are distinct outliers in levels of expression of many genes,<sup>54</sup> consistent with the idea that they originate in a earlier retinal precursor.<sup>57</sup> Another 1.5% of unilateral non-familial retinoblastomas are unexplained and have apparently normal *RB1* and *MYCN* genes.<sup>57</sup>

## [H2] The retinoblastoma cell of origin

Since RB is expressed in most if not all cells, the retina's unique sensitivity to RB loss has been perplexing. While the key to this sensitivity likely relates to the features of the cell of origin, the identity of this cell has been elusive. Studies addressing the cell of origin through analysis of tumours offered puzzling clues, as the tumours expressed diverse retinal cell type markers. Together with other evidence, the diverse cell marker expression suggested either a pluripotent cell of origin, aberrant co-expression of marker genes due to oncogenic transformation, or the presence of normal *RB1*<sup>+/+</sup> retinal cells within largely cone-like tumours.<sup>55,58</sup> Mouse models have not clarified the cell-of-origin, since such models have required an additional mutation of *Rbl1*, *Rbl2*, or *Cdkn1b* on top of *Rb1* loss,<sup>59</sup> and the resulting tumours express a different spectrum of retinal markers, raising the possibility that they might not extrapolate to humans.<sup>60</sup>

A clue to the cell of origin came from the finding that the topographic distribution of emerging retinoblastomas mimics the horizontal visual streak characteristic of red and green cones, which provided evidence that the tumours could derive from a cell in the cone photoreceptor lineage.<sup>48</sup> Consistent with this view, *RB1*<sup>-/-</sup> retinoblastomas consistently express cone photoreceptor markers but not other retinal cell type-specific proteins. In addition, normal post-mitotic maturing cone precursors have unusually high expression of pRB as well as oncoproteins (MDM2 and MYCN) that could collaborate with *RB1* loss to enable cone precursor proliferation (Figure 4a, b).<sup>46,61</sup> More recently it was shown that experimental depletion of *RB1* induces human cone precursor cell proliferation *in vitro* and pRB-depleted human cone precursors form tumours typical of differentiated retinoblastomas in orthotopic xenografts.<sup>62</sup> The proliferation of these normally post-mitotic cells depends upon their intrinsically high levels of MYCN and MDM2, cone-specific transcription factors RXR $\gamma$  and TR $\beta$ 2, and down-regulation of p27, which is associated with normal cone precursor maturation.<sup>49,62</sup> Thus, pRB may counter an oncogenic programme associated with cone precursor maturation.

Notably, the smallest tumours detected via optical coherence tomography (OCT) appear to be centered in the inner nuclear layer (INL) of the retina, not the outer nuclear layer (ONL) where mature cones reside (Figure 4c).<sup>63</sup> Possibly, the cone precursor cell that loses pRB mislocalizes to the INL. However, as they grow, the small tumors also extend into ONL and other retinal layers (Figure 4c), making it difficult to infer the retinal layer in

which tumors first form. Perhaps blood vessels and retinal astrocytes that are normally present in the inner retina promote retinoblastoma cell growth in the INL.<sup>64</sup>

## [H2] Translating knowledge of pathogenesis

Understanding retinoblastoma molecular pathways could lead to treatment and prevention opportunities. Oncoproteins such as MYCN could be targeted<sup>65</sup> in both *RBI*<sup>+/+</sup>*MYCN*<sup>A</sup> tumours<sup>57</sup> and *RBI*<sup>-/-</sup> tumours that have MYCN-dependence.<sup>61</sup> Furthermore, studies based on molecular discoveries showed that exposing retinoblastoma-prone murine foetuses to small molecule inhibitors of E2f or Cdk inhibited subsequent tumorigenesis without disrupting normal retinal development.<sup>59</sup>

These new translational opportunities require cell lines and animal models that accurately reflect retinoblastoma cell responses. Most *in vitro* studies have used two old cell lines, but many others are available.<sup>66</sup> Primary xenografts in newborn rats and immunodeficient mice<sup>67</sup> are useful<sup>53</sup> but will not reflect the cell and immune environment of natural retinoblastoma, potentially limiting translation to patients. Genetically engineered mouse models can also be used to assess novel treatments.<sup>68</sup> Whereas *Rb1*<sup>+/-</sup> mice (which are expected to lose the wild type *Rb1* allele as in *RBI*<sup>+/+</sup> humans) do not develop retinoblastoma, retinal-specific deletion of both alleles of *Rb1* (using *Pax6a*, *Nes* (Nestin), or *Chx10* promoters) in *Rbl1*, *Rbl2* or *Cdkn1b* deficient backgrounds achieves retinal tumour formation.<sup>60</sup> Models such as overexpression microRNA cluster *miR-17~92* have been used to examine genetic interactions *in vivo*.<sup>69</sup> Mice expressing the viral oncoprotein Simian Virus 40 large T-antigen develop retinoblastoma specifically in precursors of Müller cells (a type of retinal glia cells), and have been used extensively for pre-clinical therapeutic studies.<sup>70,71</sup> However, experimental murine tumours have different collaborating mutations and cell type origin, reducing their ability to predict human treatment responses. New models that more precisely recapitulate human retinoblastoma pathogenesis are needed to identify novel therapies that will target *RBI*<sup>-/-</sup> cancers.

## [H1] Diagnosis, screening and prevention

### [H2] Clinical diagnosis

Diagnosis of retinoblastoma is usually clear from presenting signs and clinical examination.<sup>3</sup> The most common sign is leukocoria (white pupil) (Figure 5). When parents report a strange reflection in the child's eye, retinoblastoma should be at the top of the differential diagnosis. The second most common sign is strabismus (misaligned eyes) when central vision is lost. Advanced disease may present with iris colour change, enlarged cornea and eye due to increased pressure, or non-infective orbital inflammation. Very late, the eye may bulge from the orbit, a common presentation where awareness and resources are inadequate.

Diagnosis of retinoblastoma does not rely on histopathologic examination since biopsy incurs risk of metastasis.<sup>72</sup> Indirect ophthalmoscopy with the pupil pharmacologically dilated is usually sufficient for diagnosis by an eye specialist. Calcification, a characteristic of retinoblastoma, is detected by ocular ultrasonography (b-scan). MRI is used to assess invasion of the optic nerve<sup>73</sup> and the presence of trilateral retinoblastoma (pinealoblastoma and primitive neuroectodermal intracranial tumours associated with *RBI*



mutations).<sup>73,74</sup> CT scans are now avoided because radiation induces second primary cancers in people carrying *RBI* mutations.<sup>75</sup>

Detailed retinal examination under general anaesthesia is required to distinguish the differential diagnoses (Coats' disease, persistent foetal vasculature, and vitreous haemorrhage) and classify the severity of intraocular disease. Accurate fundus drawing is essential to map tumour burden and location, and in some low-resource settings is the only available form of imaging. Where resources allow, the wide-angle, hand-held fundus camera is used to view and record the whole retina, while an expert depresses the sclera to bring the most anterior retina into view. At the same time that the focus is on viewing the anterior retina, care must be taken that there is not excessive pressure on the eye that could cause ischemia and would be indicated by optic nerve arterial pulsations. Very useful are high frequency (50 MHz) ultrasound biomicroscopy,<sup>76</sup> and OCT<sup>63</sup> to discover invisible tumours in infants with familial disease. Good imaging and documentation of the whole retina supports eye classification and cancer staging, documents treatment responses, supports consultation with colleagues, and helps parents understand treatment options.

## **[H2] Classification and staging**

Classification is necessary to determine appropriate management and predict outcome. The Reese-Ellsworth classification<sup>77</sup> for intraocular retinoblastoma was used to predict outcomes of external beam radiation. When new therapies became available, an international collaboration led by Murphree developed the International Classification of Intraocular Retinoblastoma (IIRC)<sup>78</sup> which better predicted responses to intravenous chemotherapy than the Reese-Ellsworth classification (Figure 6).<sup>78</sup> The IIRC classifies eyes in groups A to E, with E being the most severe. However, subsequent modification<sup>79</sup> resulted in a significantly different versions of IIRC such that severely involved (dangerous) eyes are classified with less affected eyes.<sup>80</sup> Subsequently, the Children's Oncology Group (COG) inserted a further minor variation.<sup>81</sup> The TNM cancer classification system for all cancers (defined by primary tumour (T), lymph node extension (N), and distant metastasis (M)) is yet another slightly different system for retinoblastoma.<sup>82</sup> The use of multiple classification schemes seeds confusion and undermines research in retinoblastoma. A priority to advance management strategies in retinoblastoma is to collect outcome evidence to develop one universally accepted classification based on evidence of the most important features at presentation that predict success for life and eye salvage, that will allow comparison between studies.

A number of staging systems also exist for extraocular retinoblastoma. In a coordinated action with the group that developed the original IIRC<sup>78</sup>, retinoblastoma clinicians developed the International Retinoblastoma Staging System (IRSS), focused on overall staging (different again from the standard TNM).<sup>83</sup> The American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) staging system developed the TNM classification for retinoblastoma<sup>82</sup>, which is currently under revision for the 2016 edition. Owing to its general acceptance in the oncology field, this scheme has the potential to achieve a single, collaborative, consensus classification of eyes and cancer staging, critical to clinical research.

## [H2] Pathology

Histological examination of the retinoblastoma after enucleation of the affected eye (Figure 3) is the only way to evaluate high-risk features (tumour invasion into the optic nerve beyond the confines of the eye or to the cut end of nerve; more than 3 mm of invasion of the vascular layer (choroid) under the retina; invasion of sclera) and establish pathological staging.<sup>84-86</sup> Emerging evidence indicates that high grade anaplasia (loss of differentiated characteristics) may be a risk factor for metastasis and death.<sup>87</sup> High-risk features by the Murphree IIRC<sup>78</sup> were observed in 15–33% of Group D and 50–61% of Group E eyes,<sup>85,88</sup> but by the Shields IIRC (which classifies less affected D eyes as E), only 24–39%<sup>89</sup> of Group E eyes showed high risk. Adjuvant systemic chemotherapy after high-risk features are observed in an enucleated eye may reduce risk of metastatic relapse.<sup>88-90</sup>

The preparation and examination of the enucleated eye have been optimized to evaluate all risk features.<sup>84,91</sup> Pathology specimens are staged using the pathology TNM (pTNM) and the IRSS classification schemes.<sup>82,84</sup> However, still too frequently retrospective examination finds a previously unnoticed risk after metastatic disease is diagnosed. Staining of specific markers for photoreceptor cells (the cone-rod homeobox transcription factor; CRX)<sup>92</sup> and for tumour cells (N-glycosylated ganglioside; NeuGc-GM3)<sup>93</sup> may facilitate detection of tumour cells otherwise missed or interpreted as artefact.

## [H2] Genetic diagnosis

Many patients with retinoblastoma are the first individuals to be diagnosed in a family; only 6% of patients indicated when tested for *RBI* mutations that a family member was previously diagnosed with retinoblastoma.<sup>94</sup> However, a population-based study in England suggested that 35% of bilaterally affected patients had a family history.<sup>9</sup> In three population-based studies<sup>9,11,95</sup> representing 2,738 retinoblastoma patients on three continents, 30–37% of cases were bilateral. When we add the 15–18% of unilateral cases who also carry an *RBI* mutant allele, approximately 45% of people with retinoblastoma carry one *RBI* mutation in their constitutional cells. Of the remaining patients with unilateral retinoblastoma, 98% have somatic biallelic *RBI* loss and 2% have normal *RBI* genes in the tumour, but somatic amplification of the *MYCN* oncogene instead.<sup>57</sup>

Knowledge of the patient's *RBI* mutation profile enables precise screening of relatives and subsequent generations. Without genetic testing, all children at risk (already one eye affected and/or positive family history) should undergo multiple exams under anaesthesia in the first 3 years of life to enable detection of small, easily treatable tumours. When genetic testing reveals *RBI* mutations in a family, children at risk can be tested for that mutation. Of unilateral patients, 85% will test negative with <1% residual risk for undetectable low level mosaicism, which reduces the intensity of surveillance for tumours in the normal eye and improves estimates of cancer risk of family members.<sup>8</sup> In familial retinoblastoma, prenatal screening can be performed on DNA obtained from amniotic fluid. When such prenatal testing results in a positive finding, the retinoblastoma team works with specialists to safely induce labour near term, to facilitate detection and treatment of retinoblastoma tumours as early as possible.<sup>8</sup> Pre-implantation genetic diagnosis for the known mutation of a parent offers the option for a family to have an unaffected child.<sup>96,97</sup>

Cerebrospinal fluid and bone marrow can be molecularly screened to as part of a surveillance strategy, when clinically indicated, to detect metastasis. Harvested stem cells to detect minimal residual disease. Markers that

have been used are the *RBI* tumour mutation (when mutation is not present or different in the germline alleles),<sup>98</sup> the signature of post-*RBI* genomic gains and losses<sup>99</sup> and CRX expression.<sup>92</sup>

Best outcomes in familial retinoblastoma management depend on the effectiveness of the healthcare team to identify and counsel affected families. Parents need appropriate genetic counselling and understanding of the risks and actions for each at-risk pregnancy. Alarming, a study of retinoblastoma in several developing countries observed that familial cases were diagnosed later than non-familial probands.<sup>100</sup> The authors inferred that the parents did not understand the risks to themselves and/or to their children, or were afraid that there was no treatment available. Alternatively, socioeconomic or geographic barriers may have reduced desired healthcare access. Clearly, study of social determinants of health, such as health seeking behaviour, perceptions of medical care, and sociocultural issues related to cancer inheritance would inform counselling approaches that meet the needs of families.<sup>101</sup>

## **[H2] Screening**

### **[H3] Screening through eye examination**

To detect retinoblastoma as early as possible, vision screening and eye examination have been developed. Childhood vision screening with effective training to detect signs of retinoblastoma will occasionally identify a child with retinoblastoma. Red-eye reflex examination is recommended and sometimes mandated for each doctor's visit of babies and toddlers in many parts of the world, yet this does not often identify children with retinoblastoma. A negative childhood eye screening test does not mean this child will remain tumour-free since a tumour might appear later, or may have been in the periphery and not visible (false negative).

