

# Management of Retinoblastoma in Children: Current Status

Guillermo Chantada · Paula Schaiquevich

Published online: 6 March 2015  
© Springer International Publishing Switzerland 2015

**Abstract** In recent years, there have been dramatic changes in the management of intraocular retinoblastoma. Intraocular retinoblastoma is a highly curable malignancy and current treatments are aimed to preserve vision while reducing the late effects such as treatment-induced secondary malignancies. The advent of intra-arterial chemotherapy changed the treatment paradigm from systemic treatment with chemotherapy to local treatment, and new questions emerged. While intra-arterial chemotherapy achieved encouraging results, only experience from major referral centers is reported, so its indications, advantages and risks are still to be elucidated. Many factors should be considered when choosing the appropriate conservative therapy. When the disease has extended outside the eye, the chances of cure are significantly lower and treatment should be tailored by the presence of pathology risk factors such as invasion of the choroid, the optic nerve, and the sclera. Adjuvant therapy is decided upon this information. Children with overt extraocular disease are treated with higher dose neoadjuvant therapy followed by delayed enucleation and adjuvant therapy.

## Key Points

Conservative treatment of retinoblastoma changed from systemic chemotherapy to intra-arterial chemotherapy for higher risk cases in many centers of high and middle income countries.

Enucleation of the affected eye is still needed for eyes with advanced disease potentially harboring occult metastatic dissemination.

Treatment of children with pathology risk factors for metastatic disease is encouraging with the use of tailored adjuvant therapy.

Children with overt extraocular disease may be cured when the CNS is not involved.

## 1 Introduction

Retinoblastoma is a tumor presenting in young children and it is the most common neoplasm of the eye in the pediatric age group, occurring in about 1 in 17,000 live births [1]. Its incidence may not be distributed equally around the world [2]. Reports suggested an increased incidence in some less developed countries, such as Mexico and Brazil, but also among children of Native American descent in North America [3, 4]. However, other population-based studies failed to find any difference compared with Western countries [5]. Whether these geographical variations, if they really exist, are due to ethnic or socioeconomic factors is not fully understood. Many studies

---

G. Chantada (✉)  
Hematology-Oncology Service, Hospital JP Garrahan,  
Combate de los Pozos 1881, C1245AAL Buenos Aires,  
Argentina  
e-mail: gchantada@yahoo.com

P. Schaiquevich  
Clinical Pharmacokinetics Unit, Hospital JP Garrahan/  
CONICET, Combate de los Pozos 1881,  
C1245AAL Buenos Aires, Argentina

tried to identify socioeconomic features that could explain this potentially increased incidence. Reports from Mexico suggested an association of some dietary factors such as low consumption of fruits and vegetables during pregnancy in mothers of children with an increased risk of retinoblastoma [6]. There is a long debate about the role of the human papillomavirus infection as a potential factor explaining the increased prevalence of retinoblastoma in less developed settings, but this association has not been conclusively proven to date since results from different countries led to disparate estimates [7, 8]. Other studies reported an increased risk of retinoblastoma in children born after in vitro fertilization [9], but other studies failed to find a correlation between fertility treatment and higher risk for this tumor [10].

Retinoblastoma presents in two distinct clinical forms, which critically influence treatment decisions. The first is unilateral eye involvement, accounting for about 60 % of the cases, which are sporadic in about 90 % of the cases and have germline mutations of the *RBI* gene in the remaining 10 % of cases; these children are usually diagnosed after the first year of life. The second form is bilateral or multifocal, and hereditary, accounting for the remaining 40 % of cases. This form is usually diagnosed earlier in life, frequently during the first year, and it is determined by germline mutations of the *RBI* gene, usually occurring as a new mutation in children without family history for this tumor. Less frequently, it occurs as a result of inheritance of the mutation from an affected parent. Pre-implantation diagnosis of *RBI* mutations has been reported as a method to reduce familial retinoblastoma [11]. Children carrying a germline mutation of the *RBI* gene have a higher predisposition to secondary cancers later in life [12]. This phenomenon is critically important for the management of retinoblastoma, mostly in developed countries, since secondary malignancies are currently the major cause of death of these patients [13]. Treatments such as chemotherapy and especially external-beam radiotherapy (EBRT) increase this risk [14].

The most common presenting sign of retinoblastoma is leukocoria (abnormal white pupillary reflex, Fig. 1) and less commonly with strabismus which usually denotes less advanced disease [15]. These clinical findings are normally detected by the parents, in children that otherwise look normal, and have little impact on their quality of life. However, the clinical features at presentation vary according to where the patient lives, since in less developed countries the disease is usually diagnosed after progression to glaucoma, leading to buphthalmos (increased eye size) and later to tumoral invasion of the orbit which ultimately leads to severe proptosis resulting in an orbital mass and later to metastatic dissemination [16]. These children may be severely compromised in their general health.



**Fig. 1** Leukocoria in the left eye as a presenting sign of retinoblastoma

## 2 Diagnostic Evaluation

The diagnosis of intraocular retinoblastoma is usually made by an ophthalmologist through indirect ophthalmoscopy under general anesthesia. Upon the diagnostic examination, a comprehensive intraocular disease evaluation, including the documentation of the number, location, and size of retinal tumors, as well as the presence of retinal detachment and subretinal fluid and vitreous and subretinal seeds, must be performed [17]. After this evaluation, each eye is classified according to current grouping systems that are needed to estimate the ocular prognosis and to tailor treatment [18] (Table 1).

In the same anesthetic procedure, a bi-dimensional ultrasound evaluation can be performed to further measure the tumor size. Imaging studies such as head and orbit magnetic resonance imaging are needed to evaluate extraocular extension, the involvement of the pineal or suprasellar areas, to rule out trilateral disease and to help in differentiating retinoblastoma from other causes of leukocoria. Trilateral retinoblastoma refers to the development of a primary intracranial (usually pineal or suprasellar) primitive neuroectodermal tumor in a patient with the heritable form (i.e., germline) of retinoblastoma [19].

## 3 Initial Management of Retinoblastoma: Deciding Which Eyes Need to Be Enucleated

Once extraocular extension has been ruled out clinically and by imaging studies, management decisions are focused around the possibility of eye preservation. Enucleation of the affected eye is usually curative for intraocular retinoblastoma, but in bilateral cases it will naturally lead to blindness.

### 3.1 Factors That Influence the Decision of Enucleation

The decision to preserve an eye with retinoblastoma is made by considering the extent and location of intraocular

**Table 1** The original version of the International Classification for Intraocular Retinoblastoma [18]. This table was published in Ophthalmology Clinics of North America 18:41–53, viii. Intraocular

retinoblastoma: the case for a new group classification. Linn Murphree, Copyright Elsevier (2005)

Groups	Children’s Hospital of Los Angeles version
A	Eyes with small discrete tumors away from critical structures. All tumors are ≤3 mm, confined to the retina, and located at least 3 mm from the foveola and 1.5 mm from the optic nerve. No vitreous or subretinal seeding is allowed
B	Eyes with no vitreous or subretinal seeding and retinal tumors of any size or location. Retinal tumors may be of any size or location not in group A. A small cuff of subretinal fluid extending ≤5 mm from base of the tumor is allowed
C	Eyes with only focal vitreous or subretinal seeding and discrete tumors of any size or location. Any seeding must be local, fine and limited so as to be theoretically treatable with a radioactive plaque. Retinal tumors are discrete and of any size and location. Up to one quadrant subretinal fluid may be present
D	Eyes with diffuse vitreous or subretinal seeding and/or massive non-discrete endophytic or exophytic disease. Seeding more extensive than Group C. Massive and/or diffuse intraocular disseminated disease may consist of fine or ‘greasy’ vitreous seeding or avascular masses. Subretinal seeding may be plaque-like. Includes exophytic disease and >1 quadrant retinal detachment
E	Eyes that have been destroyed anatomically or functionally by the tumor. Eyes with one or more of the following. Irreversible neovascular glaucoma, massive intraocular hemorrhage, aseptic orbital cellulitis, tumor anterior to anterior vitreous face, tumor touching lens, diffuse infiltrating retinoblastoma, phthisis or pre-phthisis

disease, which helps in predicting the likelihood of visual results, the laterality of the tumor and the resources available for conservative treatment. This is a complex decision that includes not only estimations of the likelihood of preserving vision without increasing the risk of tumor dissemination, but also tailoring therapy so that treatments used cause the least impact in inducing late effects such as sensorial nerve toxicity and especially secondary malignancies usually occurring decades later [13, 20, 21] (Table 2). In addition to these factors, familial cultural preferences for non-surgical therapies play an increasingly important role. Even though, on occasion, globe salvage does not result in improved vision, and in some cases may even be associated with an increased risk of metastatic dissemination, the preference of affected families for conservative therapies critically affects the decision process. In

some countries, family acceptance of enucleation is poor and that poses a challenge for the initial management of retinoblastoma [22]. In developing countries, its initial management is also influenced by the presence of extraocular disease which is more prevalent [23].

