Personalized Medicine in Ocular Oncology

The options for focal treatment of retinoblastoma are growing. Here is a rundown of the latest options and what the latest studies reveal.

BY VICTOR M. VILLEGAS, MD; AARON S. GOLD, OD; ANDREA WILDNER, CRA; JENNIFER THOMSON, CRA; FIONA J. EHLIES, BSc, CDOS; AND TIMOTHY G. MURRAY, MD, MBA, FACS

Retinoblastoma is the most common intraocular malignancy in children, affecting one in 15 000 live births.[1] Early diagnosis is of critical importance because small tumors have the best prognoses. Historically, leukocoria has been the most important sign,[1] but, if the primary tumor is peripheral, then leukocoria in primary position and sensory strabismus may become clinically apparent only in advanced stages of retinoblastoma and may delay ophthalmologic evaluation (Figure 1).[2]

This article reviews available and upcoming therapeutic options and offers insights into the promise of individualized therapies for retinoblastoma.

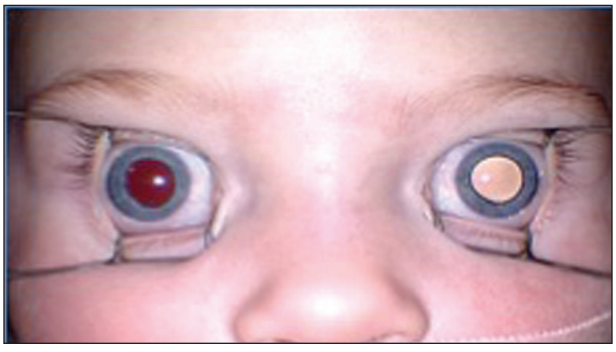


Figure 1. Child with bilateral retinoblastoma. Depending on the tumor location, the red reflex can be affected asymmetrically.

THE CHANGING TREATMENT LANDSCAPE

Therapies for retinoblastoma have dramatically advanced over the past 10 years, and a significant trend away from systemic chemotherapy and toward direct ocular and intra-arterial chemotherapy is under way. Technological changes and strategies now focus on local treatments because they result in decreased morbidity to patients and excellent tumor response. New treatments are providing new hope to patients, especially those with the most severe disease.

Management of retinoblastoma requires a multidisciplinary approach that may include an ocular oncologist, pediatric oncologist, pediatric ophthalmologist, pediatrician, interventional radiologist, and ocular pathologist. Individualized treatment, considering factors such as International Classification of Retinoblastoma (ICRB) group, laterality, location of tumors, age of patient, family history, and prior treatment must be considered.[1,3]

The ultimate goal of retinoblastoma treatment is child survival. Globe salvage and preservation of vision are secondary goals. Early diagnosis is the most crucial step in decreasing morbidity and mortality.[2]

Treatment of small tumors (Figure 2) may require only transpupillary thermotherapy.[4] These laser treatments may be repeated monthly until complete tumor regression is documented.[5] It is important to closely follow patients to monitor for recurrence. If recurrence is present, adjuvant chemotherapy may be considered.

The classic three-drug systemic chemotherapeutic treatment (carboplatin, vincristine, and etoposide) is associated with significant morbidity, and multiple

At a Glance

- Studies have found selective intra-arterial combination therapy with carboplatin, melphalan, and topotecan to be effective in the treatment of retinoblastoma.
- A trend toward direct intravitreal therapy has limited the use of periocular treatments.
- Enucleation remains the standard treatment for group E retinoblastoma.

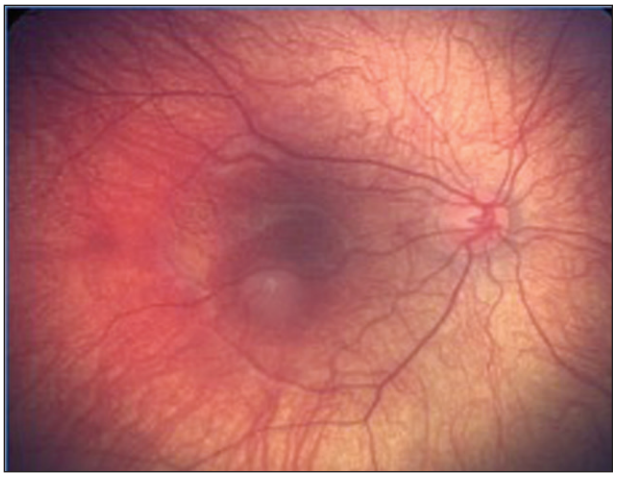


Figure 2. Small macular retinoblastoma in the right eye of the patient depicted in Figure 1.