An effective way to achieve early diagnosis is for all health professionals to be aware of retinoblastoma and the common words that parent use universally to describe the white pupil (Figure 5) that they have observed, and know that this might mean a life-threatening condition in the child that needs urgent attention. Too often health workers fail to take the parent's complaint seriously, due to lack of awareness. Photoleukocoria (the appearance of leukocoria on flash photographs, Figure 5) can bring retinoblastoma to the attention of parents, but is hindered by red-eye reduction in the camera, which constricts pupils with a pre-photograph flash, limiting photoleukocoria detection.<sup>3</sup> Red-eye and pet-eye correction tools to make portraits prettier removes photoleukocoria from photos, which might delay diagnosis. Awareness campaigns bring retinoblastoma to attention, but lose effectiveness with time. An innovative study called PhotoRed in India trained healthcare professionals to use flash photography to identify childhood eye diseases, including retinoblastoma.<sup>102</sup> Other pioneering efforts target software to enable cameras to detect photoleukocoria.<sup>103</sup> The global imaging industry could play a role in early diagnosis of retinoblastoma.

### **[H3] Surveillance**

Individuals with germline *RBI* mutation who have been treated with radiotherapy have high risk to develop specific second cancers (50% risk to develop cancer at 50 years if they received external beam radiotherapy (EBRT)), including leiomyosarcoma, osteosarcoma, melanoma, lung and bladder cancer.<sup>104</sup> Surveillance screening for second cancers with and without radiation is a pressing need in the opinion of retinoblastoma survivors.<sup>105,106</sup> The first study to evaluate annual whole body MRI surveillance for individuals with

predisposing *RBI* mutation showed it was feasible to detect second cancers, however only with 67% sensitivity and one false negative test.<sup>107</sup> Efforts to detect second cancers earlier are important to reduce mortality.

In addition, lifestyle counselling educates survivors on ways to mitigate their second cancer risk by reporting unexplained lesions, and avoiding an unnecessary radiation and carcinogens (e.g. cigarettes and excessive alcohol). The extent to which these ideas will prevent second cancers is unknown.

## **[H1] Management**

Management of retinoblastoma depends on extent of disease at diagnosis (classification of intraocular disease, stage of systemic disease), status of the opposite eye, overall health of the child, socioeconomic circumstances<sup>108</sup>, and access to expert care.<sup>3,21,109</sup>

## **[H2] Intraocular retinoblastoma**

### **[H3] Primary treatment**

Choice of primary treatment is based on likelihood of cure (patient survival), eye salvage and ultimate vision, and the status of the other eye, weighed against short term and long term complications of treatment (Table 3).<sup>109</sup> In order of approximate frequency of global use, primary treatments for intraocular disease include enucleation, intravenous chemotherapy (IVC) with focal therapy (laser therapy, cryotherapy), intra-arterial chemotherapy (IAC) with focal therapy, and focal therapy alone when tumours are small at diagnosis. External beam radiotherapy (EBRT) is no longer recommended for first line therapy for primary intraocular retinoblastoma, since radiation, especially in the first year of life, imposes a high risk of secondary cancers when the patient carries an *RBI* mutation.<sup>104,110</sup> Although there is no role for EBRT in primary treatment of intraocular retinoblastoma, for salvage of the only remaining eye that has failed other therapies, EBRT may still have a role.

Comprehensive analysis of the published literature on eye salvage by any strategy is confounded by the use of five different classification systems<sup>77-79,81,82</sup> in different studies (Figure 6), preventing a clear-cut comparison of results between centres, especially for Group D and E eyes. There is a general consensus that eyes with clinical features such as neovascular glaucoma, pthisis bulbi (a shrunken, non-functional eye), and anterior disease are not appropriate for any conservative therapy and require enucleation to know if further therapy is needed for high risk pathological features.<sup>79,85,88,89</sup> This may be particularly problematic when IAC is offered for eyes with advanced disease, since only the eye receives the chemotherapy.<sup>79,85,88,89</sup> Clinical variables strongly associated with high risk features included older age, longer lag from diagnosis to enucleation, anterior tumour or blood, scleral invasion, invasion of the post-laminar optic nerve and orbital cellulitis.<sup>89,111,112</sup> Attempted salvage by any method of an eye with features indicating high risk for extraocular extension imposes the danger of undiagnosed disease with risk for metastasis. By the Murphree IIRC<sup>78</sup> high-risk features were present in 15–33% of Group D and 50–61% of Group E eyes<sup>85,88</sup>, and by the Shields IIRC<sup>79</sup> 24–39%<sup>89</sup> of Group E eyes showed high-risk. Enucleation of the high-risk eye alerts those in the circle of care consideration of adjuvant chemotherapy to reduce risk for metastatic disease.<sup>88-90</sup> If enucleation of Group<sup>78</sup> E eyes is delayed longer than 3 months, for example by prior systemic chemotherapy, disease-specific survival is significantly lower ( $p < 0.001$ ).<sup>113</sup>

Choices for primary bilateral advanced retinoblastoma are outlined in Figure 7. However, where there is lack of expertise, equipment, resources, and difficulties with close monitoring, bilateral retinoblastoma may still be best treated with bilateral enucleation.<sup>3,21</sup> Many bilaterally enucleated retinoblastoma survivors lead active, productive and satisfying lives because they were treated by timely surgery as infants.

### **[H3] Second-line (salvage) therapy**

Second line salvage means initiating a new management plan, in a second attempt to save an eye that has failed the first attempt. A range of modalities is appropriate, but each subsequent plan has a lower success rate<sup>114</sup> and long drawn out attempts to salvage an eye incur high risks for the child and family, including metastasis and death.<sup>108,115,116</sup>

Second line treatment options include focal therapies, repeated systemic chemotherapy,<sup>117,118</sup> IAC,<sup>114,119</sup> brachytherapy (internal radiotherapy),<sup>120,121</sup> EBRT<sup>122-124</sup> and whole-eye<sup>121</sup> or proton beam<sup>125</sup> radiation. Criteria for secondary enucleation are not well defined but are dominated by refractory subretinal and vitreous seeding, vitreous haemorrhage and secondary neovascular glaucoma,<sup>117,126</sup> and socio-economic and psychological fatigue to save an eye with poor vision.<sup>108</sup>

IVC and focal therapy could avoid EBRT and enucleation in 37–47% of Group D or Reese-Ellsworth Group V eyes in three studies with long-term follow-up.<sup>124,126,127</sup> The three studies of IAC with the most patients<sup>128-130</sup> suggest that IAC achieved eye salvage in 57% of more advanced eyes, and a higher % of less advanced eyes using less focal therapy than is required to consolidate primary care following IVC. A combination of repeat IAC and intravitreal chemotherapy may play an important part in saving eyes that have failed IVC, radiation or IAC.<sup>119,130,131</sup> However, in general, extensive treatments over a long time to save an eye may increase family stress, decrease family resources,<sup>108</sup> and risk metastases and death.<sup>114,116,129</sup>

Vitreous seeding is the major cause of failure to save or salvage an eye. Pharmacokinetic studies show poor vitreous levels of drugs administered by either IVC or IAC.<sup>132</sup> The highest drug bioavailability in the vitreous is achieved by intravitreal chemotherapy using a safety-enhanced injection technique in carefully selected eligible eyes.<sup>133</sup> Following control of the source of seeds, intravitreal chemotherapy achieved two-year 98.5% control of target seeds and 90.4% event-free ocular survival.<sup>133-135</sup> The efficacy of intravitreal and IAC chemotherapy may ultimately eliminate the need for EBRT, even as second line therapy.<sup>136</sup> A combination of repeat IAC and intravitreal chemotherapy may play an important part in saving eyes that have failed IVC, radiation or IAC<sup>119,130,131</sup> However, in general, extensive treatments over a long time to save an eye may increase family stress, decrease family resources,<sup>108</sup> and risk metastases and death.<sup>114,116,129</sup>

A combination of treatments can target salvage therapies to the site of relapse. For relapse confined to the retina and/or vitreous, focal therapy and/or intravitreal chemotherapy are recommended, as long as whole-eye therapy is not required. Recurrent tumours that touch the optic nerve head and/or vision-critical regions such as the maculo-papillary bundle, or have a diffuse shallow retinal or subretinal tumour represent good indications for IAC, which might achieve better visual outcome than focal treatments.<sup>114,119</sup>

## [H2] Ocular therapies

### [H3] Enucleation

Enucleation is a first-line therapy for the majority of eyes with retinoblastoma globally; it is the fastest and least costly treatment<sup>137,138</sup>. Since the majority of children without a family history of retinoblastoma have Group<sup>78</sup> D or E disease at diagnosis, and more than 50% have unilateral disease with the other eye unaffected, cure can be achieved with enucleation (Table 3). Most Group<sup>78</sup> E eyes require enucleation because they carry risk of extraocular spreading by definition which can only be confirmed by the identification of high-risk pathological features in the enucleated eye. Good cosmetic outcome is achieved by replacement of the volume of the eye with an implant deep in the orbit, and provision of a prosthetic eye, worn in the conjunctival sac behind the eyelids (Figure 8). Many different reconstruction techniques are used worldwide.<sup>139</sup> Comparative studies have shown best artificial eye motility with a simple, cheap, polymethyl methacrylate implant and muscles sutured to the conjunctiva to form the pockets to hold the artificial eye (myoconjunctival approach), which is affordable world-wide.<sup>140,141</sup> Porous implants that become vascularized with muscles sutured to the implant are commonly used but are susceptible to infection and extrusion, and are more costly. Provision of a temporary prosthetic eye at the time of enucleation has a positive psychological impact on families,<sup>19</sup> to help the family accept enucleation for their child.

### [H3] Intravenous chemotherapy and focal therapy

Since 1996{Ferris, 1996 #7587} first-line therapy to control Groups<sup>78</sup> B, C and D disease has been IVC with different combinations, doses, schedules, and durations of carboplatin, etoposide and vincristine (CEV) followed by focal therapy to consolidate chemotherapy responses (Figures 7, 9). {Chan, 1996 #10251; Gallie, 1996 #10578; Shields, 2002 #9137; Manjandavida, 2014 #21453} One initiated RCT<sup>142</sup> includes high-dose, short-duration cyclosporine simultaneous with the chemotherapy to modulate multidrug resistance.<sup>142,143</sup> Eyes in Groups<sup>78</sup> B and C do well with CEV and focal therapy. In three studies with long-term follow-up of IVC and focal therapy, 47% of Group<sup>78</sup> D eyes<sup>124</sup>, 47% of Reese-Ellsworth Group V,<sup>126</sup> and 47% of Group{Shields, 2006 #21122} D eyes{Shields, 2006 #21122} avoided EBRT and enucleation (Figure 9).

Fundamental principles for systemic cancer therapy also apply to retinoblastoma: optimized outcomes are anticipated with high dose intensity and combination of several agents with complementary mechanisms of action and optimal delivery to the eye. This is illustrated by the reduced effectiveness of single agent, low dose carboplatin for retinoblastoma.<sup>144</sup> Acute toxicities of IVC for retinoblastoma are as for other paediatric cancers, including short-term transient pancytopenia (reduction in red blood cells, white blood cells and platelets), hair loss, vincristine-induced neurotoxicity, and infections. Long term toxicities include carboplatin-induced ototoxicity (high tone hearing loss) in some patients,<sup>145</sup> second non-ocular cancer risk with alkylating agents<sup>95,146</sup> and secondary acute myeloid leukaemia following intense chemotherapy including topoisomerase inhibitors, doxorubicin and alkylating agents.<sup>147,148</sup>

IVC alone rarely eradicates the retinoblastoma entirely, and focal therapy with repeated examinations under general anaesthesia is very important (Figure 9).<sup>126,149,150</sup> Tumours in the macular region, threatening vision, are particularly at risk of recurrence without focal therapy,<sup>151</sup> perhaps because of caution to not damage vision

results in under-treatment. Optical coherence tomography can be used to monitor macular tumours and scars for small recurrences, improving outcomes.<sup>152</sup>

Location of tumour is the most important predictor of final visual acuity (near-normal visual acuity  $\geq 0.5$  in 22% of eyes with macular tumour, 67% of eyes with extrafoveal tumour).<sup>153</sup> In children with two eyes affected, patching of the better eye to force use-dependent development of the eye with macular involvement (occlusion therapy) achieved near-normal vision in 53% and useful vision in 73% of patients.<sup>154</sup> There is no documented local toxicity of IVC to the eye (Table 3).