### 3.2 Identifying Children at Risk of Occult Disseminated Disease

Extraretinal extension to the outer layers of the eye may be present in a variable proportion of children at diagnosis, reaching up to 50 % of the affected eyes at diagnosis in some developing countries [24]. Microscopic invasion to the choroid, the sclera and the optic nerve are all associated with an increased risk of extraocular relapse [25]. The clinical identification of children with these higher risk

**Table 2** Description of various risks associated with chemotherapy for the treatment of retinoblastoma

Risk	Reported incidence	References
Secondary malignancies in irradiated areas	6–17 % (30 years)	Draper et al. [21] Marees et al. [13]
Toxic mortality of chemoreduction	Depends on setting (0–1 %)	Naseripour et al. [50]
Secondary acute myeloid leukemia	1/187 Patients 3/177 Patients	Turaka et al. [20] Chantada et al. [53]
Risk of pathology risk factors		
Eyes A–C	0 %	Kaliki et al. [29]
Eyes D	17 %	
Eyes E	24 %	
Occurrence of trilateral retinoblastoma	1.5–6 % (EBRT era) 1.5 % (Chemoreduction era)	Kivela, 1999 [19] Shields et al. [57]
Occurrence of severe ototoxicity	8/175 Patients 10/60 Patients	Jehanne et al. [56] Qaddoumi et al. [55]
Occurrence of severe vascular choroid toxicity	4/95 Patients 2/13 Patients	Gobin et al. [35] Munier et al. [65]

EBRT External-beam radiotherapy

factors is challenging and they may not always be detected by imaging studies, which have relatively low sensitivity and specificity [26]. Some clinical features at presentation may be helpful to predict which patients would have pathology risk factors warranting immediate enucleation [27, 28]. In a large series from a single institution in North America, the likelihood of harboring microscopic pathology risk factors that ultimately led to metastasis in eyes with group D or less was minimal [29]. However, for children with initially enucleated group E eyes, up to 10 % of the eyes that had pathology risk factors ultimately developed metastatic disease [29]. Other studies from developing countries found similar correlations [28]. In such series, the presence of glaucoma, buphthalmos, anterior chamber invasion, and older age at diagnosis were correlated with the presence of high risk factors on pathological examination [27, 28]. However, these associations were not so evident in other smaller series from developed countries, probably because of the lower prevalence of pathology risk factors which reduced the statistical power of the analysis [30]. Since the likelihood of extraocular relapse is directly influenced by the presence of pathology risk factors, identifying these children is important if conservative therapy is important. Minimally disseminated disease, already present at diagnosis in these higher risk patients, may lead to extraocular relapse if no treatment is given [31]. However, it is currently difficult to identify, clinically or on histopathology, those who will develop extraocular relapse if adjuvant therapy is not given since only a proportion of children with pathology risk factors will ultimately develop metastatic disease if conservative therapy is undertaken with treatments other than systemic chemotherapy. Systemic chemotherapy used for conservative therapy may reduce, but probably not completely eliminate the occurrence of metastatic relapse by treating minimally disseminated disease. From historical data, when EBRT was the predominant conservative therapy for retinoblastoma, prophylactic chemotherapy was never used to prevent occult distant dissemination in preserved eyes [32]. The occurrence of extraocular relapse in those patients was, nevertheless, very low.

### 3.3 Enucleation Versus Conservative Therapy in Eyes with Advanced Disease

In recent years, with the availability of newer local treatment modalities other than systemic chemotherapy, patients with more advanced disease are being offered globe salvage treatment. Since these advanced eyes are more likely to harbor minimally disseminated disease, some authors expressed their concerns about the potentially increased risk of metastatic dissemination [33]. This discussion concerns the use of intra-arterial chemotherapy

(IAC), also known as chemosurgery, which has recently become a popular conservative therapy. However, to date, death from metastatic disease was reported in <5 % of patients treated with IAC, which is comparable to death rates reported with other therapies in high-risk children [29, 34–36]. However, in most cases of unilateral disease, initial enucleation is the treatment preferred by most groups, especially when extraretinal disease is more likely; for example, in those group E eyes with glaucoma and invasion of the anterior chamber or rubeosis iridis [37, 38]. Eyes with less advanced disease such as those with groups A to C are treated with a conservative approach by most centers around the world [38, 39]. The most difficult decision is related to conservative therapy of group D eyes, which include those eyes with extensive retinal involvement, usually with retinal detachment and/or vitreous or subretinal seeding. Enucleation is not mandatory for these eyes because they do not present clinical risk factors associated with increased risk of occult metastatic disease; but, on the other hand, the resulting vision would be minimal. In these cases, the status of the fellow eye is a factor to consider when deciding treatment. The availability of treatment is also critical for the decision to preserve an eye with advanced disease. It is anticipated that these children would require multiple rounds of local therapy, occasionally brachytherapy with radioactive plaques, and probably more than one line of chemotherapy, usually including treatment modalities that are not available in every center. Enucleation is recommended by some groups, especially for children with unilateral disease because the visual potential would not be much different. In addition, enucleation makes it possible to perform a full pathological examination of the enucleated eye, ruling out the rare occurrence of pathology risk factors that would put the patient at risk of occult metastatic disease [40]. However, other groups would consider conservative therapy for these eyes, even in unilateral cases (summarized in Table 3) [39]. In these cases, no obvious visual functional advantage would be added, but the cosmetics and psychological adjustment may be better compared with those enucleated. Children who were treated only with enucleation for retinoblastoma presented declining cognitive and adaptive skills over time and a lower chance of finding jobs and partners in adulthood [41, 42]. The risk of presenting pathology risk factors in group D eyes is lower than 20 % and these are frequently choroidal invasion or intralaminar optic nerve invasion [43], which lead to <5 % of extraocular relapse [44]. In addition, extraocular relapse may occur in children with enucleated eyes that show no pathology risk factors or in enucleated eyes with such factors that were given adjuvant therapy, so the actual risk of metastatic relapse in preserved eyes of group D disease that did not receive systemic treatment compared with

**Table 3** Schematic comparison of treatment modalities for unilateral retinoblastoma. Eyes with glaucoma, buphthalmos and/or anterior chamber invasion are treated by enucleation [35, 38, 52]

	Enucleation	Systemic chemotherapy	Intra-arterial chemotherapy
Availability	Always	High	Limited
Inherent potentially severe acute toxicity	Related to general anesthesia	Infection Transfusion Secondary leukemia Central line	Procedure related Occult disseminated disease Stroke
Long-term quality-of-life related risks	Cosmetic	Ototoxicity	Ocular toxicity
Major weakness	Cosmetic sequelae	Poor results in advanced eyes Acute and long-term sequelae	Potentially missing occult tumor dissemination
Major advantage	Safety	Availability	Cosmetic

those initially enucleated in whom adjuvant therapy is tailored according to the pathology has not been estimated. In many developing countries, enucleation may not be initially accepted by some families [22, 45]. This problem accounts for at least half of the deaths in those settings, so some clinicians use pre-enucleation chemotherapy in order to bide time and prevent abandonment while efforts are made to persuade the families to accept enucleation [46]. However, when this approach is undertaken, timely enucleation should be done since prolonged administration of chemotherapy in eyes that should have been initially enucleated may lead to tumor progression after initial response, followed by extraocular dissemination and death [47]. Nevertheless, in countries with limited resources, initial enucleation of group D and E eyes with retinoblastoma should be considered as a life-saving procedure and strategies to improve the acceptance of affected families to this surgical procedure should be available to decrease treatment refusal. In these settings, investing in early diagnosis, pathology services, prostheses, and good focal therapy may probably be a priority.