cycles are routinely needed. Additionally, bone marrow suppression, ototoxicity, nephrotoxicity, and risk of induction of secondary cancers have been reported.[6,7] Trilateral retinoblastoma may be prevented in hereditary cases by treatment with systemic chemotherapy.[8,9] Combined therapy has been shown to have better globe salvage rates than chemotherapy alone in both early and advanced retinoblastoma.[10-13]

A recent study of macular retinoblastoma outcomes showed that chemoreduction with transpupillary thermotherapy of both foveal and extrafoveal tumors achieved tumor control in 83% of Reese-Ellsworth (RE) group 5 tumors.[14] In all tumors less than RE group 5 tumors, 100% control was achieved. Despite ablative foveal laser treatment, 56% of eyes had better than 20/80 visual acuity.

Enucleation remains the standard treatment for retinoblastoma of ICRB group E.[15] Histopathologic analysis may determine whether adjuvant treatment is necessary, depending on high-risk criteria at the time of enucleation.[16] Adjuvant therapy following enucleation has been shown to decrease metastasis in advanced retinoblastoma from 24% of children to 4%.[17]

INTRA-ARTERIAL CHEMOTHERAPY

In 2004, Japanese physicians revolutionized the treatment of retinoblastoma by introducing the infusion of melphalan into the ophthalmic artery, a technique dubbed *intra-arterial chemotherapy*.[18] Their technique consisted of catheterization of the internal carotid artery and occlusion with a microballoon distal to the ophthalmic artery. During the temporary occlusion, they infused melphalan directly into the ophthalmic artery. The study authors performed 563 intra-arterial chemotherapy

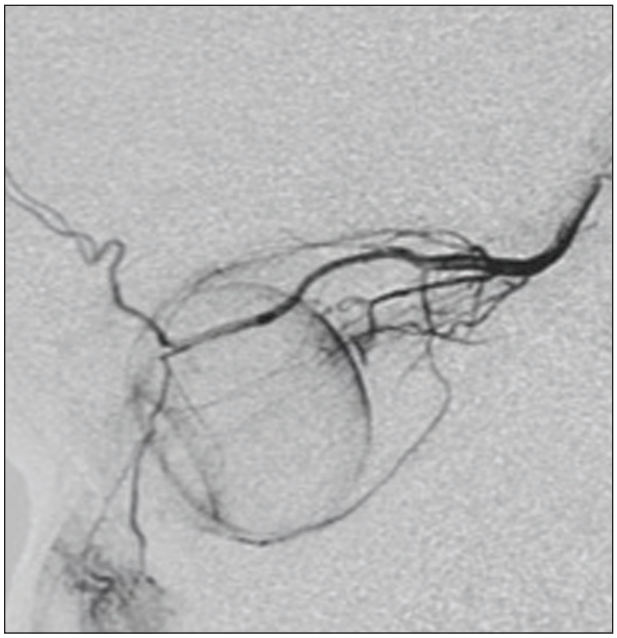


Figure 3. Digitally subtracted selective arteriogram image of the ophthalmic artery without balloon occlusion.

procedures in 187 patients with no reported serious complications, including stroke. The youngest patient to be treated was 1 month old. The most common complications were mild transient bradycardia, periorbital erythema, and swelling. The study concluded that melphalan could be successfully administered to the ophthalmic artery 97% of the time with significant efficacy.[18]

After the initial description of the intra-arterial procedure, many large centers had significant interest in expanding this procedure for use in patients with advanced retinoblastoma. Subsequently, Abramson and associates developed a technique that allowed repeated cannulation of the ophthalmic artery in young children with advanced retinoblastoma without the need to occlude the distal cerebral blood flow at the time of infusion (Figure 3).[19] Initial studies using this technique reported significant tumor control and stabilization of vision in children with RE group 5 tumors without severe side effects.[19,20] Only one patient from these studies had disease progression that required enucleation. No patient received systemic chemotherapy or radiation. The same group later reported results in four patients with bilateral RE group 5 who were initially treated bilaterally.[20] All patients avoided enucleation or radiation, and no significant adverse effects were observed.