### **[H3] Intra-arterial chemotherapy**

IAC has been used for eye salvage therapy for decades in Japan<sup>129</sup> and has been recently refined to achieve a more selective delivery to the eye.<sup>128,155</sup> Interventional radiologists pass a micro-catheter through the femoral artery up to the orifice of the ophthalmic artery of the eye with retinoblastoma, and chemotherapy (single drug or combination; melphalan, topotecan, carboplatin) is infused in a pulsatile fashion over 30 minutes. Different interventional approaches are used for specific anatomic situations.<sup>156</sup>

The three papers with mean follow-up 13 to 79 months and a total of 488 patients<sup>128-130</sup> suggest that IAC achieved eye salvage for 65% of all eyes (Figure 10). Overall globe (eyeball) salvage was 74% when IAC was used as first-line treatment and 67% when used as second-line treatment.<sup>130</sup> In the North American experience,<sup>130,157</sup> globe salvage without needing EBRT (definition of “success”) was 96-100% for less advanced eyes (IIRC A-C or RE I-III) and 94% for Group<sup>79</sup> D eyes and 36% of selected Group E eyes<sup>79</sup> with a median follow-up time <24 months; 80% of RE V that received OAC as primary treatment could be saved (n=36). The longest follow-up study (median 74 months; range 0–252 months) for IAC salvaged the eye in 88% of Group B, 65% of Group C, and 45% of Group D eyes with vision  $\geq 0.5$  (Vision decimal system, 1 = normal, 0.1=legal blindness) in 50% of the eyes that had no foveal involvement.<sup>129</sup> This evidence suggests that IAC is a reasonable approach to primary therapy for eye salvage, with good evidence for a role in secondary eye salvage.<sup>114,119</sup>

From the IAC publications, the total number of patients with metastases is unclear, but at least 14 are reported.<sup>116,128,129,158</sup> Since many other treatments were given simultaneously with IAC, the 8 deaths from metastases of 343 patients with long follow-up<sup>129</sup> are not clearly assigned to IAC alone. From the IAC publications with high patient number and longer follow-up, metastatic relapse occurred in <2% of the children receiving IAC, but the relapsed patients often had received extensive, multimodal therapies<sup>116,128,129,158</sup>. The long term Japanese experience<sup>129</sup> and the more recent New York experience<sup>128</sup> have not reported an increase in metastases or metastatic deaths to date.

Ocular complications of IAC included vitreous haemorrhage (2%), branch retinal artery obstruction (1%), ophthalmic artery spasm with reperfusion (2%), ophthalmic artery obstruction (2%), partial choroidal ischaemia (2%) and optic neuropathy (< 1%).<sup>130</sup> At the time of IAC, minor transient complications included transient eyelid oedema (5%), blepharoptosis (droopy eyelid 5%), and forehead increased blood flow, 2%).<sup>130</sup> Electroretinogram showed minor evidence of retinal toxicity which lasted up to 12 months, which was not considered clinically relevant.<sup>159</sup>

### [H3] Focal therapy

Focal therapy (laser therapy, cryotherapy, local chemotherapy) is the local application of therapy to the eye, under direct visualization through the pharmacologically dilated pupil. Focal therapy is the primary treatment for Group<sup>78</sup> A eyes and is used to consolidate responses of Group<sup>78</sup> B, C, D eyes after IVC or IAC with the aim to physically destroy residual or recurrent small-volume, active retinoblastoma. In general, focal therapy is repeated monthly until the tumour is completely atrophic or calcified (Figures 8, 9).

Two types of laser treatments are applied monthly until the tumour is flat, atrophic or calcified. Transpupillary thermotherapy involves a 810 nm diode laser through the dilated pupil to heat tumour, rather than precisely coagulate the tumour for 3–5 minutes per spot. Photocoagulation lasers with 532 nm, 810 nm or continuous wave 1064 nm laser beams are directly applied by multiple short (0.7 s) burns to small volume active or suspicious tumour, gradually increasing power from sub-coagulation power intensity until the tumour is coagulated opaque white. Cryotherapy, on the other hand, involves freezing the tumour through the sclera with a nitrous oxide probe; the tumour is directly visualized and duration of freeze judged to completely eliminate the tumour. Since tumour cells die when thawing, one minute is allowed for each thaw between freezing cycles. Cryotherapy effectively destroys small primary tumour(s) or recurrences in the periphery of the retina. Plaque radiotherapy involves positioning a radioactive probe on the eye to deliver trans-scleral radiotherapy with an apex dose of 35–40 Gy over 4–7 days. Plaque focal radiation has not been associated with new primary tumours and is effective to treat a single primary or recurrent tumour in a location that will not compromise vision. Finally, paraocular chemotherapy has been useful for small volume recurrences and vitreous seeds.<sup>118,160-162</sup>

### [H3] Intravitreal Chemotherapy

Primary treatments mainly fail due to the presence of vitreous seeds.<sup>118</sup> Intravitreal chemotherapy is adjunctive to other treatments, and is only initiated after the source of the seeds is controlled. The use of intravitreal chemotherapy to control vitreous seeds — most difficult to control form of retinoblastoma — has been promising, especially in combination with a safety-enhanced injection technique and well defined eligibility criteria.<sup>133,135,136,163,164</sup> Under anaesthesia, the intraocular pressure is lowered by removing a small volume of fluid from the anterior chamber or by digital massage. Melphalan (or combined with topotecan) is injected into the vitreous behind the lens, through the conjunctival, sclera, and pars plana with a small-gauge needle. On needle withdrawal, the injection site is sealed and sterilized with cryotherapy and the eye is shaken gently to distribute the drug throughout the vitreous. Three clinically defined classes of vitreous seeds have significantly different median times to regression, mean number of injections and cumulative and mean melphalan dose.<sup>135</sup> Ultrasound biomicroscopy may be used to evaluate the otherwise hidden ciliary region behind the iris<sup>76</sup> to confirm that the injection site is tumour-free prior to intravitreal chemotherapy treatment. The toxicity of intravitreal chemotherapy is limited to technique-dependent localized peripheral retinal toxicity.<sup>136</sup>



## [H2] Extraocular retinoblastoma

### [H3] Extraocular at presentation

Retinoblastoma may present with evident extraocular disease, especially in low-income countries. Orbital extension of an intraocular retinoblastoma may be detected clinically in children with proptosis or a fungating mass or occasionally by imaging studies. Treatment includes neo-adjuvant chemotherapy, including carboplatin, etoposide and vincristine, as for intra-ocular retinoblastoma; other agents that are useful include cisplatin, cyclophosphamide and anthracyclines.<sup>21</sup> This is followed by enucleation and limited excision of affected tissues without mutilation, orbital radiation and adjuvant chemotherapy. Intrathecal chemotherapy (injection into the cerebrospinal fluid space) may also be used and high dose chemotherapy with stem cell rescue may be added in metastatic disease. Children with orbital disease or metastatic spread without central nervous system invasion may be cured with intensive therapy. Long term outcomes for retinoblastoma patients presenting with extraocular disease are complicated by late-developing effects, but overall they are moderate.<sup>165</sup>

## [H2] Palliation

Palliation includes pain management, symptom relief, nutritional support, and psychosocial support for the child and families.<sup>21</sup> Untreated retinoblastoma is highly sensitive to most chemotherapy agents. Children presenting with orbital retinoblastoma are usually in severe pain and discomfort that may be alleviated with judicious use of anticancer therapy, including conventional chemotherapy, even when no curative intent is pursued. These children often present with severe emaciation (severe weight loss) needing prompt medical treatment. Radiotherapy may also be helpful, especially for a central nervous system relapse or for the treatment of massive orbital extension, but it is often unavailable in low-income countries.<sup>166</sup>

## [H1] Quality of life

Quality of life (QoL) describes the level of physical, emotional and psychological wellbeing experienced by an individual. Cancer, its treatment and effects on the developing body, brain and mind can significantly decrease QoL, which has implications for treatment decisions and supportive and long-term follow-up care.

## [H2] Measured life-long impact

In one study, children with retinoblastoma followed from diagnosis to age 5 years showed decline in developmental functioning over time.<sup>167</sup> However, survivors diagnosed under 1 year of age performed significantly better in short-term and long-term verbal memory, verbal learning and verbal reasoning abilities compared with those diagnosed at over 1 year of age.<sup>167,168</sup> Surprisingly, verbal IQ was significantly above both age-matched sighted and non-retinoblastoma blind persons.<sup>169</sup> This unexpected higher performance might relate to the heritable *RBI* mutant allele in the younger children, since the opposite outcome is expected in this group.

Adults who lost one eye to retinoblastoma in early-life have altered development of visual, auditory and multisensory brain morphology, suggesting that the remaining eye acquired increased contralateral visual and other cortical connections, also measured as enhanced functioning of the remaining eye, if the other eye is removed at a young age.<sup>170-172</sup> However, clinical and animal studies suggest that repeated anaesthesia of young children, such as is necessary for eye salvage and to discover and treat small tumours, may impair

neurocognitive development.<sup>138,173,174</sup> Clearly the long-term development of retinoblastoma survivors is an important area for future study, to both guide future care of newly diagnosed children and survivors, and learn about human development.

## **[H2] Direct insight from survivors**

Social media have brought parents of patients with retinoblastoma and survivors into peer-support communities that share experiences and insights with each other. It is not yet clear how to reference and include the unique and rich data of social media in formal research.

Children's perception of pain and medical interventions changes over time.<sup>175-177</sup> Since children with retinoblastoma have to undergo many repeated procedures, normal anticipation anxiety around procedures might be magnified, resulting in intolerance of even minimally invasive experiences and mild pain. The child's initial strong emotions may be suppressed as the child gives up, and re-emerge as depression, post-traumatic stress or developmental trauma disorder.<sup>115,178-180</sup> Child Life interventions at any age and with any treatment help children cope and even thrive during treatment, reduce treatment costs, ease family stress and improve long-term mental health (Figure 11).<sup>5,75,181-184</sup>

Retinoblastoma treatment is often the childrens' only life experience and forms the centerpiece of their earliest memories. Adult survivors may not remember being anaesthetized, however, many describe acute fear of their mouth and nose being covered. Extended isolation during therapy may impact social functioning. Although most adult survivors perform well socially, many report low confidence and intense anxiety, especially in large groups and crowded environments. Most survivors are high cognitive performers.<sup>168</sup> However, reduced vision causes some children to become frustrated by their inability to keep up with peers, damaging self-esteem and confidence.<sup>165,185,186</sup>

## **[H2] Late effects of radiotherapy**

Although radiotherapy is nowadays rarely used for primary treatment of retinoblastoma, thousands of adult survivors live with its long-term effects. Many feel neglected and demoralized by lack of follow-up and prospective management. Facial deformity induced by radiation blocking tissue growth causes low self-confidence and social anxiety. Reconstructive surgery is a painful process that may impact remaining vision, but its cosmetic effects can dramatically improve QoL. Dry eye is very painful, and corneal vascularization reduces already limited vision. Use of ocular lubricants may prevent complications, if started early before pain and vision loss occur.

## **[H2] Second cancer risk**

Individuals at risk of second primary cancers as a consequence of *RBI* gene mutation require life-long follow-up.<sup>95,104,187</sup> Lack of agreed-upon protocols causes confusion, frustration and fear as adults struggle to access informed care, compounded by primary doctors who are not aware of late effects and life-long implications of *RBI* mutations. Looking forward, full information about the patient's cancer history, genetic status, life-long risks and action plan when concerns arise will empower survivors to be advocates for their own and their children's health.<sup>188-191</sup>

## **[H2] Family planning**

Many adult survivors have little knowledge of retinoblastoma genetics, genetic counselling or testing, their own status, or options for their baby. Genetic testing provides knowledge of inheritance of the parental *RBI* mutant alleles, empowering several options.<sup>8</sup> Pre-implantation genetic diagnosis is expensive but available for parents with retinoblastoma in some countries<sup>96,97</sup>. Choices for an affected foetus are complex and dependent on social contexts. Parents seek an agreed-upon screening protocol for at-risk babies, and the discussion and planning may cause, or remove anxiety among survivor-parents. Anger and guilt about somehow being responsible for the child's cancer is amplified when diagnosis is delayed. Screening protocols for at-risk children will reduce survivor-parent anxiety and enhance early diagnosis to achieve minimally invasive therapy.

## **[H1] Outlook**

Retinoblastoma is curable if diagnosed early. We propose that within 10 years retinoblastoma can be a ZERO-death cancer. Our anti-retinoblastoma arsenal will consist not only of curative treatments, but also measures to prevent development of disease in predisposed individuals. To achieve this vision, critical efforts are needed to build upon the current state-of-the-art for retinoblastoma as presented in this Primer. Here, we outline the key action items, respond to the immediate obvious targets for improvements, and set the stage for innovative discoveries in retinoblastoma (Box 1). We are confident that the 8,000 children that will be diagnosed each year will have optimized timely diagnosis, stage-appropriate care, and survive and have productive, happy lives. We are also inspired by the principles that define a "High Reliability Organization"<sup>192</sup> such as decision support tools based on evidence; deep analysis of unexpected events (such as metastasis unexpected for the stage at presentation, technical errors, failure to achieve parental compliance) and action to prevent such events; and full, honest and open communication.

We propose to determine the most likely cause of every death from retinoblastoma. Similar in concept to the groundbreaking Million Death Study,<sup>193</sup> each death will be an opportunity to avoid more deaths. The identified key risk factors will lead to systematic guidelines to avert preventable deaths. These guidelines will fall into categories such as public awareness (public and health system understanding of the urgency of leukocoria, use of social media), health services<sup>194</sup> (infant screening, financial support for every child, regional retinoblastoma expertise, etc.), clinical research (co-operative groups for clinical trials, outcomes analysis from learning health records, etc.) and basic research (define in retinoblastoma tumour cells targets for new therapy, define molecular targetable pathways for treatment and prevention of *RBI*<sup>-/-</sup> cancers, starting with heritable retinoblastoma).