#### 4 Deciding the Chemotherapy Strategy for Conservative Therapy

##### 4.1 Systemic Chemotherapy

Except for selected cases with very limited disease, categorized as group A eyes (usually detected by family screening), which may be treated solely with focal treatments such as laser or cryotherapy only, most other cases would need an initial period of tumor reduction with chemotherapy. The role of chemoreduction is to decrease tumor size and make the tumors suitable for subsequent local therapy. Local therapy is influenced by many factors such

as quality of the equipment and skill of the treating group, all of which are critical for the success of this strategy. In the mid-1990s, systemic chemoreduction with carboplatin-based regimens, usually in combination with vincristine and etoposide, were used extensively by most groups in many parts of the world [48]. With this treatment, most intraocular tumors usually show dramatic shrinkage allowing for consolidation with local treatment after a number of cycles (usually less than six). This treatment was successful in avoiding EBRT as conservative therapy in more than 90 % of those with eyes in the less advanced groups A to C [49]. However, even though the toxicity profile of systemic chemoreduction clearly favors that of EBRT, changing the paradigm from a globe-localized treatment devoid of systemic toxicity such as EBRT to a systemic treatment highlighted the chemotherapy toxicity. Despite the vast majority of patients not presenting any significant systemic long-term side effects associated with chemotherapy, severe toxicities were reported in a minority of patients [50]. The toxicities observed were similar to other pediatric tumors, but in the case of tumor chemoreduction for retinoblastoma, they should be considered from a different perspective since disease-free survival is 100 %, so chemoreduction is used only to reduce long-term toxicity. Hence, what may be an acceptable toxicity for a life-threatening tumor may be unacceptable for retinoblastoma. There have been reports about deaths related to systemic chemotherapy used for chemoreduction [50], but reports on toxicity with this and other therapies such as IAC are scant [51, 52]. The causes of mortality in children receiving chemoreduction for intraocular retinoblastoma included toxicity of the chemotherapy used [50] and secondary malignancies such as etoposide-related acute myeloblastic leukemia [53, 54]. Other non-fatal, but potentially severe long-term toxicities of systemic chemotherapy included carboplatin-induced ototoxicity [55, 56]. An initial report,



based on a retrospective cohort analysis, suggested that systemic carboplatin-based chemotherapy might prevent the occurrence of trilateral retinoblastoma [57]. However, other studies with a similar design could not confirm this observation [53]. These estimations are summarized in Table 2. Alternatives to conventional three-drug (carboplatin-etoposide-vincristine) chemoreduction include the addition of intravenous topotecan and periocular carboplatin as reported from St Jude Children's Research Hospital in the US, which showed very good results in children with advanced tumors [58], or synchronized thermochemotherapy as pioneered by the Institut Curie in France [59]. The Hospital for Sick Children in Toronto, Canada added cyclosporine to the three-drug regimen in an attempt to maximize its effect by blocking multidrug resistance proteins [60]. Their comparison with standard regimens has not been published thus far.

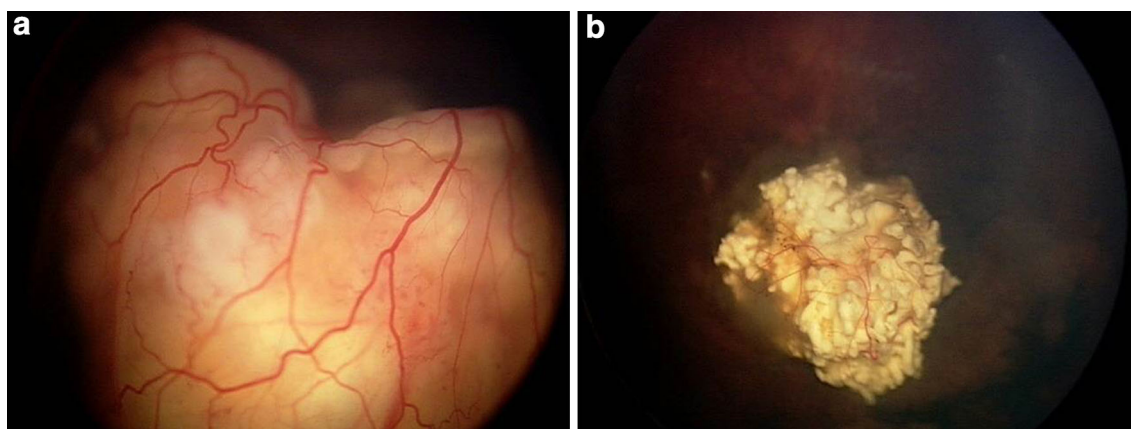
For patients with advanced intraocular tumors (group D), especially those with vitreous seeds, ocular salvage rates were still not satisfactory with systemic chemoreduction [53, 61]. In many cases, additional EBRT was required. A sizable proportion of these eyes were ultimately enucleated after failing both therapies, which results in a high exposure to mutagenic agents for such children, who usually receive multiple cycles of chemotherapy and EBRT. The addition of different treatment modalities such as periocular administration of carboplatin [38] or topotecan did not improve results in this population [53].

#### 4.2 Intra-Arterial Chemotherapy

IAC via superselective catheterization of the ophthalmic artery through a catheter inserted through the iliac artery has been pioneered by the New York group refining a treatment strategy originally developed in Japan [62, 63]. The levels of chemotherapy in the eyes after IAC are significantly

higher than those of systemic chemotherapy, with significantly lower systemic exposure as reported in preclinical work [64]. This improved ocular pharmacokinetics also came with a predictable increased ocular toxicity that would occasionally cause severe vascular damage to the choroid [65]. Other concerns about the use of this treatment are related to the exposure to ionizing radiation during the procedure; however, it was reported to be at safe levels if a limited number of applications are performed by an experienced group [66]. Another drawback is related to the lack of protection against occult metastatic and trilateral disease, but the evidence supporting this fact is limited when patients are appropriately selected for this treatment, as discussed above. Even though it was not reported in the literature, CNS stroke is one of the most serious potential complications related to IAC, however no case has been reported so far. Patients with thrombophilia may be at higher risk for this complication, so IAC is not recommended [67].

IAC yielded the best results in advanced tumors reported to date according to results in large referral centers (Fig. 2); however, long-term follow-up is necessary [34, 35, 68, 69]. The chemotherapy agents used for this treatment originally included melphalan, as pioneered by Japanese investigators, but use of other agents such as topotecan and carboplatin soon became common [35, 70, 71]. Following these outstanding results, many patients with unilateral retinoblastoma and group D disease are currently offered IAC by many groups [38, 72]. However, it should be noted that a high degree of expertise including the availability of a special interventional radiology and qualified personnel and a trained pharmacy facility are needed for the successful administration of IAC. Traditionally, initial enucleation was used in these children, however, there is still controversy about this practice and there is no unanimous opinion or randomized studies supporting this recommendation [37].



**Fig. 2** **a** Extensive intraocular retinoblastoma at diagnosis. **b** The same tumor after two cycles of intra-arterial chemotherapy (photographs courtesy of Dr. David H. Abramson)

### 4.3 Current Practice

Currently, most groups offer systemic chemoreduction for group A–C disease [38] and IAC is used as secondary treatment for relapsed disease to avoid EBRT. Other groups would offer upfront IAC based on its lower systemic toxicity profile [73], but its ocular side effects may counterbalance this improved toxicity profile, especially in less advanced disease where visual outcome is usually excellent. In group D eyes, many groups are moving to upfront IAC [35, 37], especially for unilateral disease where systemic chemoreduction led to disappointing results. Others will still favor enucleation, except in those cases with bilateral involvement [37]. For eyes with group E disease, most groups would offer initial enucleation because the risk of occult metastatic disease is relatively higher [37]. This is especially true in less developed countries where pathology risk factors are more prevalent [74]. However, despite its widespread use, IAC has not yet been tested in formal prospective randomized trials.

## 5 Second Line Chemotherapy for Preserving Eyes with Relapsed–Refractory Retinoblastoma

After treatment with chemoreduction (systemic or intra-arterial), previously treated tumors or vitreous or subretinal seeds may relapse or progress. Less frequently, new tumors appear, occasionally later, after many years of follow-up. Since these patients are usually at very close follow-up, these recurrences are detected early and frequently no further chemoreduction is needed for tumor control. Treatment should be highly individualized. However, especially in the case of vitreous and subretinal seeding which are usually not amenable to focal treatment alone, further chemotherapy and occasionally EBRT may be needed [75]. The decision to give further conservative therapy after failure of first-line chemoreduction and focal therapy depends on many factors, but mostly on the status of the contralateral eye and the visual potential. Delaying enucleation after long attempts with salvage therapies has been associated with an increased risk of tumor dissemination [76]. However, in certain situations, especially when a low number of cycles were used for initial chemoreduction and if IAC is not available, second-line systemic chemoreduction may be considered [77]. There is no recommended standard regimen in this situation and the same carboplatin-etoposide-vincristine may be used (limiting the cumulative dose of etoposide) as well as other regimens including topotecan, another active drug [58]. In centers where IAC is available and it was not used for prior therapy, it may be an alternative especially for subretinal seeding or treatment of relapsed retinal tumors not

amenable to focal therapy [77]. In a retrospective analysis, it was significantly more effective than periocular and sequential systemic chemotherapy [77]. In cases where vitreous seeding predominates, recent experience favors the use of intravitreal injection of chemotherapy [78]. This treatment, despite it being initially described decades ago, gained renewed interest thanks to an improved administration schedule minimizing its risk of orbital tumoral seeding [79]. The most commonly used agent is melphalan, but thiotepea [80, 81] and more recently, topotecan [82] have also been used. Retinal toxicity is the limiting toxicity in these usually heavily pretreated eyes [83]. Even though the results of this treatment have been encouraging, many questions regarding the toxicity, dose, and schedule of melphalan are yet to be elucidated. EBRT may also be used as rescue therapy for chemotherapy refractory cases [75].