A recent study performed at Wills Eye Hospital further validated the efficacy of intra-arterial chemotherapy.[21] The study authors analyzed 70 eyes

CASE PRESENTATION UTILIZING INTRAVITREAL CHEMOTHERAPY

A 5-year-old male with diffuse retinoblastoma was treated with six cycles of intra-arterial three-drug chemotherapy. Despite treatment, persistent globular vitreous seeding was still present (Figure 1). Although further treatment modality options included enucleation, systemic chemotherapy, or continued intra-arterial chemotherapy, the decision was made to proceed with intravitreal chemotherapy. After two intravitreal injections of melphalan, tumoral involution was evident (Figures 2 and 3). The patient's 12-month follow-up exam revealed that his visual acuity was 20/20 in the treated eye. There has been no evidence of tumor recurrence.

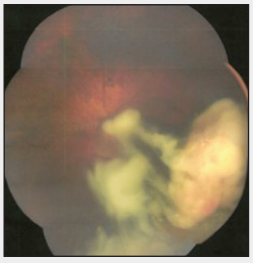


Figure 1. Initial presentation of patient with globular seeding of the vitreous.

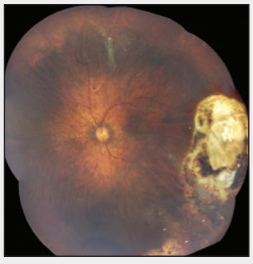


Figure 2. Follow-up examination after intra-arterial chemotherapy and two adjuvant intravitreal melphalan injections.

Figure 3. Intravitreal melphalan injection preparation while the patient is under anesthesia in the operating room.

of 67 patients following ophthalmic artery chemotherapy infusion under fluoroscopic guidance. The mean patient age at initiation of treatment was 30 months. The treatment was primary in 36 eyes and secondary in 34 eyes. Globe salvage was achieved in 72% of primary cases and in 62% of secondary cases. Specifically, primary therapy achieved globe salvage for ICRB group B (100%), group C (100%), group D (94%), and group E (36%) tumors. Common complications included transient eyelid edema, blepharoptosis, and forehead hyperemia. No significant systemic adverse events were reported, including stroke, seizure, neurologic impairment, limb ischemia, secondary leukemia, metastasis, or death. A similar study performed at the Bascom Palmer Eye Institute evaluated selective ophthalmic artery infusion with melphalan in patients with RE group 5 tumors that had failed to respond to prior systemic chemotherapy and laser consolidation; comparable results were reported.[22]

> "Sequential intravenous chemotherapy followed by intra-arterial chemotherapy ... for young infants with retinoblastoma may be considered in eyes in which cannulation of the ophthalmic artery is not possible."

Significant interest in intra-arterial delivery of other chemotherapeutic agents has prompted several small studies. This strategy has been investigated to avoid melphalan dose restriction during bilateral therapy. Francis et al recently reported the use of single-agent carboplatin at doses ranging from 25 mg to 40 mg, and cumulative doses from 25 mg to 100 mg, in three cases in which high-dose melphalan was needed in the contralateral eye and systemic toxicity limited the use of melphalan to one eye.[23] Tumor regression was seen with as little as one cycle, and no systemic adverse effects were seen. Similar results have been shown with intra-arterial infusion of both carboplatin and topotecan.[24] Analysis of electroretinogram (ERG) responses following infusions containing carboplatin only and carboplatin with topotecan revealed no statistically significant changes.[23,24]

Recently, the use of three-drug intra-arterial treatment with carboplatin, melphalan, and topotecan has been reported.[25] Twenty-six eyes of 25 patients received the three-drug chemotherapy for treatment of advanced retinoblastoma. Dose ranges were 2.5 mg to 7.5 mg for melphalan, 0.3 mg to 0.6 mg for topotecan, and 25 mg to 50 mg for carboplatin, and median infusions per eye was two (range, 1-4). Kaplan-Meier estimate of ocular survival at 24 months was 75%. ERG showed improvement greater than 25 µV in four eyes (15%), loss greater than 25 µV in 12 eyes (46%), and no change greater than 25 µV in 10 eyes (39%). A large study by Shields and colleagues has also reported successful treatment using this regimen.[21] These findings suggest that selective intra-arterial combination therapy with carboplatin, melphalan, and topotecan is effective in the treatment of retinoblastoma and decreases the toxic window during treatment, especially in patients who require bilateral therapy.