## **[H2] Patient-centered, global research**

Retinoblastoma will benefit from clinical science at the level that has achieved dramatic change for paediatric cancer. In retinoblastoma, we have lagged behind in systematic accumulation of evidence on which to base therapy. Today we are faced with sweeping change in treatment of retinoblastoma using IAC and salvage of unresponsive vitreous seeds by intravitreal chemotherapy. However, we do not have full data on which to judge patient eligibility, long-term effectiveness or outcomes. In-depth systematic reviews and meta-analyses of therapies and outcomes for retinoblastoma can capture retrospective evidence on which to base present

recommendations for therapy. Global collaboration will empower RCTs of many aspects of care for children, families and survivors of retinoblastoma.

We have opportunities now to learn from initiatives and innovations everywhere, and to listen to the patient's voice to become an inclusive, participatory global network. Patients and families, empowered by social media, will be heard in the research approaches, making these approaches relevant, ethical, and appropriate. Stakeholders from low-and-middle income countries will be active innovators, assuring that protocols are feasible and acceptable. Equality for retinoblastoma will come by education (internet access), with shared collaborative care coordinated online. Retinoblastoma expertise will be developed locally (proportional to the local burden of retinoblastoma) but derived globally, with best practices drawing on rigorous molecular and clinical research. Broad inclusion in high quality studies of all aspects of care, including traditional, complementary and alternative medicine, will enhance outcomes.<sup>195</sup> Retinoblastoma RCTs will be collaborative, high-enrolment studies. Effective innovations will include all outcomes (survival, eye salvage, short- and long-term toxicities, social impact, etc.) and sustainability (cost, uptake by health ministries, social acceptability of therapy, etc.). The global burden of retinoblastoma shows the inherent inequity in how patients are diagnosed and cared for around the world.<sup>5</sup> Documentation of human and material resources required for optimal care, drawn from established clinical care guidelines, represents a first step towards health equity. National strategies in areas currently under represented in the retinoblastoma literature are poised to fill the knowledge gap.

We need a universally endorsed single classification in order to avoid confusion regarding eligibility and salvage rates for new treatments. Progress in therapy is critically dependent on achieving consensus on a standard classification of intraocular disease. The ongoing AJCC/UICC international survey of features of eyes at diagnosis, treatments given and outcomes will be used to derive one evidence-based classification to replace the current clinical staging schemes that confuse analysis, eligibility and outcomes.<sup>78-80,82</sup>

We are poised to collect uniform data on all children with retinoblastoma. The 1RBW.org map documents “staff, stuff systems and spaces”<sup>196</sup> and provides an alternative system of global collaboration, perhaps unique to small neglected diseases, but possibly a fore-runner for future learning health systems. The eCancerCare<sup>RB</sup> (eCC<sup>RB</sup>) point-of-care database summarizes the health record from diagnosis (including features of eyes and patient, treatments, complications, and outcomes) and is available to all retinoblastoma centres.<sup>197,198</sup> eCC<sup>RB</sup> also supports the caregivers and families by graphically displaying this information ensuring high motivation to collect good data quality by clinicians who directly benefit from efficient and accurate patient care (Figures 5, 8, 9). The next phase of this online project (aiming at 2020) will combine the 1RBW map (<http://www.1rbw.org>) with eCC<sup>RB</sup> de-identified patient data to develop a ‘learning health system’ which will provide real-time analysis of outcomes and will guide clinicians to apply evidenced-based care.<sup>199</sup>

## **[H2] Targeted therapies**

Our knowledge of the cell of origin of retinoblastoma and its unique sensitivity to *RBI* inactivation is steadily increasing, and may reveal strategies to prevent tumourigenesis. Moreover, ongoing work to define the molecular signature of retinoblastoma will continue to yield novel therapeutic targets. Advances in therapeutic development and prevention depend on these molecular studies, but we do not yet have preclinical models that accurately reflect retinoblastoma pathogenesis and treatment.

One of the earliest targeted therapies envisioned for use in retinoblastoma, based on preclinical information generated in transgenic mice, was Nutlin-3.<sup>200</sup> Nutlin-3 blocks the interaction of MDM2 and MDM4 with p53, resulting in stabilization of p53 and stimulation of apoptotic cell death. Nutlin-3 showed promising activity in combination with topotecan for retinoblastoma control in preclinical models. Although the SYK antagonist R406 was also considered a promising candidate based on preclinical studies,<sup>53</sup> its ocular pharmacokinetic profile following peri-ocular administration rendered it unfavourable for clinical use.<sup>201</sup> Preclinical studies further suggest that retinoblastoma initiation and tumor growth might be suppressed by inhibitors of MYCN, SKP2, E2F, or CDKs (Figure 12).

Preclinical studies have also sought to identify and evaluate pharmacokinetics of new agents alone or in combination<sup>202</sup> before clinical use. A few translational studies have resulted in changes in retinoblastoma clinical practice.<sup>203</sup> Innovative delivery systems to target small-volume subretinal tumours and vitreous seeding include episcleral implants<sup>204</sup> and nanoparticles<sup>205</sup>. Approaches adjusting chemotherapy schedules and dose intensity (metronomic therapy)<sup>206</sup> may particularly play an important role in palliative care for retinoblastoma. New biomarkers for molecular dissemination of retinoblastoma outside the eye may lead to earlier diagnosis of metastasis and targeted treatments.<sup>92,93</sup>

## [H2] Quality of life

Without thinking about the lifelong outlook for patients, however, all of the above endeavours become moot. Quality of life begins in infancy, assisted by Child Life<sup>181</sup> and respect for the child's need to play. Patient-oriented research will develop and validate life-long survivor follow-up care protocols, including centralized specialist eye care, genetic testing, second cancer screening, ongoing psychological support and fertility care. The education of primary physicians and development of survivor clinics will reduce morbidity and facilitate research to inform patient management with a major outcome being good quality of life during treatment and throughout life.

## Display Item Legends

### Box 1: Outstanding research questions

Patient-centered, global research focus

- Collect outcome evidence to develop one universally accepted classification based on clinical features of the eye at presentation that predict success for life and eye salvage, that will allow comparison between studies.
- Address the reluctance of health care workers to explain the true risks (such as in Jordan where doctors do not want to destroy families), and the choice of families who know and understand the risks but choose not to follow through because of stigma.
- Define treatment success: cure (life), salvage of vision (one eye), salvage of eye (vision and cosmetic), fewest examinations under anaesthesia (cumulative toxicity of anaesthesia).
- Meta-analysis of all children treated primarily with intra-arterial chemotherapy to quickly establish that this treatment is both safe and effective.

- Develop a “retinoblastoma index” to predict outcome and include levels of awareness, access to centres with the necessary resources and expertise, and application of evidence-based care.
- Collect evidence about the quality of life following retinoblastoma, and develop action plans to address the issues that most affect survivors.
- Determine what failures led to death of children from retinoblastoma, and focus global efforts on these targets to make retinoblastoma a ZERO death cancer.
- Implement a global retinoblastoma health record, such as eCC<sup>RB</sup>, to underpin a Learning Health System bringing real-time evidence to treatment choices.

#### Pathogenesis

- What underlies the genomic progression post *RB1* loss?
- What molecular changes promote (early) metastasis?
- Are there other *RB1*<sup>+/+</sup> genetic subtypes beyond *MYCN*-amplified retinoblastoma?
- What dictates the tissue specificity of *RB1* loss leading to cancer?
- What are the molecular features of second cancers in *RB1* mutation carriers?
- Develop drugs with low systemic toxicity and high effectiveness against retinoblastoma.

**Figure 1 | Progression of retinoblastoma.** **a)** Anatomical features of a healthy eye. Genomic damage (orange lightning bolt) leads to mutation of *RB1* resulting in biallelic functional loss of *RB1* in a developing retinal cell (possibly a cone photoreceptor precursor cell that is dependent on pRB to stop proliferation). **b)** Genomic instability leads to a benign retinoma; only 5% of patients show retinoma without retinoblastoma. Inset shows a small retinoma/tumour not visible except by optical coherence tomography. **c)** Intraretinal retinoblastoma arises as additional genomic changes promote uncontrolled cell proliferation; the tumour grows and seeds become independent, floating under the retina and into vitreous. **d)** Retinoblastoma can invade adjacent tissues: into the optic nerve, uvea, or sclera to constitute high-risk pathologic features. **e)** Eventually, retinoblastoma can extend extraocularly into orbit and metastasize especially to the bone marrow, or into the brain (direct or via the cerebrospinal fluid).

**Figure 2 | Global retinoblastoma treatment centres and patient distribution relative to resources.** One Retinoblastoma World ([www.1rbw.org](http://www.1rbw.org)) shows Retinoblastoma Centres of Excellence, providing a resource for affected families to access expert care. The majority of patients reside in low and middle income countries, while the majority of Retinoblastoma Centres are in high income countries. Images from [www.1rbw.org](http://www.1rbw.org).

**Figure 3 | Genetic origins of retinoblastoma.** Three genetic subtypes of retinoblastoma are known. Heritable retinoblastoma patients have a constitutive inactivating mutation (M1) in the *RB1* tumor suppressor gene in all cells of their body. A second, somatic mutation (M2) in a susceptible retinal cell can lead to benign retinoma. Further genetic and/or epigenetic events (M3...Mn) are required to transform to retinoblastoma. Non-heritable, *RB1*<sup>-/-</sup> retinoblastomas progress similarly, except both M1 and M2 occur in one susceptible retinal cell. *RB1*<sup>+/+</sup>*MYCN*-amplified (*RB1*<sup>+/+</sup>*MYCN*<sup>A</sup>) retinoblastoma is a rare, non-heritable retinoblastoma subtype driven by amplification of *MYCN* with normal *RB1*; other changes in these tumours remain uncharacterized. Retinoma histology shows distinct photoreceptor-like fleurettes, whereas *RB1*<sup>-/-</sup> retinoblastoma can show Flexner-Wintersteiner (insert) and Homer Wright rosettes (not shown). *RB1*<sup>+/+</sup>*MYCN*<sup>A</sup> retinoblastoma have a distinct morphology with rounded nuclei and prominent nucleoli related to the high *MYCN* protein.

**Figure 4 | Retinoblastomas originate in the retina.** **a,** The retina has a complex structure and contains multiple cell types. Haematoxylin and eosin staining of post-fertilization week 19 retina shows three post-mitotic nuclear layers in the central retina near the fovea (*left*) and two nuclear layers in the less mature periphery of the same histologic section (*right*). Cell types in each layer are indicated. **b,** Cone precursors normally have high expression of the RB protein (pRB). Immunofluorescence staining of post-fertilization week 19 retina shows especially strong pRB signal (pink) in maturing cone precursors in the central retina (*left*, counterstained for cone arrestin, green) and in retinal progenitor cells in the peripheral retina of the same histologic section (*right*). pRB staining is less intense in DAPI-stained nuclei (blue) in other retinal cell types. **c,** Wide-angle retinal image (*left image*) shows no tumor. In the plane of the green line, optical coherence tomography (OCT; *right image*) shows a tiny intra-retinal tumor in a 2.5 month old infant, which seems to extend from the inner nuclear layer to the outer nuclear layer. The tumour has an uncertain epicentre making it difficult to infer the retinal layer from which the tumor arose. The layer-of-origin may be defined in the future with more images of higher resolution OCT of retina in very young children carrying an inherited germline *RB1* mutation. Abbreviations: ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer; NBL, neuroblastic layer; RPE, retinal pigment epithelium.

**Figure 5 | Online diagnosis of retinoblastoma.** **a**, Detection of photoleukocoria (white pupil) on this digital image led the parents to the diagnosis of retinoblastoma. The left eye was determined by examination under anaesthetic to have IIRC<sup>78</sup> Group D retinoblastoma and was enucleated two days later; CT scan was scheduled to check for trilateral tumour. (Today, MRI would be recommended to reduce radiation exposure.) The pathologic examination of the eye showed no high-risk features. The other eye was normal at diagnosis but on the next examination a small tumour was detected and treated with only laser. **b**, One month after surgery, the child presented with high intracranial pressure. A large intracranial midline tumour (trilateral disease) was diagnosed on CT scan, which was treated with chemotherapy, high-dose chemotherapy with hematopoietic rescue by stem cell transplant, and intrathecal chemotherapy injections (directly into the cerebro-spinal fluid) through an implanted intraventricular catheter. **c**, Follow-up MRI at age 8 years shows no residual disease and **d**, the child is well with one good-looking artificial eye and one eye with normal vision; he wears polycarbonate lenses to protect his only eye. **e**, The eCC<sup>RB</sup> timeline of treatments; not all intrathecal chemotherapy injections (\*) are shown.

**Figure 6 | Different classification schemes for intraocular retinoblastoma confound comparison of outcomes.** The features listed determine the overall classification, ranging from small tumours not threatening vision (“Group A”) to tumours clinically noted to have features suggesting potential spread outside the eye (“Group E”). Most importantly, size of tumour alone does not make an eye dangerous by Murphree,<sup>78</sup> Children’s Oncology Group (COG),<sup>81</sup> or TNM classification;<sup>82</sup> but any eye with tumour >50% of eye volume is E (advanced-stage disease) by Shields classification.<sup>79</sup> The consequence is widespread confusion in the literature undermining clinical research, since studies using the different classifications cannot be compared. The red boxes indicate the critical differences between the different classifications.