## 6 Which Patients Have a Higher Risk of Extraocular Relapse?

After enucleation, a thorough pathological examination should be done including a careful evaluation of structures of the eye that are critical to predict extraocular extension. Retinoblastoma tends to invade the optic nerve from where it may find its way into the CNS. It also frequently invades the choroid and after filling its whole width, it may also invade the sclera on its way to the orbit. It can also give rise to systemic metastasis after gaining access to the choroidal circulation or other yet unidentified mechanisms since distant metastases were also reported in patients without choroidal invasion. However, it is important to note the limitations of assessing the risk of metastasis entirely by histological risk factors since only a limited portion of the eyeball can be effectively evaluated; therefore choroidal invasion may be missed through insufficient sampling, even by expert and comprehensive evaluations [84]. The most common metastatic sites of retinoblastoma include the CNS, bones and the bone marrow [85]. Occasionally, it can metastasize to other organs such as the liver or distant lymph nodes. Thus, in eyes showing invasion to the choroid, the optic nerve, or the sclera, most groups use adjuvant therapy to reduce the risk of extraocular relapse [25]. Other pathological features such as invasion to the anterior segment, tumor differentiation or others were not conclusively found to be associated with a significantly higher risk by many groups [86, 87], although they are used by other groups for tailoring adjuvant therapy [87, 88]. Despite there being general agreement that invasion to the choroid, optic nerve, and sclera are risk factors for extraocular relapse, there is still some discussion about the inclusion of different subgroups in the higher-risk population [25]. On occasions, there is a microscopic

residue after enucleation, either when the tumor extends beyond the resection margin of the optic nerve or when it extends to the orbit through the sclera [84]. Such cases should be treated with intensive adjuvant therapy in order to prevent relapse [89].

### 6.1 Uveal Invasion

The extent of uveal invasion is important to assess the risk for extraocular relapse [44, 84]. After a worldwide consensus, massive choroidal invasion has been defined as that greater than 3 mm in any dimension [84]. Less than massive choroidal invasion has not been significantly associated with an increased risk of extraocular relapse [90, 91]. Children initially enucleated whose eyes showed massive choroidal invasion have an estimated 6 % risk of extraocular relapse. Even though they have a significantly lower probability of event-free survival (94.2 %) compared with those with focal invasion (99.2 %) ( $p = 0.04$ ), there was no significant difference in survival (98.7 vs. 99.2 %, respectively;  $p = 0.29$ ) because some patients with an extraocular relapse may be cured with second-line therapy [44]. Hence, some groups recommend adjuvant therapy to further reduce the occurrence of extraocular relapse [88, 92], and others recommend only observation if high-dose therapy is available to rescue those who relapse [93]. So, if all these children are given adjuvant therapy, about 95 % of them would be exposed to unnecessary chemotherapy in order to potentially prevent extraocular relapse in the remaining 5 % [44]. Salvage therapy for extraocular relapse is highly intensive and, though effective in more than 50 % of the cases, it is associated with a high frequency of severe late effects [29, 94]. Therefore, the use of adjuvant therapy should consider other factors including the availability of effective salvage therapy, adequate follow up and reliability of the pathological examination as well as supportive care during the course of chemotherapy.

### 6.2 Optic Nerve Invasion

When optic nerve invasion is considered as a risk factor for extraocular relapse, there is general agreement that those with prelaminar involvement are not at a significantly higher risk [25, 95]. However, when the tumor extends beyond the lamina cribrosa (which is technically considered a microscopically extraocular extension), the risk of extraocular relapse is higher and adjuvant chemotherapy has been recommended by most groups [88, 92]. However, the exact risk for extraocular relapse is not known since relatively few patients were treated with observation alone. There is some evidence to suggest that the risk of extraocular relapse may be additive when concomitant massive choroidal invasion or scleral compromise

is present [25]. However, even in these higher risk populations, >95 % of the children survive with adequate therapy [88, 96, 97]. Those with microscopic, completely resected scleral invasion usually present with other risk factors, and most groups agree that adjuvant therapy is needed [98]. A study reported that the extraocular relapse rate may be significantly lower when higher dose adjuvant therapies are used [98]. However, there is no agreement on this topic, so some groups report encouraging results using standard doses of carboplatin-based regimens and others propose that higher intensity regimens, usually also including alkylating agents, may yield better results in higher risk populations [98, 99]. The major limitation of most studies is the low number of patients included, which make it difficult to estimate the efficacy of an adjuvant regimen in cohorts with relatively low risk of extraocular relapse.

### 6.3 Other Pathology Risk Factors

In addition to massive choroidal, scleral, and post-laminar optic nerve extension, some groups recommend adjuvant chemotherapy to other children with putative higher risk factors such as anterior chamber invasion or combination of prelaminar optic nerve and focal choroidal invasion [92]. However, the benefit of this approach is difficult to determine because their risk for extraocular relapse is lower than 3 % without therapy other than enucleation [90]. Most of the available evidence about the role of pathology risk factors as predictors of extraocular relapse is based upon initially enucleated cases, so their impact in cases that have been secondarily enucleated after chemoreduction is not known. Most groups follow the same guidelines for the indication of adjuvant therapy [25]. However, because of the need to limit high cumulative doses of carboplatin and etoposide, on occasions the use of alternative regimens using topotecan or alkylating agents to reduce long-term toxicity is recommended [76].

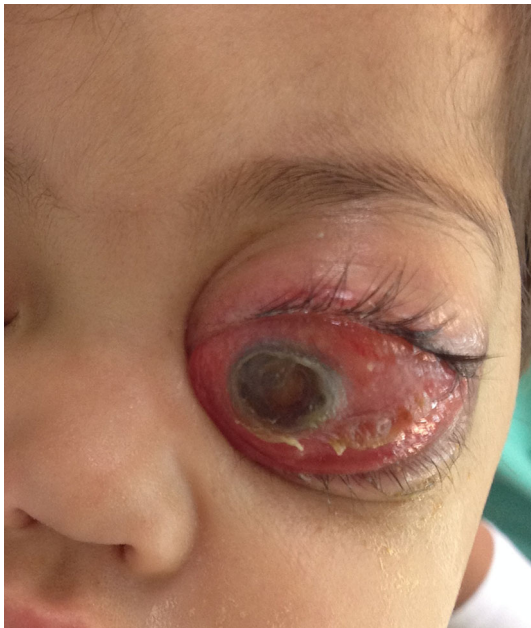
## 7 Management of Patients Presenting Overt Extraocular Disease

Patients with overt extraocular dissemination are seen more frequently in developing countries and virtually never seen in developed countries (Fig. 3) [23]. Delayed diagnosis is probably the reason for this discrepancy [23].

### 7.1 Orbital Retinoblastoma

Orbital extension is usually evident on physical examination of the patient but occasionally it may be recognized only by imaging studies [100]. With newer and more sensitive imaging modalities, more subtle orbital invasion,





**Fig. 3** Massive orbital dissemination as a presenting sign in retinoblastoma

especially to the optic nerve or to the sclera, may be more accurately detected making it difficult to categorize these patients as with orbital extension [101].

The standard treatment for retinoblastoma with regionally disseminated disease includes preoperative chemotherapy followed by resection of any residual orbital mass and adjuvant chemotherapy and radiotherapy [102, 103]. Extensive and mutilating surgical procedures such as orbital exenteration should be avoided since the orbital masses usually regress after neoadjuvant chemotherapy. About two thirds of patients with orbital retinoblastoma may be cured with this approach, however, those presenting with massive optic nerve enlargement still do poorly and more intensive therapies may be necessary [104].

## 7.2 Metastatic Retinoblastoma

When distant or CNS metastasis are present, the prognosis is not so favorable. In fact, until the use of high-dose chemotherapy and autologous stem cell rescue (ASCR) for the treatment of these children became available, virtually none survived [85]. According to reported series including a limited patient number, consolidation with high-dose chemotherapy and ASCR provides a chance for cure to about 60–70 % for stage 4a patients (metastatic disease outside the CNS) [105], even in middle-income countries with lesser resources [106]. However, even with this treatment, those with invasion to the CNS (stage 4b disease) have a dismal prognosis [107].

There are yet unresolved controversies in the management of children with metastatic retinoblastoma with high-

dose chemotherapy and ASCR. The role of thiotepa in the preparative regimens as opposed to other protocols with less toxic agents is not well defined. The need for post-ASCR radiotherapy to involved bulky sites, especially the CNS, is currently not determined. Recent studies showed that minimal disease is present in the cerebrospinal fluid (CSF) in children with high risk of CSF relapse, such as those with massive optic nerve involvement [31]. The use of intrathecal or intraventricular chemotherapy using new agents such as topotecan or radioimmunotherapy agents including radio-labelled GD2 may be considered for these patients [108].