Sequential intravenous chemotherapy followed by intra-arterial chemotherapy (bridge chemotherapy) for young infants with retinoblastoma may be considered in eyes in which cannulation of the ophthalmic artery is not possible.[26] Further studies will elucidate the optimal timing for bridging.

"Patients with hereditary retinoblastoma may benefit the most from systemic chemotherapy to prevent late-onset intracranial malignancies."

Intra-arterial chemotherapy delivers high-dose chemotherapy to the eyes of children with retinoblastoma. The 5-year experience has demonstrated the effectiveness of this novel therapy both as salvage and primary management. No deaths or strokes have been observed, but vision-threatening vascular complications have been reported to date. Long-term studies evaluating selective intra-arterial chemotherapy are needed to determine safety and efficacy in patients with retinoblastoma. Minimizing systemic adverse events in patients with retinoblastoma using local chemotherapy may benefit a specific subset of patients. Patients with hereditary retinoblastoma may benefit the most from systemic chemotherapy to prevent late-onset intracranial malignancies.[9]

INTRAVITREAL CHEMOTHERAPY

The significant tumoricidal effects reported with intra-arterial melphalan generated enough enthusiasm to warrant the study of intravitreal delivery of the drug for vitreous seeding. However, the potential for tumor dissemination through the needle tract following intravitreal penetration has limited its use.

In 2012, Jules-Gonin Eye Hospital reported the first clinically documented case series of patients with retinoblastoma treated with intravitreal melphalan. The study included 122 injections in 23 eyes that had significant active vitreous seeding following primary therapy. Globe retention was achieved in 20 of 23 (87%) cases. Despite the confounding effects of concomitant chemotherapy, the authors concluded that intravitreal melphalan achieved unprecedented control of vitreous seeding.[27]

A recent cohort study at two institutions evaluated the vitreous seed response following 475 intravitreal injections of melphalan.[28] The study included 87 eyes treated weekly (median dose, 30 µg) with a median of five treatments per eye (range, 1-12 times). The 2-year Kaplan-Meier estimates for ocular and patient survival were 90.4% and 100%, respectively. Other authors have also reported on the efficacy of intravitreal melphalan for the treatment of retinoblastoma.[29-31]

The risk of tumor dissemination subsequent to intravitreal injection was evaluated by researchers at Columbia University; their literature review included

304 patients following therapeutic intravitreal melphalan injections for retinoblastoma.[32] Only one patient had extraocular tumor spread; for one other patient, intravitreal treatment could not be excluded as a contributor to metastatic disease. In these combined reports, the proportion of subjects with extraocular tumor spread potentially caused by intravitreal treatment was 0.007 (95% CI, 0.0008-0.0236), with a mean follow-up of 72.1 months. In a subset of 61 patients receiving intravitreal treatment via safety-enhancing injection techniques (347 injections, 19.6 months mean follow-up), there were no reports of tumor spread. The authors of this study concluded that retinoblastoma metastasis following intravitreal therapy is rare and should not preclude its clinical use in appropriately selected cases.

Data regarding toxicity of intravitreal melphalan are limited. A recent study evaluating retinal and systemic toxicity of intravitreal melphalan in a rabbit model concluded that weekly injections of 30 µg of melphalan can result in a decreased ERG response.[33] Previous studies have also shown that 50 µg of intravitreal melphalan is toxic to the eye, causing persistent hypotonia and phthisis bulbi.[31] In contrast, 20/40 visual acuity has been reported in a patient who received four doses (30, 30, 30, and 20 µg.) of intravitreal melphalan with no change in ERG amplitudes before or after therapy.[30]

Effective intravitreal combination of melphalan (40 µg in 0.04 mL of diluent) and topotecan (8-20 µg in 0.04 mL of balanced salt solution) has also recently been reported in nine eyes.[34] In this study, no cases of episcleral or orbital retinoblastoma extension or remote retinoblastoma metastasis were reported. There was no change in the a- and b-waves of bright-flash ERGs.

Further studies are required to assess long-term safety of intravitreal therapy and to better delineate its role in the management of retinoblastoma. The adoption of specific guidelines for intravitreal treatment case selection and additional data on potential ocular toxicity are essential to enabling more widespread use of this treatment (see Case Presentation Utilizing Intravitreal Chemotherapy on page 70 as an example).