**Figure 7 | Primary treatment choices based on the Murphree IIRC.** Treatment depends on the combined severity of each of the affected eyes (Eye 1 | Eye 2); the preferred option for each eye is depicted in the blue boxes. IIRC Group<sup>78</sup> A eyes can be treated with only laser or cryotherapy (focal therapy consolidation). Group<sup>78</sup> B and C eyes require several cycles of systemic intravenous chemotherapy (IVC) or intra-arterial chemotherapy (IAC) followed by focal therapy and intravitreal chemotherapy with melphalan and/or topotecan for residual or recurrent vitreous seeds. Isolated single tumours in Group<sup>78</sup> B or C eyes may occasionally be appropriate for primary radioactive plaque therapy. Group<sup>78</sup> D eyes require either IVC (with focal therapy consolidation) or IAC (with focal therapy consolidation). All eyes with features suggesting imminent extraocular extension (Group<sup>78</sup> E) should be removed so that accurate pathological examination can be performed to determine risk of metastasis requiring adjuvant chemotherapy. IIRC, International Intraocular Retinoblastoma Classification.<sup>78</sup>

**Figure 8 | Triplets with retinoblastoma.** These triplets developed retinoblastomas in all six eyes illustrating the full expressivity and penetrance of *RBI*<sup>-/-</sup> germline mutations that completely abolish pRB expression. The pedigree indicates that the three children all have the same *RBI* mutation, which is not detected in their parents or older brother. The eCC<sup>RB</sup> timelines reveal that all eyes had individualized therapy, with choices considering the overall impact on each child. Each child lost one eye, has a good-looking artificial eye and an eye with normal vision, and has disease control in less than one year. The number of eye exams under anaesthesia (EUA) was least for the child who had primary enucleation. OD, right eye; OS, left eye. Eye involvement at diagnosis is indicated in by the International Intraocular Retinoblastoma Classification;<sup>78</sup> the eye labelled “Group 0” had no tumour at initial diagnosis but developed a tumour 6 weeks later.



**Figure 9 | Retinoblastoma treated with IVC.** **a**, Detection of photoleukocoria in both eyes at age 8 months. **b**, Retinal photography at diagnosis showed total retinal detachment with underlying large tumours in the right (left image) and left eyes (right image). **c**, Both eyes have calcified tumor remnants and attached retinas 20 years after diagnosis, with 0.25 vision, right eye and 0.1 vision, left eye (Vision decimal system, 1 = normal, 0.1=legal blindness). **d**, eCC<sup>RB</sup> time line of shows treatment with IVC and focal therapy, then only follow-up visits.

**Figure 10 | Retinoblastoma treated with IAC:** **a**, Before IAC for a massive exophytic (outward growing tumour) retinoblastoma with total retinal detachment; and **b**, after IAC, showing complete response and eye salvage, leaving a 6 mm calcified scar. **c**, Before IAC for an endophytic (inward growing tumour) retinoblastoma tumour with extensive vitreous seeding; and **d**, after IAC showing complete control with clinically intact fovea and visual acuity 20/25. **e**, Before IAC for an eye with macular retinoblastoma demonstrating excellent tumor regression away from the macula; and **f**, after IAC leaving intact fovea and hope for vision.

**Figure 11 | Child Life promotes effective coping through play, preparation, education, positive-touch and self-expression activities, based on natural child development.** Child Life<sup>181</sup> helps children and their families cope with challenging healthcare issues, hospitalization and therapeutic interventions. This child plays doctor and nurse, wearing gloves and stethoscopes to examine Puppet Kevin who had one eye removed for retinoblastoma. They also learn how to put in his artificial eye, to better understand their own artificial eye. Play (medical play and just general play) promotes a sense of control for children within their medical experience.

#### **Figure 12 | Proposed roles for cone precursor circuitry in retinoblastoma tumorigenesis and targeted therapy**

Signalling circuitry specific to maturing L/M-cone precursors is uniquely conducive to oncogenic transformation in response to pRB loss.<sup>49,61,62</sup> Specifically, these cone precursors express the transcription factors RXR $\gamma$  and thyroid hormone receptor (TR)  $\beta$ 2, and high levels of MYCN, MDM2, and pRB, and down regulate p27 via a SKP2-related mechanism. pRB depletion in maturing cone precursors blocks cone photoreceptor differentiation and enables proliferation and survival dependent upon *MYCN*, *SKP2*, and *MDM2*. Mouse models suggest that E2Fs and CDKs contribute to the proliferative response to pRB loss<sup>59</sup>; these genes/proteins are also needed for proliferation and survival of human retinoblastoma cells.<sup>61,207</sup> Identifying the molecular mechanism mediating the cone precursor proliferative response to pRB loss provides opportunities to prevent tumor initiation, at least in preclinical models. Potential drugs are inhibitors of MYCN expression (JQ1),<sup>208</sup> inhibitors of MDM2-mediated p53 degradation (Nutlin 3a),<sup>200</sup> inhibitors of SKP2-mediated p27 degradation (C25),<sup>209</sup> and inhibitors of E2F and CDK activities (HLM006474 and R547, respectively)<sup>59</sup>. In addition, SYK was highly expressed in retinoblastoma but not normal retina.<sup>53</sup> Additional targeted agents may be effective against growing tumours, such as improved versions of the SYK inhibitor R406.<sup>53,201</sup>

<b>Table 1   Estimated global distribution of retinoblastoma.</b>							
<b>Category</b>	<b>Population, total</b>	<b>Birth rate, crude (per 1,000 people)</b>	<b>Mortality rate, infant (per 1,000 live births)</b>	<b>Forecast live birth total</b>	<b>Retinoblastoma incidence (high) 1:16,000</b>	<b>Retinoblastoma incidence (low) 1:18000</b>	<b>% of world retinoblastoma</b>
World	7 x 10 <sup>9</sup>	19.4	34.6	131 10 <sup>6</sup>	8,200	7,300	100%
<b>High income</b>	<b>1,299,489,520</b>	<b>11.6</b>	<b>5.5</b>	<b>14,941,320</b>	<b>934</b>	<b>830</b>	<b>11.4%</b>
<i>High income: non-OECD</i>	<i>249,927,994</i>	<i>13.8</i>	<i>10.3</i>	<i>3,418,872</i>	<i>214</i>	<i>190</i>	<b>not applicable</b>
<i>High income: OECD</i>	<i>1,049,561,526</i>	<i>11.0</i>	<i>4.4</i>	<i>11,518,845</i>	<i>720</i>	<i>640</i>	<b>not applicable</b>
<b>Low &amp; middle income</b>	<b>5,743,616,071</b>	<b>21.1</b>	<b>38.1</b>	<b>116,699,203</b>	<b>7,294</b>	<b>6,483</b>	<b>88.7%</b>
<b>Middle income</b>	<b>4,913,580,797</b>	<b>19.2</b>	<b>33.7</b>	<b>91,300,546</b>	<b>5,706</b>	<b>5,072</b>	<b>69.4%</b>
<i>Upper middle income</i>	<i>2,390,210,774</i>	<i>14.8</i>	<i>16.3</i>	<i>34,737,317</i>	<i>2,171</i>	<i>1,930</i>	<b>26.4%</b>
<i>Lower middle income</i>	<i>2,523,370,023</i>	<i>23.4</i>	<i>45.3</i>	<i>56,490,388</i>	<i>3,531</i>	<i>3,138</i>	<b>42.9%</b>
<b>Low income</b>	<b>830,035,274</b>	<b>32.3</b>	<b>54.5</b>	<b>25,373,890</b>	<b>1,586</b>	<b>1,410</b>	<b>19.3%</b>
<p>Forecast births were calculated using most recent data (2012) on each World Bank Category for population, birth rate and mortality rate (World Bank (<a href="http://data.worldbank.org">http://data.worldbank.org</a>) accessed on 5 February 2015. Low (1:18,000 live births) and high estimates (1:16,000 live births) for retinoblastoma were calculated, following the example of Kivela.<sup>5</sup> Since retinoblastoma arises before and near birth, the usual cancer incidence rate calculations (number of cases diagnosed in a given population, sometimes adjusted for the under-5<sup>14</sup> or under-14<sup>11</sup> populations in children's cancers) do not provide accurate data for retinoblastoma. OECD, Organisation for Economic Co-operation and Development</p>							

**Table 2 | Availability of treatment and imaging at retinoblastoma centres worldwide.**

<b>Countries</b>	<b>Total number of centres</b>	<b>Enucleation (%)</b>	<b>Laser therapy (%)</b>	<b>Cryotherapy (%)</b>	<b>IVC (%)</b>	<b>Retinal imaging (%)</b>
High income	47	100%	91%	89%	85%	89%
Middle income	75	100%	91%	91%	69%	63%
Low income	10	100%	50%	20%	5 (50%)	30%
Total	132	100%	88%	85%	97 (73%)	70%

Retinoblastoma centres worldwide were surveyed and featured on the One Retinoblastoma World Map ([1rbw.org](http://1rbw.org), accessed on 25 March 2015). Information is available for 54 countries (22 high-income countries, 28 middle-income countries and 4 low-income countries, following World Bank Classification). The percentages depict the percentage of centres offering the specific treatment or diagnostic tools. IVC, intravenous chemotherapy.

**Table 3 | Qualitative comparison of enucleation, IVC and IAC for unilateral retinoblastoma (Murphree IIRC Groups B, C, D).**

	Enucleation	IVC and focal therapy	IAC and focal therapy
Treatment	Major muscles removed, optic nerve cut, eye removed; orbital volume reconstituted with implant; muscles sutured to gain motility of the artificial eye, held in place by the eyelids.	Chemotherapy (carboplatin, vincristine, and etoposide) is given intravenously, best through an implanted "port", every 3-4 weeks; tumour response consolidated by focal therapy (laser and cryotherapy) every 1-4 months for 3 years.	Chemotherapy (melphalan, and/or carboplatin, and/or topotecan) infused via micro-catheter through femoral artery, near, or into the ophthalmic artery with radiographic confirmation; tumour response consolidated by focal therapy (laser and cryotherapy), every 1-4 months for 3 years.
Success rate to save eye (%)	0%	47% Group <sup>78</sup> D; >90% Group B & C	45 <sup>129</sup> to 95 <sup>130%</sup> Group <sup>78</sup> D*; >90% Group B & C
Acute toxicity	Loss of eye; parental distress waiting for artificial eye fitting; transient bruising, swelling and headache	Hair loss; low white cell count; fevers require IV antibiotics and hospital admission; protective isolation at a time of social development; parental distress over prolonged treatment	Groin haematoma; transient low white cell count; potential carotid vascular spasm and stroke; vascular compromise of the ophthalmic artery, retinal artery, or choroidal vessels; headache
No. of Procedures	1 surgery	2-8 chemotherapy cycles every 3 weeks	2-8 treatments, every 4 weeks
No. of EUAs	1 (unless patients are <i>RB1</i> +/-)	>20 for consolidation and monitoring	>10 since only 30% need consolidation
Patient survival	>99%	>98%	high unless undetected high risk features
High risk pathology detected	Yes	No	No
Hidden metastases treated	No	Yes	No
Identification of tumour genetics	Yes	No	No
Duration of treatment	2 months (if not <i>RB1</i> +/-)	> 1 year	> 1 year
Invasive surgery	Loss of eye	1 central venous catheter	2-8 intracranial arterial canulations
Long term toxicity	Loss of eye	High tone hearing loss ~20% (Carboplatin); <1% AML (etoposide)	4% severe vascular choroidal toxicity; long term unknown vision risk
psychological harms	Possibly low self-esteem due to loss of the eye or artificial eye; distress of fitting and maintenance of artificial eye	Years of social isolation for treatments; delay physical, emotional, social development; long term separations of families; parental relationship breakdown; hospital-associated anxiety that hinders future medical care; post-traumatic stress disorder; Developmental Trauma Disorder; parental anxiety over illnesses; PTSD in parents or siblings; financial distress	
Radiation	none	No Group <sup>78</sup> D get EBRT; enucleation preferred	Fluoroscopy; No Group <sup>78</sup> D to need EBRT
Socio-economic impact <sup>108</sup>	minimal	profound	very profound
Disease-specific mortality	<<1%	<1%	unknown
Resource	Anaesthesia; ophthalmic surgery, ocularist; artificial eye	Anaesthesia; retinoblastoma and oncology expertise; drugs; laser and cryotherapy equipment; imaging equipment; Child Life support; <sup>181</sup> long term follow-up	Anaesthesia; retinoblastoma-specific interventional radiology and oncology expertise; drugs; laser and cryotherapy equipment; imaging equipment; Child Life support; <sup>181</sup> long term follow-up
Long term follow-up	Minimal unless inherited	Every 2-6 months for 4 years after tumour control	Every 2-6 months for 4 years after tumour control
<b>Availability:</b>			
Available in high income countries	Yes	Yes	Yes
Available in middle income countries	Yes	Yes	Limited
Available in low income countries	Yes	No	No

AML, acute myeloid leukaemia; EUA, examination under anaesthesia; IV, intravenous; IVC, intravenous chemotherapy; IAC, intra-arterial chemotherapy; \* very wide range reflects different classifications and different follow-up durations.

## **Competing interests**

There is NO competing interest.