## 7.3 Patients with Impending Extraocular Dissemination

An additional higher-risk patient cohort includes those with severe buphthalmos caused by glaucoma, but without conclusive imaging features of extraocular disease [53, 109]. In this particular population, enucleation is mandatory for tumor control and most of these patients will show pathology risk factors [28]. However, enucleation may be difficult in these patients, even in experienced hands. These severely swollen eyeballs may make it difficult for the surgeon to obtain an adequate optic nerve stump and occasionally a tumoral residue may be left [28], which would make intensive therapy including orbital radiotherapy necessary for treatment. Additionally, these eyeballs are more susceptible to perforation which would also require intensive therapy, including radiotherapy as recommended for cases with prior intraocular surgery [110]. Recent evidence suggests that some of these children present minimally disseminated disease in the CSF which would increase their risk of CSF relapse [31]. Hence, extrapolating the encouraging experience with the use of neoadjuvant chemotherapy for overt orbital disease, some groups introduced this treatment for these children with the aim of facilitating secondary enucleation after chemotherapy induces tumor regression following a limited number of cycles [109]. In addition, in children initially treated with chemotherapy, it may be introduced earlier compared with those initially enucleated who would need to wait some weeks to recover from the procedure [53]. This may provide a prompt treatment of minimally disseminated disease. However, with this treatment, risk estimation based upon pathology would be limited by the fact that pre-enucleation chemotherapy causes tumor regression, which occasionally may even be complete, and invasion to critical coats may go undetected [111]. Hence, this treatment would aim at reducing the incidence of globe perforation and incomplete tumor resection in the optic nerve. So, the groups currently using this strategy recommend planned enucleation after two or three chemotherapy cycles using

agents in doses capable of obtaining a good penetration to the CNS and continuing adjuvant chemotherapy after enucleation regardless of the pathology findings [53]. In cases where invasion to the resection margin is evident (even if cells look necrotic), orbital radiotherapy should be considered. Other groups are not using this strategy with the rationale that not all of these patients actually have pathology risk factors needing adjuvant therapy which may be better tailored by initial enucleation and pathology examination [111] in an untreated eye and that neoadjuvant chemotherapy and secondary enucleation may cause tumor progression.

## 8 Investigational Therapies

Even though many translational research projects including preclinical models have been developed for retinoblastoma treatment, their contribution to current patient management is relatively limited. Topotecan and carboplatin are probably the drugs that were evaluated more thoroughly in preclinical models and their ocular pharmacology has been characterized in detail [112, 113]. More recently, melphalan ocular pharmacology was fully characterized [64]; however, most of the studies were done after it was introduced for patient use. For IAC, the pharmacokinetic assessment of the ocular and systemic disposition of intra-arterial melphalan and topotecan was carried out in non-tumor-bearing swine [114] and primate [115, 116] models due to the size of the animal and the size of the ophthalmic artery that technically allowed for catheterization. Despite being the best possible animal models to perform the studies on, the anatomical and physiological differences between the species only provide estimative data. Also, it has to be acknowledged that there are no tumor-bearing animal models whose size allows for super-selective infusion into the ophthalmic artery.

Besides the characterization of the ocular pharmacology and in vitro and in vivo antitumor activity of conventional drugs, preclinical models may play a critical role in finding new treatment strategies for retinoblastoma in two additional fields [117]. One is the development of improved delivery systems to the vitreous in order to treat vitreous seeding in a more effective way. Devices for sustained-release preparations for periocular and intravitreal routes were evaluated [118]. One of them, embedding chemotherapy to fibrin sealant to increase the dose and achieve a longer exposure, made its way to the clinic [119, 120]. However, many interesting targeted therapies such as anti-angiogenic [121] or hypoxia-targeting [122] agents, for example, have not yet progressed further from animal models. Additionally, preclinical work is critical to identify new targets based on molecular mechanisms for

tumorigenesis in retinoblastoma. The earliest experience in this field was reported for nutlins [123–125]. Nutlins, and specifically nutlin-3, are selective inhibitors of the *p53*–*MDM2* interaction where *MDM2* is a negative regulator of *p53*. Thus, by targeting the results of the extra copies of the *MDM2* gene in retinoblastoma cells, nutlin-3 could induce cell death mediated by *p53*. Nutlin-3 showed promising activity in combination with topotecan in retinoblastoma cell lines and tumor-bearing animals [123]. However, because of pharmacological and ocular bioavailability limitations, this drug was not explored further. More recently, spleen tyrosine kinase (*SYK*), another new target identified by sequencing the whole genome and the epigenome of retinoblastoma tumors, became of interest for further clinical use [126]. Specifically, *SYK* is a proto-oncogene required for retinoblastoma cell survival and evidenced to be upregulated in retinoblastoma samples. Taking into account that this target has already been related to other malignancies, it would be of interest to have a deeper understanding of its implications and therapeutics in retinoblastoma. *SYK* inhibitors such as fostamatinib were evaluated in transgenic and xenograft models showing an encouraging activity, but limitations in its ocular pharmacology made it difficult to translate to the clinic [127]. The recent identification of *MYCN* as a driver for retinoblastoma tumorigenesis in cases with no *RBI* gene mutation also provides an opportunity for targeted therapy [128]. All these developments highlight that for a new agent to become incorporated in the clinics, issues like ocular pharmacology including pharmacokinetics and safety assessment are essential for their feasibility. Thus, defining therapeutic doses and schedules of treatment is a very sensitive task that should be performed in conjunction with different preclinical models. Limitations in animal models include the fact that transgenic mice do not entirely recapitulate the tumorigenic steps of human retinoblastoma and xenografts are created by injecting human retinoblastoma cells in the vitreous or the subretinal space. This mechanism is not the same as that of human retinoblastoma where the tumor grows from the retina to these spaces. Major changes in the blood–retinal barrier make it difficult to translate the results to the human situation.

New knowledge generated from genomic studies may provide more specific clues for assessing the risk of extraocular relapse than conventional pathology. The recent characterization of retinoblastoma subtypes with potential differences in their malignant potential may be the first step in identifying genetically higher risk populations [129]. Another aspect of recent research that is of great interest is based on the need for identification of patients with advanced disease with extraocular dissemination but before metastasis is present. As for other pediatric malignancies, the study of minimally disseminated disease by molecular

techniques may provide new information on the patterns of dissemination of retinoblastoma and their clinical implications. In this area, new data about the detection of molecular markers showed promising results for identifying these patients at high risk of metastasis [31, 130]. Finally, non-invasive imaging studies capable of recognizing viable retinoblastoma cells would also provide important information potentially affecting the management of this tumor [131].

**Acknowledgments** The authors wish to acknowledge Dr. David Abramson for his constant and unconditional intellectual support to their group. The support of the Fund for Ophthalmic Knowledge (NY, USA), the Fundacion Natali Dafne Flexer (Buenos Aires, Argentina), the CONICET (Buenos Aires, Argentina) and the Hospital JP Garrahan (Buenos Aires, Argentina) is acknowledged. G. L. Chantada and P. S. Schaiquevich have no conflicts of interest to declare. No sources of funding were used in the preparation of this manuscript.