PERIOCULAR CHEMOTHERAPY

Focal therapies are aimed at increasing the tumoricidal effects in retinoblastoma-affected tissues while minimizing systemic toxicity. Periocular chemotherapy has been investigated by multiple authors as adjuvant to systemic, intra-arterial, and intravitreal chemotherapy.[35-38]

Abramson et al evaluated subconjunctival carboplatin (1.4-2.0 mL in 10-mg/mL solution) in ICRB group C and D eyes, but the results were limited.[35] Toxicities associated with treatment in this study included transient periorbital edema in four eyes and optic atrophy in one eye that also received laser photocoagulation and cryotherapy.

Leng and colleagues reported a case that responded to focal subconjuctival carboplatin chemotherapy as primary chemotherapy.[36] Side effects of periocular carboplatin include strabismus, optic neuropathy, periocular inflammation, and fat atrophy.[35-38] Inflammation associated with periocular carboplatin has limited its use in children with retinoblastoma.

Periocular topotecan has also been investigated as adjuvant therapy in patients with retinoblastoma.[39] Eight patients (10 eyes) were enrolled in a study in which one to four injections of periocular topotecan in fibrin sealant, with or without concomitant laser, were performed. Six children with ICRB group A and B tumors responded favorably to treatment. Two children with ICRB group D tumors were not controlled by periocular topotecan as an adjuvant to systemic chemotherapy. Hematologic toxic effects were self-limiting and included decreased hemoglobin, absolute neutrophil, white blood cell, and platelet counts.

Murray et al evaluated the effects of intravitreal and subconjunctival melphalan on tumor burden, hypoxia, and vasculature in an LHbeta-TAg murine retinoblastoma model. They reported a significant decline in hypoxia at 1 week following intravitreal injection and after maximum dosage of subconjunctival melphalan.[40] There was a significant decrease in tumor burden following serial subconjunctival injections of melphalan, showing an 86% reduction. No toxicities were seen on histology following treatments.

Prospective studies are needed to assess the role of periocular chemotherapies in the treatment of retinoblastoma. However, the recent trend toward direct intravitreal therapy has limited the use of periocular treatments.

UP-AND-COMING TREATMENT OPTIONS

The new frontier in oncology includes the development of genetic therapies and viral vectors for the treatment of various malignancies including retinoblastoma. In a recent study, investigators used viral vectors to infect retinoblastoma cells from enucleated eyes.[41] This technique is being investigated as a possible mechanism to directly target retinoblastoma cells. Other developments include the application of extended-release implants that may deliver chemotherapeutic agents directly to the globe.[42]

Another recent study evaluated the efficacy of subconjunctival nanoparticle carboplatin in the treatment of retinoblastoma in transgenic mice.[43] Dendrimeric

nanoparticles loaded with carboplatin were injected into the subconjuctival space in these mouse models. Mean tumor burden in treated eyes was significantly less than in untreated eyes. Other investigators have also been able to load carboplatin into nanoparticles and show tumoricidal effects.[44]

Kang et al recently developed a rabbit model of retinoblastoma from human retinoblastoma cell lines that were implanted into rabbit eyes.[45] This model is currently being used to investigate periocular injection of nanoparticles containing carboplatin and intravitreal topotecan.[46]

Carcinogenesis and tumor microenvironment represent new frontiers in scientific developments.[47] Angiogenesis plays a key role in the development of malignant tumors. During tumor growth, VEGF levels increase, and the protein stimulates vessel growth to provide metabolic needs. There is growing evidence that the p53 tumor suppressor gene downregulates VEGF expression; however, the exact mechanism by which p53 interacts with VEGF remains unknown.[48]

Inhibition of glycolysis with 2-deoxy-d-glucose (2-DG) targets the cellular mechanism that hypoxic tumoral cells use for survival. Thus, the inhibition of glucose metabolism in hypoxic microenvironments is currently being studied as a possible target for cancer treatment. 2-DG competes with glucose for cellular transporters during glycolysis and, as a result, it inhibits the metabolic machinery of tumoral cells. Studies have shown that 2-DG decreases angiogenesis and hypoxia in vitro and in vivo.[49-51] Also, researchers have shown that 2-DG has synergistic tumoricidal effects when used in combination with periocular carboplatin in an animal model of retinoblastoma.[51]