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## **Author contributions**

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Figure 1

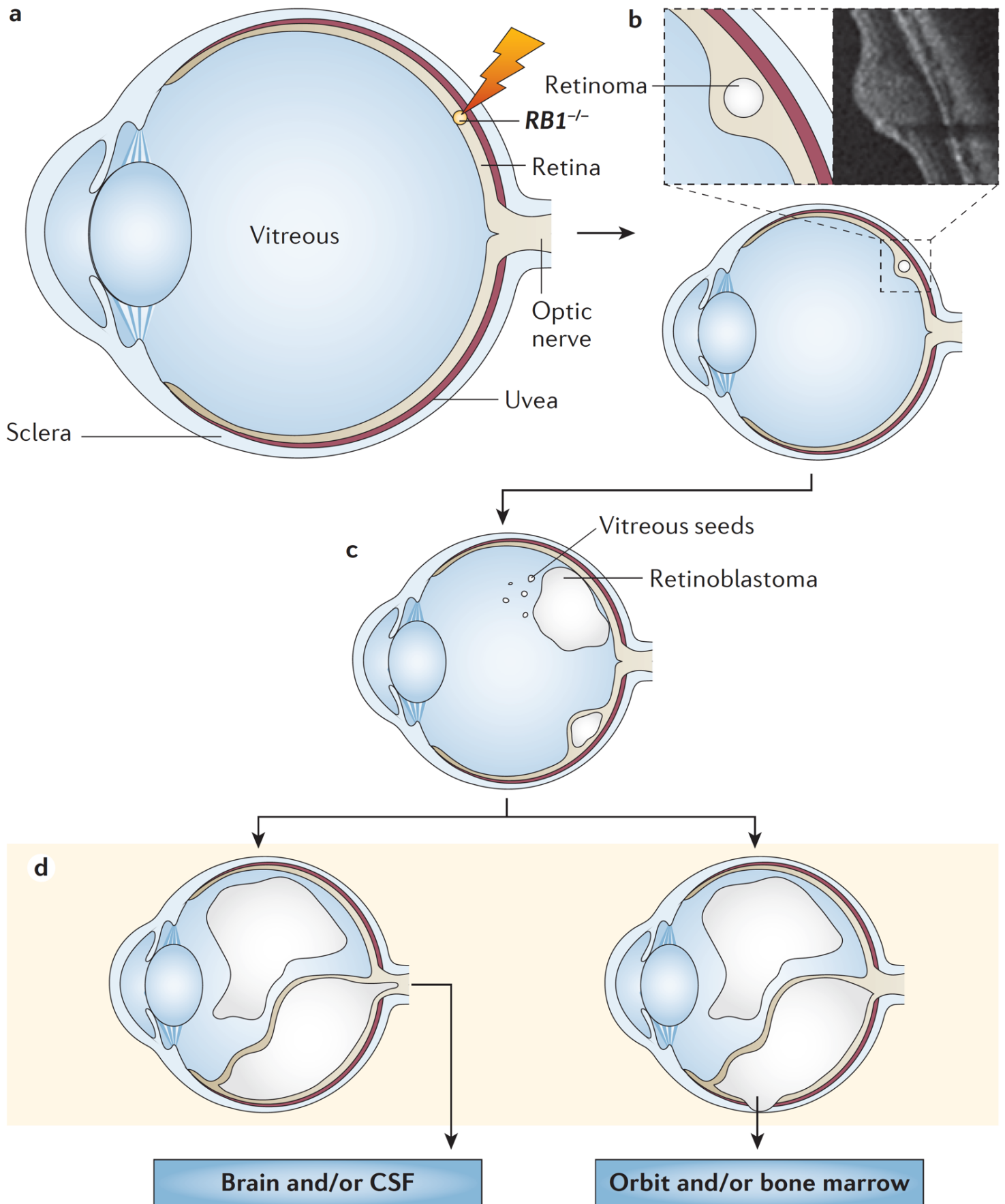


Figure 2

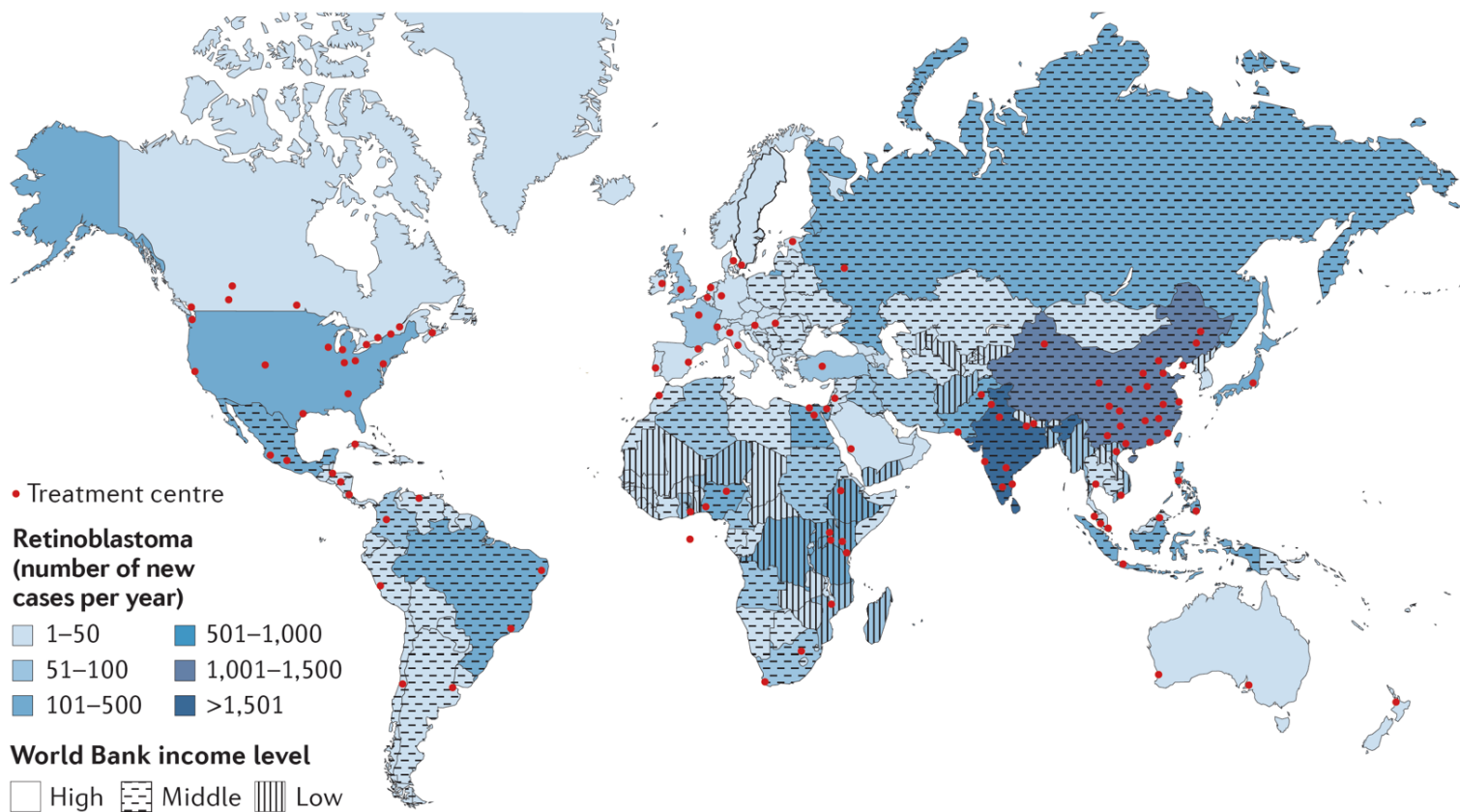


Figure 3

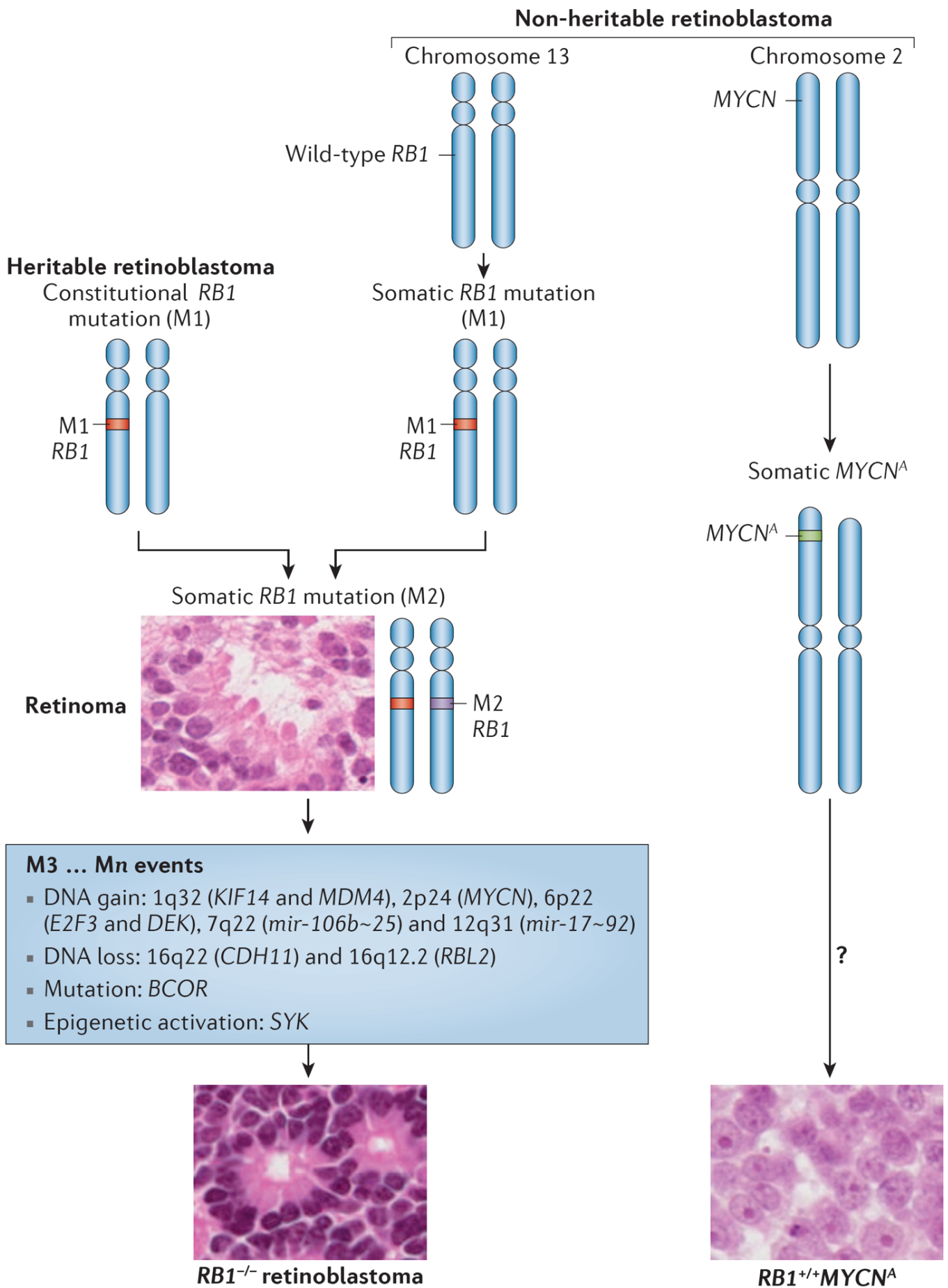


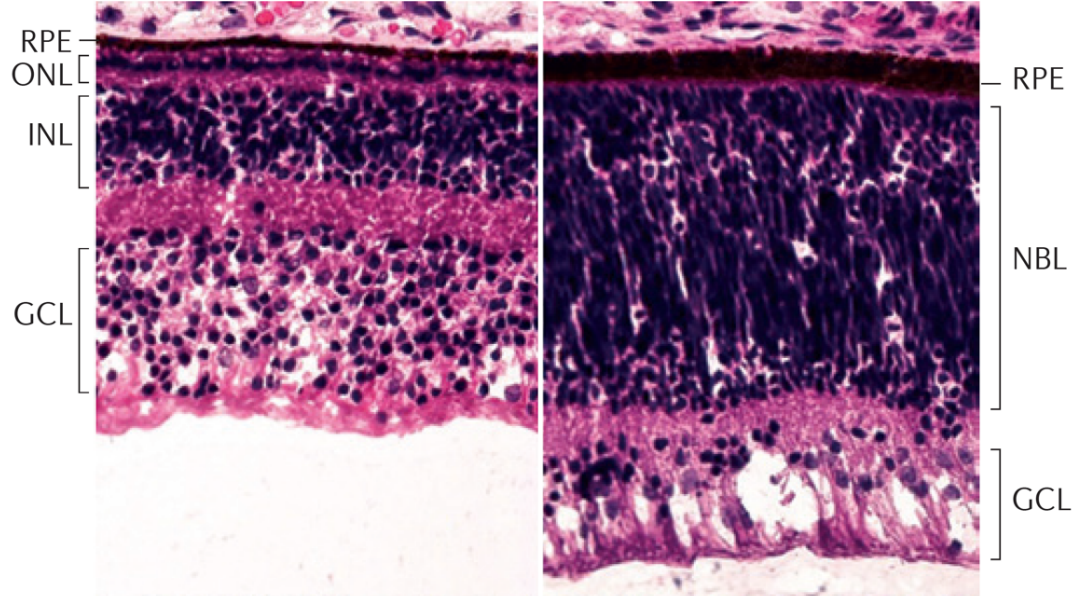
Figure 4

a

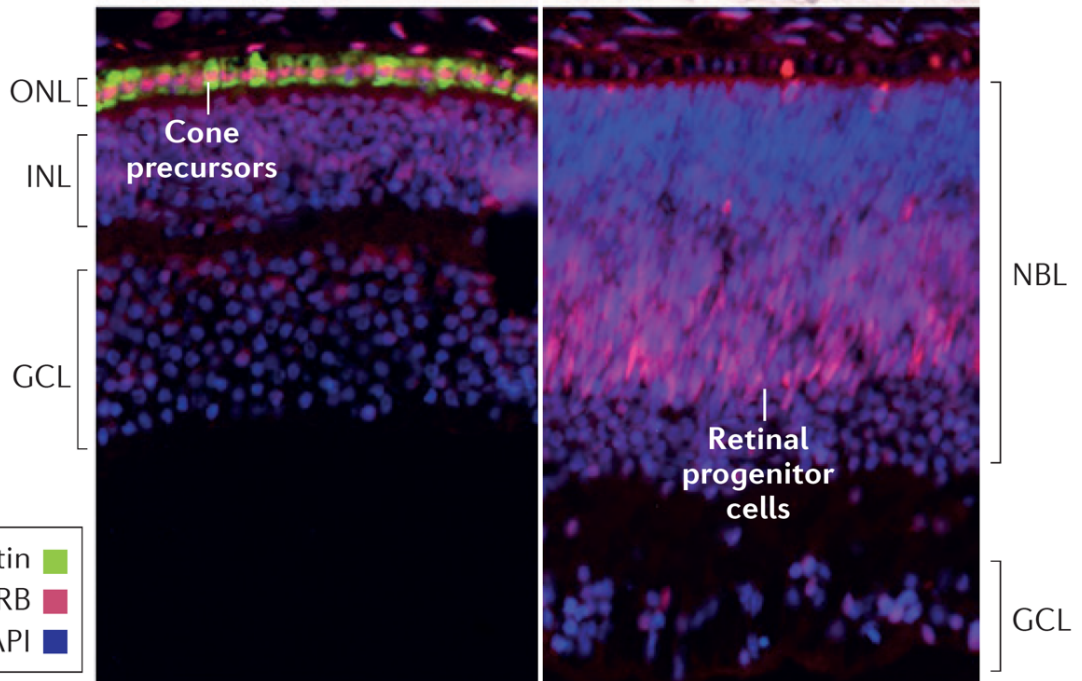
Embryonic retina

Central retina

Peripheral retina



b



c

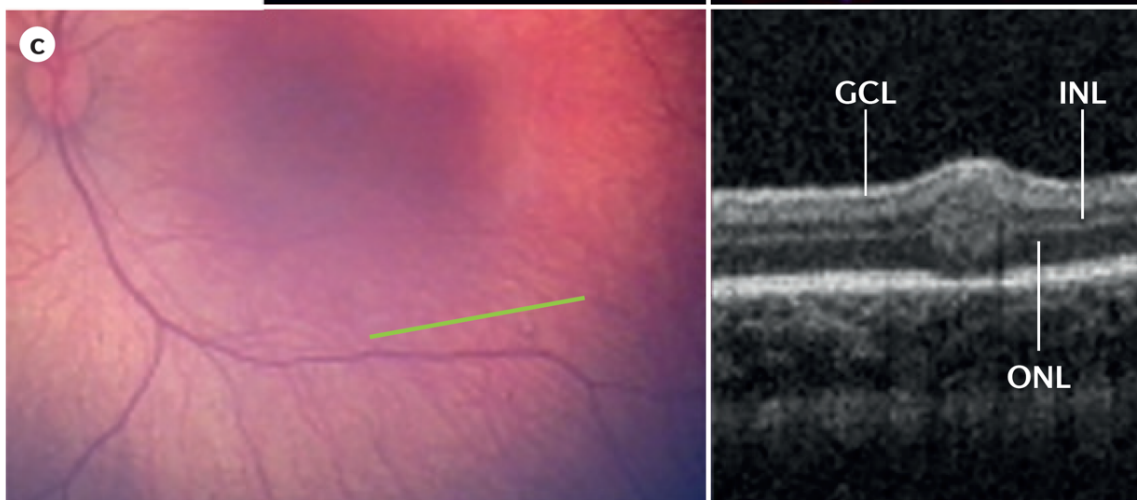
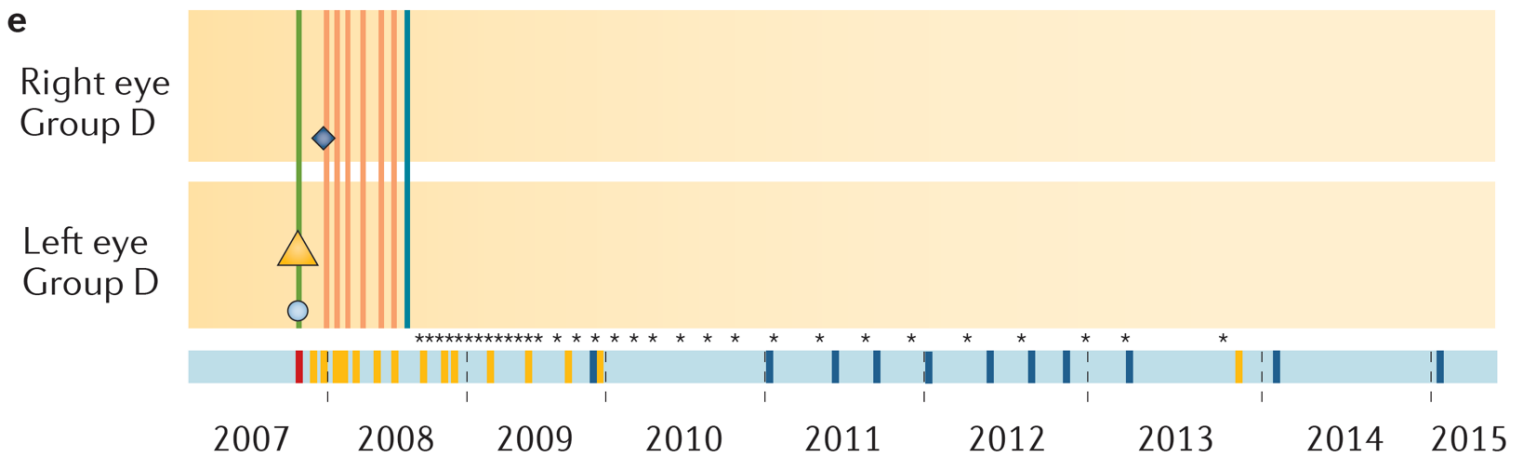