## References

- Moll AC, Kuik DJ, Bouter LM, et al. Incidence and survival of retinoblastoma in The Netherlands: a register based study 1862–1995. *Br J Ophthalmol*. 1997;81:559–62.
- Dean M, Bendfeldt G, Lou H, et al. Increased incidence and disparity of diagnosis of retinoblastoma patients in Guatemala. *Cancer Lett*. 2014;351:59–63.
- Lanier AP, Holck P, Ehram Day G, Key C. Childhood cancer among Alaska Natives. *Pediatrics*. 2003;112:e396.
- Amozorrutia-Alegria V, Bravo-Ortiz JC, Vazquez-Viveros J, et al. Epidemiological characteristics of retinoblastoma in children attending the Mexican Social Security Institute in Mexico City, 1990–94. *Paediatr Perinat Epidemiol*. 2002;16:370–4.
- Moreno F, Sinaki B, Fandino A, Dussel V, Orellana L, Chantada G. A population-based study of retinoblastoma incidence and survival in Argentine children. *Pediatr Blood Cancer*. 2014;61:1610–5.
- Orjuela MA, Titievsky L, Liu X, et al. Fruit and vegetable intake during pregnancy and risk for development of sporadic retinoblastoma. *Cancer Epidemiol Biomarkers Prev*. 2005;14:1433–40.
- Palazzi MA, Yunes JA, Cardinali IA, et al. Detection of oncogenic human papillomavirus in sporadic retinoblastoma. *Acta Ophthalmol Scand*. 2003;81:396–8.
- Antoneli CB, Ribeiro KB, Sredni ST, et al. Low prevalence of HPV in Brazilian children with retinoblastoma. *J Med Virol*. 2011;83:115–8.
- Marees T, Dommering CJ, Imhof SM, et al. Incidence of retinoblastoma in Dutch children conceived by IVF: an expanded study. *Hum Reprod*. 2009;24:3220–4.
- Foix-L'Heliás L, Aerts I, Marchand L, et al. Are children born after infertility treatment at increased risk of retinoblastoma? *Hum Reprod*. 2012;27:2186–92.
- Xu K, Rosenwaks Z, Beaverson K, Cholst I, Veck L, Abramson DH. Preimplantation genetic diagnosis for retinoblastoma: the first reported liveborn. *Am J Ophthalmol*. 2004;137:18–23.
- Kleinerman RA, Yu CL, Little MP, et al. Variation of second cancer risk by family history of retinoblastoma among long-term survivors. *J Clin Oncol*. 2012;30:950–7.
- Marees T, van Leeuwen FE, de Boer MR, Imhof SM, Ringens PJ, Moll AC. Cancer mortality in long-term survivors of retinoblastoma. *Eur J Cancer*. 2009;45:3245–53.
- Moll AC, Imhof SM, Schouten-Van Meeteren AY, Kuik DJ, Hofman P, Boers M. Second primary tumors in hereditary retinoblastoma: a register-based study, 1945–1997: is there an age effect on radiation-related risk? *Ophthalmology*. 2001;108:1109–14.
- Abramson DH, Frank CM, Susman M, Whalen MP, Dunkel IJ, Boyd NW 3rd. Presenting signs of retinoblastoma. *J Pediatr*. 1998;132:505–8.
- Kaimbo WK, Mvitu MM, Missotten L. Presenting signs of retinoblastoma in Congolese patients. *Bull Soc Belge Ophtalmol*. 2002;282:37–41.
- Parulekar MV. Retinoblastoma—current treatment and future direction. *Early Hum Dev*. 2010;86:619–25.
- Linn Murphree A. Intraocular retinoblastoma: the case for a new group classification. *Ophthalmol. Clin. North Am*. 2005;18:41–53, viii.
- Kivela T. Trilateral retinoblastoma: a meta-analysis of hereditary retinoblastoma associated with primary ectopic intracranial retinoblastoma. *J Clin Oncol*. 1999;17:1829–37.
- Turaka K, Shields CL, Meadows AT, Leahey A. Second malignant neoplasms following chemoreduction with carboplatin, etoposide, and vincristine in 245 patients with intraocular retinoblastoma. *Pediatr Blood Cancer*. 2012;59:121–5.
- Draper GJ, Sanders BM, Kingston JE. Second primary neoplasms in patients with retinoblastoma. *Br J Cancer*. 1986;53:661–71.
- Sitorus RS, Moll AC, Suhardjono S, et al. The effect of therapy refusal against medical advice in retinoblastoma patients in a setting where treatment delays are common. *Ophthalmic Genet*. 2009;30:31–6.
- Canturk S, Qaddoumi I, Khetan V, et al. Survival of retinoblastoma in less-developed countries impact of socioeconomic and health-related indicators. *Br J Ophthalmol*. 2010;94:1432–6.
- Gupta R, Vemuganti GK, Reddy VA, Honavar SG. Histopathologic risk factors in retinoblastoma in India. *Arch Pathol Lab Med*. 2009;133:1210–4.
- Chantada GL, Dunkel IJ, de Davila MT, Abramson DH. Retinoblastoma patients with high risk ocular pathological features: who needs adjuvant therapy? *Br J Ophthalmol*. 2004;88:1069–73.
- de Graaf P, Gorricke S, Rodjan F, et al. Guidelines for imaging retinoblastoma: imaging principles and MRI standardization. *Pediatr Radiol*. 2012;42:2–14.
- Kashyap S, Meel R, Pushker N, et al. Clinical predictors of high risk histopathology in retinoblastoma. *Pediatr Blood Cancer*. 2012;58:356–61.
- Chantada GL, Gonzalez A, Fandino A, et al. Some clinical findings at presentation can predict high-risk pathology features in unilateral retinoblastoma. *J Pediatr Hematol Oncol*. 2009;31:325–9.
- Kaliki S, Shields CL, Rojanaporn D, et al. High-risk retinoblastoma based on international classification of retinoblastoma: analysis of 519 enucleated eyes. *Ophthalmology*. 2013;120:997–1003.
- Wilson MW, Qaddoumi I, Billups C, Haik BG, Rodriguez-Galindo C. A clinicopathological correlation of 67 eyes primarily enucleated for advanced intraocular retinoblastoma. *Br J Ophthalmol*. 2011;95:553–8.
- Laurent VE, Sampor C, Solernou V, et al. Detection of minimally disseminated disease in the cerebrospinal fluid of children with high-risk retinoblastoma by reverse transcriptase-polymerase chain reaction for GD2 synthase mRNA. *Eur J Cancer*. 2013;49:2892–9.
- McCormick B, Ellsworth R, Abramson D, LoSasso T, Grabowski E. Results of external beam radiation for children with

- retinoblastoma: a comparison of two techniques. *J Pediatr Ophthalmol Strabismus*. 1989;26:239–43.
33. Levin MH, Gombos DS, O'Brien JM. Intra-arterial chemotherapy for advanced retinoblastoma: is the time right for a prospective clinical trial? *Arch Ophthalmol*. 2011;129:1487–9.
  34. Suzuki S, Yamane T, Mohri M, Kaneko A. Selective ophthalmic arterial injection therapy for intraocular retinoblastoma: the long-term prognosis. *Ophthalmology*. 2011;118:2081–7.
  35. Gobin YP, Dunkel IJ, Marr BP, Brodie SE, Abramson DH. Intra-arterial chemotherapy for the management of retinoblastoma: four-year experience. *Arch Ophthalmol*. 2011;129:732–7.
  36. Lee V, Hungerford JL, Bunce C, Ahmed F, Kingston JE, Plowman PN. Globe conserving treatment of the only eye in bilateral retinoblastoma. *Br J Ophthalmol*. 2003;87:1374–80.
  37. Grigorovski N, Lucena E, Mattosinho C, et al. Use of intra-arterial chemotherapy for retinoblastoma: results of a survey. *Int J Ophthalmol*. 2014;7:726–30.
  38. Shields CL, Fulco EM, Arias JD, et al. Retinoblastoma frontiers with intravenous, intra-arterial, periocular, and intravitreal chemotherapy. *Eye (Lond)*. 2013;27:253–64.
  39. Abramson DH. Retinoblastoma: saving life with vision. *Annu Rev Med*. 2014;65:171–84.
  40. Shields CL, Shields JA. Retinoblastoma management: advances in enucleation, intravenous chemoreduction, and intra-arterial chemotherapy. *Curr Opin Ophthalmol*. 2010;21:203–12.
  41. Willard VW, Qaddoumi I, Chen S, et al. Developmental and adaptive functioning in children with retinoblastoma: a longitudinal investigation. *J Clin Oncol*. 2014;32:2788–93.
  42. van Dijk J, Imhof SM, Moll AC, et al. Quality of life of adult retinoblastoma survivors in The Netherlands. *Health Qual Life Outcomes*. 2007;5:30.
  43. Eagle RC Jr, Shields CL, Bianciotto C, Jabbour P, Shields JA. Histopathologic observations after intra-arterial chemotherapy for retinoblastoma. *Arch Ophthalmol*. 2011;129:1416–21.
  44. Bosaleh A, Sampor C, Solernou V, et al. Outcome of children with retinoblastoma and isolated choroidal invasion. *Arch Ophthalmol*. 2012;130:724–9.
  45. Olteanu C, Dimaras H. Enucleation refusal for retinoblastoma: a global study. *Ophthalmic Genet*. 2014;31:1–7.
  46. Chantada GL, Qaddoumi I, Canturk S, et al. Strategies to manage retinoblastoma in developing countries. *Pediatr Blood Cancer*. 2011;56:341–8.
  47. Zhao J, Dimaras H, Massey C, et al. Pre-enucleation chemotherapy for eyes severely affected by retinoblastoma masks risk of tumor extension and increases death from metastasis. *J Clin Oncol*. 2011;29:845–51.
  48. Kingston JE, Hungerford JL, Madreperla SA, Plowman PN. Results of combined chemotherapy and radiotherapy for advanced intraocular retinoblastoma. *Arch Ophthalmol*. 1996;114:1339–43.
  49. Shields CL, Mashayekhi A, Au AK, et al. The International Classification of Retinoblastoma predicts chemoreduction success. *Ophthalmology*. 2006;113:2276–80.
  50. Naseripour M, Nazari H, Bakhtiari P, Modarres-zadeh M, Vosough P, Ausari M. Retinoblastoma in Iran: outcomes in terms of patients' survival and globe survival. *Br J Ophthalmol*. 2009;93:28–32.
  51. Shields CL, Bianciotto CG, Jabbour P, et al. Intra-arterial chemotherapy for retinoblastoma: report No. 2, treatment complications. *Arch Ophthalmol*. 2011;129:1407–15.
  52. Dunkel IJ, Shi W, Salvaggio K, et al. Risk factors for severe neutropenia following intra-arterial chemotherapy for intraocular retinoblastoma. *PLoS One*. 2014;9:e108692.
  53. Chantada GL, Fandino AC, Schwartzman E, Raslawski E, Schaiquevich P, Manzitti J. Impact of chemoreduction for conservative therapy for retinoblastoma in Argentina. *Pediatr Blood Cancer*. 2014;61:821–6.
  54. Gombos DS, Hungerford J, Abramson DH, et al. Secondary acute myelogenous leukemia in patients with retinoblastoma: is chemotherapy a factor? *Ophthalmology*. 2007;114:1378–83.
  55. Qaddoumi I, Bass JK, Wu J, et al. Carboplatin-associated ototoxicity in children with retinoblastoma. *J Clin Oncol*. 2012;30:1034–41.
  56. Jehanne M, Lumbroso-Le Rouic L, Savignoni A, et al. Analysis of ototoxicity in young children receiving carboplatin in the context of conservative management of unilateral or bilateral retinoblastoma. *Pediatr Blood Cancer*. 2009;52:637–43.
  57. Shields CL, Shields JA, Meadows AT. Chemoreduction for retinoblastoma may prevent trilateral retinoblastoma. *J Clin Oncol*. 2000;18:236–7.
  58. Qaddoumi I, Billups CA, Tagen M, et al. Topotecan and vincristine combination is effective against advanced bilateral intraocular retinoblastoma and has manageable toxicity. *Cancer*. 2012;118:5663–70.
  59. Lumbroso L, Doz F, Urbietta M, et al. Chemothermotherapy in the management of retinoblastoma. *Ophthalmology*. 2002;109:1130–6.
  60. Greenwald MJ, Strauss LC. Combining cyclosporin with chemotherapy controls intraocular retinoblastoma without requiring radiation. *Clin Cancer Res*. 1997;3:491–2.
  61. Berry JL, Jubran R, Kim JW, et al. Long-term outcomes of Group D eyes in bilateral retinoblastoma patients treated with chemoreduction and low-dose IMRT salvage. *Pediatr Blood Cancer*. 2013;60:688–93.
  62. Abramson DH, Dunkel IJ, Brodie SE, Kim JW, Gobin YP. A phase III study of direct intraarterial (ophthalmic artery) chemotherapy with melphalan for intraocular retinoblastoma initial results. *Ophthalmology*. 2008;115:1398–404, 404 e1.
  63. Suzuki S, Kaneko A. Management of intraocular retinoblastoma and ocular prognosis. *Int J Clin Oncol*. 2004;9:1–6.
  64. Schaiquevich P, Buitrago E, Taich P, et al. Pharmacokinetic analysis of melphalan after superselective ophthalmic artery infusion in preclinical models and retinoblastoma patients. *Invest Ophthalmol Vis Sci*. 2012;53:4205–12.
  65. Munier FL, Beck-Popovic M, Balmer A, Gaillard MC, Bovey E, Binaghi S. Occurrence of sectoral choroidal occlusive vasculopathy and retinal arteriolar embolization after superselective ophthalmic artery chemotherapy for advanced intraocular retinoblastoma. *Retina*. 2011;31:566–73.
  66. Gobin YP, Rosenstein LM, Marr BP, Brodie SE, Abramson DH. Radiation exposure during intra-arterial chemotherapy for retinoblastoma. *Arch Ophthalmol*. 2012;130:403–4 (author reply 4–5).
  67. Francis JH, Gobin YP, Nagiel A, et al. Thrombophilia in patients with retinoblastoma receiving ophthalmic artery chemosurgery. *Arch Ophthalmol*. 2012;130:1605–8.
  68. Shields CL, Manjandavida FP, Lally SE, et al. Intra-arterial chemotherapy for retinoblastoma in 70 eyes: outcomes based on the international classification of retinoblastoma. *Ophthalmology*. 2014;121:1453–60.
  69. Venturi C, Bracco S, Cerase A, et al. Superselective ophthalmic artery infusion of melphalan for intraocular retinoblastoma: preliminary results from 140 treatments. *Acta Ophthalmol*. 2013;91:335–42.
  70. Francis JH, Gobin YP, Brodie SE, Marr BP, Dunkel IJ, Abramson DH. Experience of intra-arterial chemosurgery with single agent carboplatin for retinoblastoma. *Br J Ophthalmol*. 2012;96:1270–1.
  71. Marr BP, Brodie SE, Dunkel IJ, Gobin YP, Abramson DH. Three-drug intra-arterial chemotherapy using simultaneous carboplatin, topotecan and melphalan for intraocular