Antiangiogenic agents have also been shown to be effective antitumoral agents. In a recent study, the antiangiogenic agent anecortave acetate significantly controlled tumor burden in a murine model of retinoblastoma when used as monotherapy or adjuvant therapy.[52] A recent study that evaluated the potential effect of the VEGF inhibitor bevacizumab (Avastin, Genentech) on angiogenesis and tumor growth of retinoblastoma in vitro and in vivo showed a 75% reduction in tumor growth without significant systemic toxicity.[53]

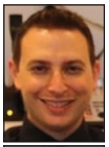
THE FUTURE IS INDIVIDUALIZED

Ocular oncology is currently undergoing a therapeutic revolution in the application of individualized therapies. Most of the associated changes in management have occurred without the support of clinical trials. Clinical experience remains the most important tool in the

management of patients with retinoblastoma. We look forward to large randomized clinical trials that will better delineate how to use the available therapeutic options more efficiently. ■

Fiona J. Ehlies, BSc, CDOS, is director of echography at Murray Ocular Oncology and Retina in Miami, Florida.

Aaron S. Gold, OD, is director of optometric services at Murray Oncology and Retina in Miami, Florida. He may be reached at agold@murraymd.com.



Timothy G. Murray, MD, MBA, FACS, is the founder of Murray Ocular Oncology and Retina in Miami, Florida. He is a member of the Retina Today *Editorial Board. Dr. Murray may be reached at +1-305-487-7470 or tmurray@murraymd.com.*

Jennifer Thomson, CRA, is an ophthalmic photographer at Murray Ocular Oncology and Retina.

Victor M. Villegas, MD, is an ophthalmologist in Miami, Florida.

Andrea Wildner, CRA, is director of photography at Murray Oncology and Retina in Miami, Florida.

No conflicting relationship exists for any author.