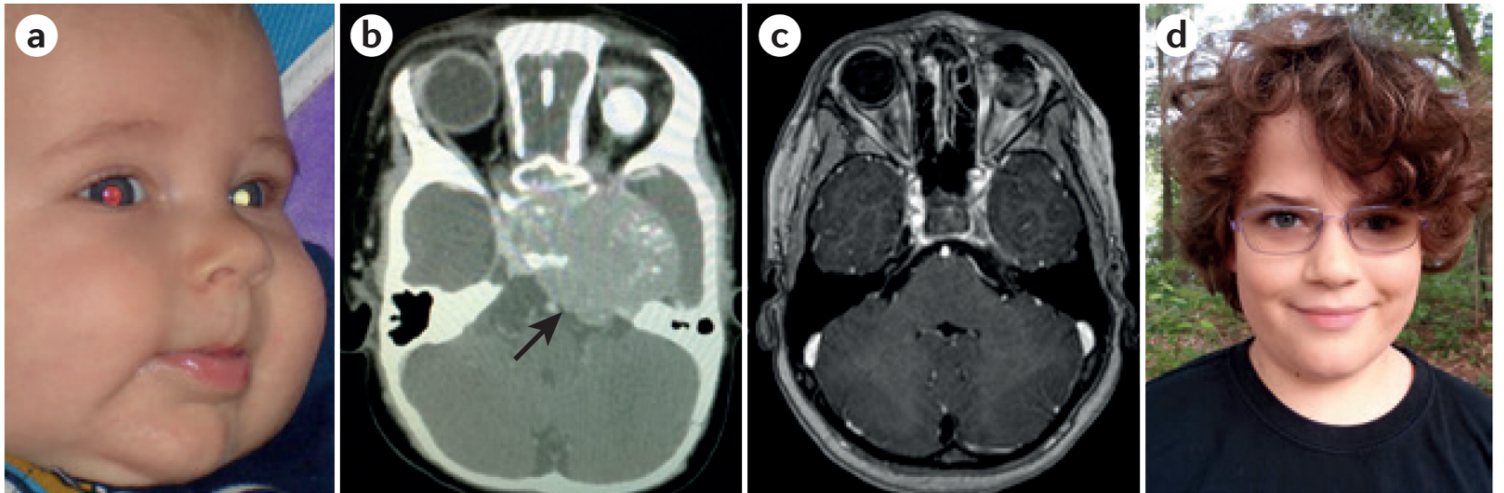




Figure 5



# Figure 6

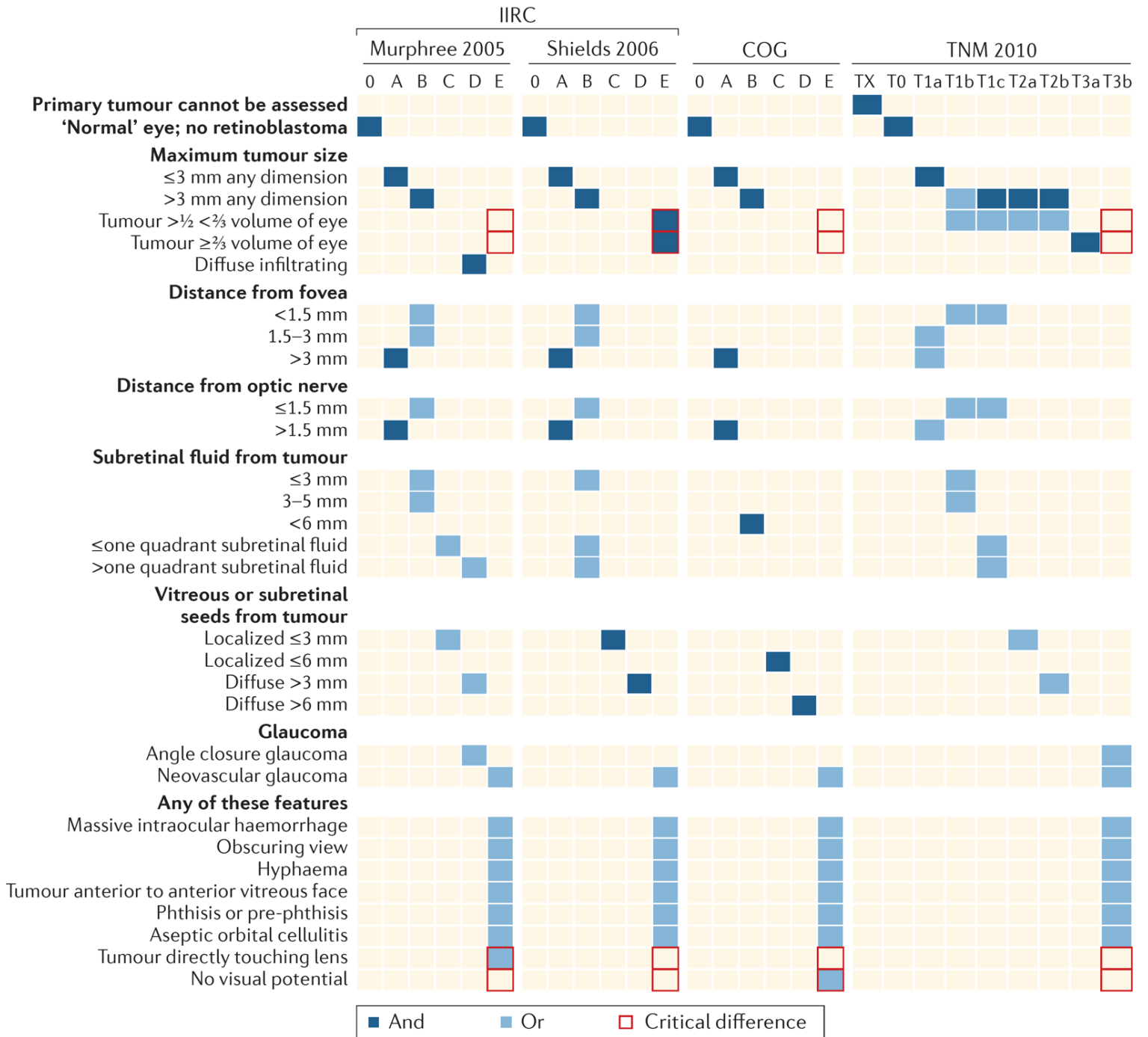


Figure 7

Eye 1	Eye 2	A	A	A,B,C	B,C	A,B,C	D	D	D	A	E	B,C	E	D	E	N	B,C	N	D	N	E	E	E	
<b>Focal</b>		A	A	A		A				A														
<b>IVC +/- focal</b>				B	B	B						B					B							
Fewer cycles				C	C	C						C					C							
More cycles							D	D	D					D					D					
<b>IAC +/- focal</b>				B	B	B						B					B							
				C	C	C	D	D	D			C					C		D					
<b>Enucleation</b>										E			E		E		D		D		E	E	E	