- retinoblastoma: preliminary results. *Br J Ophthalmol.* 2012;96:1300–3.
72. Palioura S, Gobin YP, Brodie SE, Marr BP, Dunkel IJ, Abramson DH. Ophthalmic artery chemosurgery for the management of retinoblastoma in eyes with extensive (>50 %) retinal detachment. *Pediatr Blood Cancer.* 2012;59:859–64.
  73. Abramson DH, Marr BP, Brodie SE, Dunkel I, Palioura S, Gobin YP. Ophthalmic artery chemosurgery for less advanced intraocular retinoblastoma: five year review. *PLoS One.* 2012;7:e34120.
  74. Biswas J, Das D, Krishnakumar S, Shanmugam MP. Histopathologic analysis of 232 eyes with retinoblastoma conducted in an Indian tertiary-care ophthalmic center. *J Pediatr Ophthalmol Strabismus.* 2003;40:265–7.
  75. Chan MP, Hungerford JL, Kingston JE, Plowman PN. Salvage external beam radiotherapy after failed primary chemotherapy for bilateral retinoblastoma: rate of eye and vision preservation. *Br J Ophthalmol.* 2009;93:891–4.
  76. Chantada GL, Dunkel IJ, Antoneli CB, et al. Risk factors for extraocular relapse following enucleation after failure of chemoreduction in retinoblastoma. *Pediatr Blood Cancer.* 2007;49:256–60.
  77. Schaiquevich P, Ceciliano A, Millan N, et al. Intra-arterial chemotherapy is more effective than sequential periocular and intravenous chemotherapy as salvage treatment for relapsed retinoblastoma. *Pediatr Blood Cancer.* 2013;60:766–70.
  78. Shields CL, Manjandavida FP, Arepalli S, Kaliki S, Lally SE, Shields JA. Intravitreal melphalan for persistent or recurrent retinoblastoma vitreous seeds: preliminary results. *JAMA Ophthalmol.* 2014;132:319–25.
  79. Munier FL, Gaillard MC, Balmer A, Beck-Popovic M. Intravitreal chemotherapy for vitreous seeding in retinoblastoma: recent advances and perspectives. *Saudi J Ophthalmol.* 2013;27:147–50.
  80. Seregard S, Kock E, af Trampe E. Intravitreal chemotherapy for recurrent retinoblastoma in an only eye. *Br J Ophthalmol.* 1995;79:194–5.
  81. Kaneko A, Suzuki S. Eye-preservation treatment of retinoblastoma with vitreous seeding. *Jpn J Clin Oncol.* 2003;33:601–7.
  82. Ghassemi F, Shields CL, Ghadimi H, Khodabandeh A, Roohipour R. Combined intravitreal melphalan and topotecan for refractory or recurrent vitreous seeding from retinoblastoma. *JAMA Ophthalmol.* 2014;132:936–41.
  83. Francis JH, Schaiquevich P, Buitrago E, et al. Local and systemic toxicity of intravitreal melphalan for vitreous seeding in retinoblastoma: a preclinical and clinical study. *Ophthalmology.* 2014;121:1810–7.
  84. Sastre X, Chantada GL, Doz F, et al. Proceedings of the consensus meetings from the International Retinoblastoma Staging Working Group on the pathology guidelines for the examination of enucleated eyes and evaluation of prognostic risk factors in retinoblastoma. *Arch Pathol Lab Med.* 2009;133:1199–202.
  85. Leal-Leal CA, Rivera-Luna R, Flores-Rojo M, Juarez-Echenique JC, Ordaz JC, Amador-Zarco J. Survival in extra-orbital metastatic retinoblastoma: treatment results. *Clin Transl Oncol.* 2006;8:39–44.
  86. Baroni LV, Sampor C, Fandino A, et al. Anterior segment invasion in retinoblastoma: is it a risk factor for extraocular relapse? *J Pediatr Hematol Oncol.* 2014;36:509–12.
  87. Khelifaoui F, Validire P, Auperin A, et al. Histopathologic risk factors in retinoblastoma: a retrospective study of 172 patients treated in a single institution. *Cancer.* 1996;77:1206–13.
  88. Aerts I, Sastre-Garau X, Savignoni A, et al. Results of a multicenter prospective study on the postoperative treatment of unilateral retinoblastoma after primary enucleation. *J Clin Oncol.* 2013;31:1458–63.
  89. Chantada GL, Gutter MR, Fandino AC, et al. Treatment results in patients with retinoblastoma and invasion to the cut end of the optic nerve. *Pediatr Blood Cancer.* 2009;52:218–22.
  90. Chantada GL, Sampor C, Bosaleh A, Solernou V, Fandino A, de Davila MT. Comparison of staging systems for extraocular retinoblastoma: analysis of 533 patients. *JAMA Ophthalmol.* 2013;131:1127–34.
  91. Shields CL, Shields JA, Baez KA, Cater J, De Potter PV. Choroidal invasion of retinoblastoma: metastatic potential and clinical risk factors. *Br J Ophthalmol.* 1993;77:544–8.
  92. Kaliki S, Shields CL, Shah SU, Eagle RC Jr, Shields JA, Leahey A. Postenucleation adjuvant chemotherapy with vincristine, etoposide, and carboplatin for the treatment of high-risk retinoblastoma. *Arch Ophthalmol.* 2011;129:1422–7.
  93. Chantada GL, Fandino AC, Gutter MR, et al. Results of a prospective study for the treatment of unilateral retinoblastoma. *Pediatr Blood Cancer.* 2010;55:60–6.
  94. Friedman DN, Sklar CA, Oeffinger KC, et al. Long-term medical outcomes in survivors of extra-ocular retinoblastoma: the Memorial Sloan-Kettering Cancer Center (MSKCC) experience. *Pediatr Blood Cancer.* 2013;60:694–9.
  95. Magrann I, Abramson DH, Ellsworth RM. Optic nerve involvement in retinoblastoma. *Ophthalmology.* 1989;96:217–22.
  96. Sullivan EM, Wilson MW, Billups CA, et al. Pathologic risk-based adjuvant chemotherapy for unilateral retinoblastoma following enucleation. *J Pediatr Hematol Oncol.* 2014;36:e335–40.
  97. Chantada GL, Casco F, Fandino AC, et al. Outcome of patients with retinoblastoma and postlaminar optic nerve invasion. *Ophthalmology.* 2007;114:2083–9.
  98. Cuenca A, Giron F, Castro D, et al. Microscopic scleral invasion in retinoblastoma: clinicopathological features and outcome. *Arch Ophthalmol.* 2009;127:1006–10.
  99. Antoneli CB, Ribeiro KB, Rodriguez-Galindo C, et al. The addition of ifosfamide/etoposide to cisplatin/teniposide improves the survival of children with retinoblastoma and orbital involvement. *J Pediatr Hematol Oncol.* 2007;29:700–4.
  100. Ali MJ, Honavar SG, Reddy VA. Orbital retinoblastoma: present status and future challenges—a review. *Saudi J Ophthalmol.* 2011;25:159–67.
  101. Brisse HJ, de Graaf P, Galluzzi P, et al. Assessment of early-stage optic nerve invasion in retinoblastoma using high-resolution 1.5 Tesla MRI with surface coils: a multicentre, prospective accuracy study with histopathological correlation. *Eur Radiol.* 2014. doi:10.1007/s00330-014-3514-1.
  102. Doz F, Khelifaoui F, Mosseri V, et al. The role of chemotherapy in orbital involvement of retinoblastoma. The experience of a single institution with 33 patients. *Cancer.* 1994;74:722–32.
  103. Ali MJ, Reddy VA, Honavar SG, Naik M. Orbital retinoblastoma: where do we go from here? *J Cancer Res Ther.* 2011;7:11–4.
  104. Radhakrishnan V, Sharma S, Vishnubhatla S, Bakhshi S. MRI findings at baseline and after neoadjuvant chemotherapy in orbital retinoblastoma (IRSS stage III). *Br J Ophthalmol.* 2013;97:52–8.
  105. Dunkel IJ, Khakoo Y, Kernan NA, et al. Intensive multimodality therapy for patients with stage 4a metastatic retinoblastoma. *Pediatr Blood Cancer.* 2010;55:55–9.
  106. Palma J, Sasso DF, Dufort G, et al. Successful treatment of metastatic retinoblastoma with high-dose chemotherapy and autologous stem cell rescue in South America. *Bone Marrow Transplant.* 2012;47:522–7.
  107. Dunkel IJ, Chan HS, Jubran R, et al. High-dose chemotherapy with autologous hematopoietic stem cell rescue for stage 4B retinoblastoma. *Pediatr Blood Cancer.* 2010;55:149–52.
  108. Dimaras H, Heon E, Budning A, et al. Retinoblastoma CSF metastasis cured by multimodality chemotherapy without radiation. *Ophthalmic Genet.* 2009;30:121–6.