1. Shields CL, Shields JA. Basic understanding of current classification and management of retinoblastoma. *Curr Opin Ophthalmol*. 2006;17(3):228-234.
2. Leander C, Fu LC, Pena A, et al. Impact of an education program on late diagnosis of retinoblastoma in Honduras. *Pediatr Blood Cancer*. 2007;49(6):817-819.
3. Shields CL, Shields JA. Retinoblastoma management: advances in enucleation, intravenous chemoreduction, and intra-arterial chemotherapy. *Curr Opin Ophthalmol*. 2010;21(3):203-212.
4. Shields CL, Santos MC, Diniz W, et al. Thermotherapy for retinoblastoma. *Arch Ophthalmol*. 1999;117(7):885-893.
5. Schefler AC, Cicciarelli N, Feuer W, et al. Macular retinoblastoma: evaluation of tumor control, local complications, and visual outcomes for eyes treated with chemotherapy and repetitive foveal laser ablation. *Ophthalmology*. 2007;114(1):162-169.
6. 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(5):637-643.
7. Bartuma K, Pal N, Kosek S, Holm S, All-Ericsson C. A 10-year experience of outcome in chemotherapy-treated hereditary retinoblastoma. *Acta Ophthalmol*. 2014;92(5):404-411.
8. De Potter P, Shields CL, Shields JA. Clinical variations of trilateral retinoblastoma: a report of 13 cases. *J Pediatr Ophthalmol Strabismus*. 1994;31(1):26-31.
9. Shields CL, Meadows AT, Shields JA, Carvalho C, Smith AF. Chemoreduction for retinoblastoma may prevent intracranial neuroblastic malignancy (trilateral retinoblastoma). *Arch Ophthalmol*. 2001;119(9):1269-1272.
10. Shields CL, Mashayekhi A, Au AK, et al. The international classification of retinoblastoma predicts chemoreduction success. *Ophthalmology*. 2006;113(12):2276-2280.
11. Rodriguez-Galindo C, Wilson MW, Haik BG, et al. Treatment of intraocular retinoblastoma with vincristine and carboplatin. *J Clin Oncol*. 2003;21(10):2019-2025.
12. Gombos DS, Kelly A, Coen PG, et al. Retinoblastoma treated with primary chemotherapy alone: the significance of tumour size, location, and age. *Br J Ophthalmol*. 2002;86(1):80-83.
13. Shields CL, Mashayekhi A, Cater J, et al. Chemoreduction for retinoblastoma: analysis of tumor control and risks for recurrence in 457 tumors. *Trans Am Ophthalmol Soc*. 2004;102:35-44. discussion 44-35.
14. Schefler AC, Cicciarelli N, Feuer W, et al. Macular retinoblastoma: evaluation of tumor control, local complications, and visual outcomes for eyes treated with chemotherapy and repetitive foveal laser ablation. *Ophthalmology*. 2007;114(1):162-169.
15. Honavar SG, Singh AD. Management of advanced retinoblastoma. *Ophthalmol Clin North Am*. 2005;18(1):65-73,viii.
16. Eagle RC Jr. High-risk features and tumor differentiation in retinoblastoma: a retrospective histopathologic study. *Arch Pathol Lab Med*. 2009;133(8):1203-1209.
17. Honavar SG, Singh AD, Shields CL, et al. Postenucleation adjuvant therapy in high-risk retinoblastoma. *Arch Ophthalmol*. 2002;120(7):923-931.
18. Yamane T, Kaneko A, Mohri M. The technique of ophthalmic arterial infusion therapy for patients with intraocular retinoblastoma. *Int J Clin Oncol*. 2004;9(2):69-73.
19. Abramson DH, Dunkel IJ, Brodie SE, et al. A phase I/II study of direct intraarterial (ophthalmic artery) chemotherapy with melphalan for intraocular retinoblastoma initial results. *Ophthalmology*. 2008;115(8):1398-1404. 1404, e1391.
20. Abramson DH, Dunkel IJ, Brodie SE, et al. Superselective ophthalmic artery chemotherapy as primary treatment for retinoblastoma (chemosurgery). *Ophthalmology*. 2010;117(8):1623-1629.
21. 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(7):1453-1460.
22. Vajzovic LM, Murray TG, Aziz-Sultan MA, et al. Supraselective intra-arterial chemotherapy: evaluation of treatment-related complications in advanced retinoblastoma. *Clin Ophthalmol*. 2011;5:171-176.
23. Francis JH, Gobin YP, Brodie SE, et al. Experience of intra-arterial chemosurgery with single agent carboplatin for retinoblastoma. *Br J Ophthalmol*. 2012;96(9):1270-1271.
24. Francis JH, Gobin YP, Dunkel IJ, et al. Carboplatin +/- topotecan ophthalmic artery chemosurgery for intraocular retinoblastoma. *PLoS One*. 2013;8(8):e72441.
25. Marr BP, Brodie SE, Dunkel IJ, et al. Three-drug intra-arterial chemotherapy using simultaneous carboplatin, topotecan and melphalan for intraocular retinoblastoma: preliminary results. *Br J Ophthalmol*. 2012;96(10):1300-1303.
26. Gobin YP, Dunkel IJ, Marr BP, et al. Combined, sequential intravenous and intra-arterial chemotherapy (bridge chemotherapy) for young infants with retinoblastoma. *PLoS One*. 2012;7(9):e44322.
27. Munier FL, Gaillard MC, Balmer A, et al. Intravitreal chemotherapy for vitreous disease in retinoblastoma revisited: from prohibition to conditional indications. *Br J Ophthalmol*. 2012;96(8):1078-1083.