Figure 8

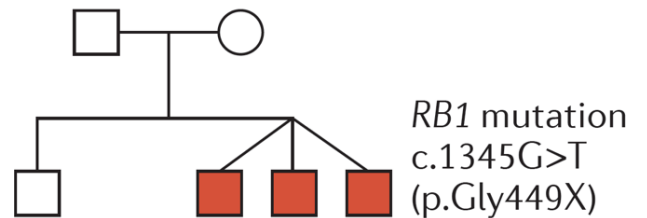
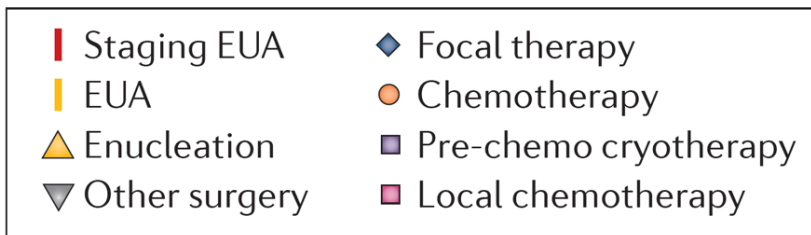
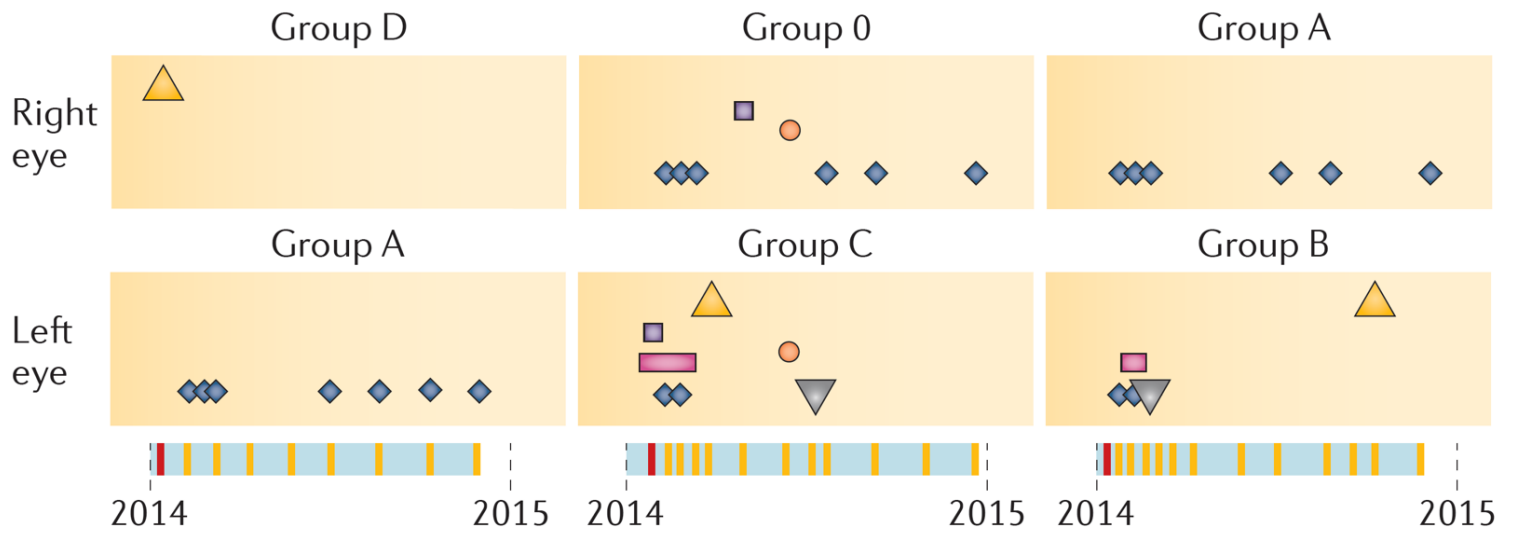


Figure 9

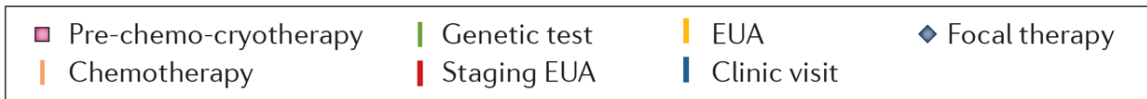
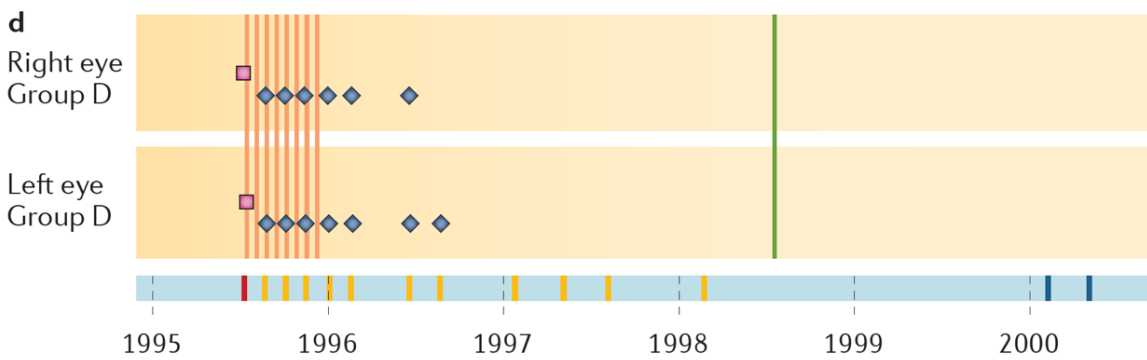
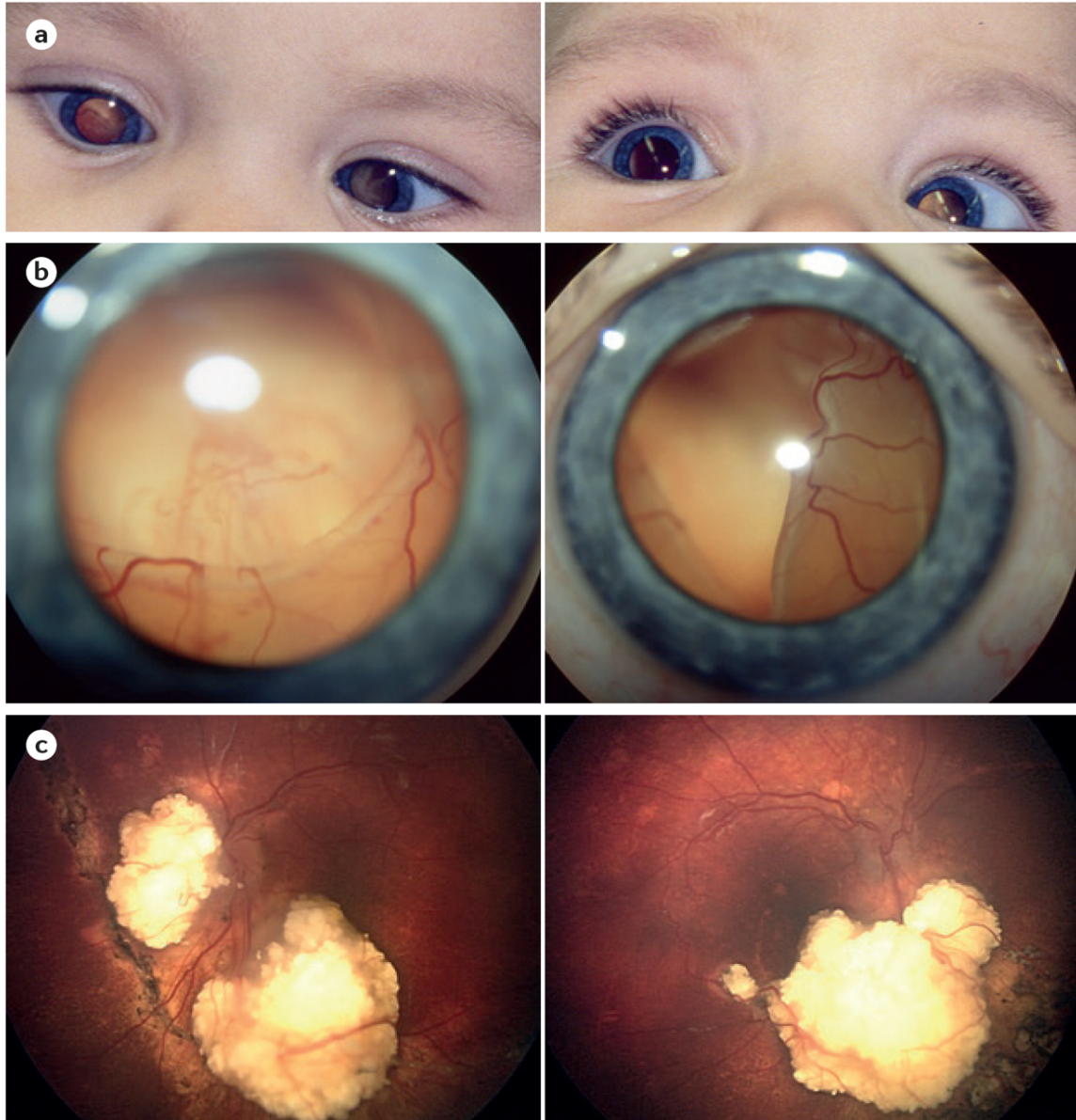


Figure 10

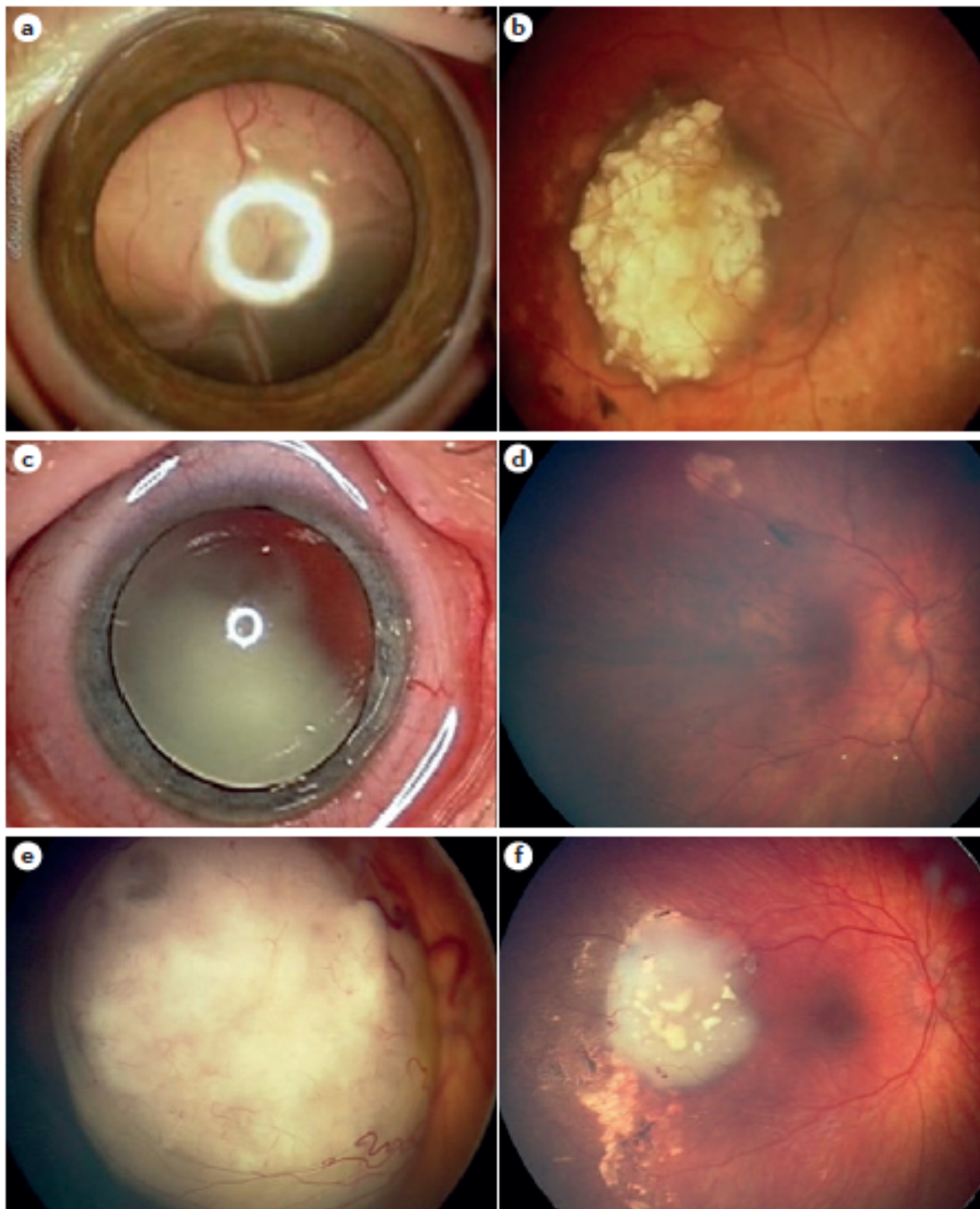


Figure 11



Figure 12