109. Bellaton E, Bertozzi AI, Behar C, et al. Neoadjuvant chemotherapy for extensive unilateral retinoblastoma. *Br J Ophthalmol*. 2003;87:327–9.
110. Shields CL, Honavar S, Shields JA, Demirci H, Meadows AT. Vitrectomy in eyes with unsuspected retinoblastoma. *Ophthalmology*. 2000;107:2250–5.
111. Chantada G, Leal-Leal C, Brisse H, et al. Is it pre-enucleation chemotherapy or delayed enucleation of severely involved eyes with intraocular retinoblastoma that risks extraocular dissemination and death? *J Clin Oncol*. 2011;29:3333–4 (author reply 5–6).
112. Schaiquevich P, Carcaboso AM, Buitrago E, et al. Ocular pharmacology of topotecan and its activity in retinoblastoma. *Retina*. 2014;34:1719–27.
113. Hayden BC, Jockovich ME, Murray TG, et al. Pharmacokinetics of systemic versus focal Carboplatin chemotherapy in the rabbit eye: possible implication in the treatment of retinoblastoma. *Invest Ophthalmol Vis Sci*. 2004;45:3644–9.
114. Requejo F, Sierre S, Marelli J, et al. Ophthalmic artery microcatheterization for research purposes in pigs. A technical note. *J Invest Surg*. 2014;27:291–3.
115. Steinle JJ, Zhang Q, Thompson KE, et al. Intra-ophthalmic artery chemotherapy triggers vascular toxicity through endothelial cell inflammation and leukostasis. *Invest Ophthalmol Vis Sci*. 2012;53:2439–45.
116. Tse BC, Steinle JJ, Johnson D, Haik BG, Wilson MW. Superselective intraophthalmic artery chemotherapy in a nonhuman primate model: histopathologic findings. *JAMA Ophthalmol*. 2013;131:903–11.
117. Dyer MA, Rodriguez-Galindo C, Wilson MW. Use of pre-clinical models to improve treatment of retinoblastoma. *PLoS Med*. 2005;2:e332.
118. Pontes de Carvalho RA, Krausse ML, Murphree AL, Schmitt EE, Campochiaro PA, Maumenee IH. Delivery from episcleral explants. *Invest Ophthalmol Vis Sci*. 2006;47:4532–9.
119. Martin NE, Kim JW, Abramson DH. Fibrin sealant for retinoblastoma: where are we? *J Ocul Pharmacol Ther*. 2008;24:433–8.
120. Mallipatna AC, Dimaras H, Chan HS, Heon E, Gallie BL. Periocular topotecan for intraocular retinoblastoma. *Arch Ophthalmol*. 2011;129:738–45.
121. Houston SK, Pina Y, Murray TG, et al. Novel retinoblastoma treatment avoids chemotherapy: the effect of optimally timed combination therapy with angiogenic and glycolytic inhibitors on LH(BETA)T(AG) retinoblastoma tumors. *Clin Ophthalmol*. 2011;5:129–37.
122. Boutrid H, Jockovich ME, Murray TG, et al. Targeting hypoxia, a novel treatment for advanced retinoblastoma. *Invest Ophthalmol Vis Sci*. 2008;49:2799–805.
123. Brennan RC, Federico S, Bradley C, et al. Targeting the p53 pathway in retinoblastoma with subconjunctival Nutlin-3a. *Cancer Res*. 2011;71:4205–13.
124. Laurie NA, Shih CS, Dyer MA. Targeting MDM2 and MDMX in retinoblastoma. *Curr Cancer Drug Targets*. 2007;7:689–95.
125. Zhang F, Tagen M, Throm S, et al. Whole-body physiologically based pharmacokinetic model for nutlin-3a in mice after intravenous and oral administration. *Drug Metab Dispos Biol Fate Chem*. 2011;39:15–21.
126. Zhang J, Benavente CA, McEvoy J, et al. A novel retinoblastoma therapy from genomic and epigenetic analyses. *Nature*. 2012;481:329–34.
127. Pritchard EM, Stewart E, Zhu F, et al. Pharmacokinetics and efficacy of the spleen tyrosine kinase inhibitor R406 after ocular delivery for retinoblastoma. *Pharm Res*. 2014;31:3060–72.
128. Rushlow DE, Mol BM, Kennett JY, et al. Characterisation of retinoblastomas without RB1 mutations: genomic, gene expression, and clinical studies. *Lancet Oncol*. 2013;14:327–34.
129. Kapatai G, Brundler MA, Jenkinson H, et al. Gene expression profiling identifies different sub-types of retinoblastoma. *Br J Cancer*. 2013;109:512–25.
130. Dimaras H, Rushlow D, Halliday W, et al. Using RB1 mutations to assess minimal residual disease in metastatic retinoblastoma. *Transl Res*. 2010;156:91–7.
131. de Graaf P, Pouwels PJ, Rodjan F, et al. Single-shot turbo spin-echo diffusion-weighted imaging for retinoblastoma: initial experience. *AJNR Am J Neuroradiol*. 2012;33:110–8.