28. Francis JH, Abramson DH, Gaillard MC, et al. The classification of vitreous seeds in retinoblastoma and response to intravitreal melphalan. *Ophthalmology*. 2015;122(6):1173-1179.
29. Sun YB, Hui P, Punyara K, et al. Intravitreal injection of melphalan in the treatment of retinoblastoma with vitreous cavity seeding. *Chin Med J (Engl)*. 2013;126(8):1587.
30. Brodie SE, Munier FL, Francis JH, et al. Persistence of retinal function after intravitreal melphalan injection for retinoblastoma. *Doc Ophthalmol*. 2013;126(1):79-84.
31. Ghassemi F, Shields CL. Intravitreal melphalan for refractory or recurrent vitreous seeding from retinoblastoma. *Arch Ophthalmol*. 2012;130(10):1268-1271.
32. Smith SJ, Smith BD. Evaluating the risk of extraocular tumour spread following intravitreal injection therapy for retinoblastoma: a systematic review. *Br J Ophthalmol*. 2013;97(10):1231-1236.
33. 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(9):1810-1817.
34. Ghassemi F, Shields CL, Ghadimi H, et al. Combined intravitreal melphalan and topotecan for refractory or recurrent vitreous seeding from retinoblastoma. *JAMA Ophthalmol*. 2014;132(8):936-941.
35. Abramson DH, Frank CM, Dunkel IJ. A phase I/II study of subconjunctival carboplatin for intraocular retinoblastoma. *Ophthalmology*. 1999;106(10):1947-1950.
36. Leng T, Cebulla CM, Schefler AC, Murray TG. Focal periocular carboplatin chemotherapy avoids systemic chemotherapy for unilateral, progressive retinoblastoma. *Retina*. 2010;30(Suppl 4):S66-68.
37. Mulvihill A, Budning A, Jay V, et al. Ocular motility changes after subtenon carboplatin chemotherapy for retinoblastoma. *Arch Ophthalmol*. 2003;121(8):1120-1124.
38. Schmack I, Hubbard GB, Kang SJ, et al. Ischemic necrosis and atrophy of the optic nerve after periocular carboplatin injection for intraocular retinoblastoma. *Am J Ophthalmol*. 2006;142(2):310-315.
39. Mallipatna AC, Dimaras H, Chan HS, et al. Periocular topotecan for intraocular retinoblastoma. *Arch Ophthalmol*. 2011;129(6):738-745.
4. Shah NV, Pham DG, Murray TG, et al. Intravitreal and subconjunctival melphalan for retinoblastoma in transgenic mice [published online March 10, 2014]. *J Ophthalmol*. 2014;2014:829879. doi: 10.1155/2014/829879.
41. Subramanian N, Navaneethakrishnan S, Biswas J, et al. RNAi mediated Tiam1 gene knockdown inhibits invasion of retinoblastoma. *PLoS One*. 2013;8(8):e70422.
42. Edelhauser HF, Rowe-Rendleman CL, Robinson MR, et al. Ophthalmic drug delivery systems for the treatment of retinal diseases: basic research to clinical applications *Invest Ophthalmol Vis Sci*, 2010;51(11):5403-5420.
43. Kang SJ, Durairaj C, Kompella UB, et al. Subconjunctival nanoparticle carboplatin in the treatment of murine retinoblastoma. *Arch Ophthalmol*. 2009;127(8):1043-1047.
44. Tanmoy Sadhukha, Swayam Prabha. Encapsulation in nanoparticles improves anti-cancer efficacy of carboplatin. *AAPS PharmSciTech*. 2014; 15(4):1029-1038.
45. Kang SJ, Yang H, Grossniklaus HE. Rabbit model of retinoblastoma. *J Biomed Biotechnol*. 2013;97(7):929-933.
46. Grossniklaus HE. Retinoblastoma. Fifty years of progress. The LXXI Edward Jackson Memorial Lecture. *Am J Ophthalmol*. 2014;158(5):875-891.
47. Houston SK, Lampidis TJ, Murray TG. Models and discovery strategies for new therapies of retinoblastoma. *Expert Opin Drug Discov*. 2013;8(4):383-394.
48. Farhang Ghahremani M, Goossens S, Nittner D, et al. p53 promotes VEGF expression and angiogenesis in the absence of an intact p21-Rb pathway. *Cell Death Differ*. 2013;20(7):888-897.
49. Piña Y, Decatur C, Murray TG, et al. Retinoblastoma treatment: utilization of the glycolytic inhibitor, 2-deoxy-2-fluoro-D-glucose (2-FG), to target the chemoresistant hypoxic regions in LH(BETA)T(AG) retinal tumors. *Invest Ophthalmol Vis Sci*. 2012;53(2):996-1002.
50. Piña Y, Houston SK, Murray TG, et al. Retinoblastoma treatment: impact of the glycolytic inhibitor 2-deoxy-d-glucose on molecular genomics expression in LHBETATAG retinal tumors. *Clin Ophthalmol*. 2012;6:817-830.
51. Boutrid H, Jockovich ME, Murray TG, et al. Targeting hypoxia, a novel treatment for advanced retinoblastoma. *Invest Ophthalmol Vis Sci*. 2008;49:2799-2805.
52. Jockovich ME, Murray TG, Escalona-Benz E, et al. Anecortave acetate as single and adjuvant therapy in the treatment of retinal tumors of LHBETATAG mice. *Invest Ophthalmol Vis Sci*. 2006;47:1264-1268.
53. Lee SY, Kim DK, Cho JH, Koh JY, Yoon YH. Inhibitory effect of bevacizumab on the angiogenesis and growth of retinoblastoma. *Arch Ophthalmol*. 2008;126(7):953-958.