



Published in final edited form as:

Int Ophthalmol Clin. 2025 October 01; 65(4): 35–41. doi:10.1097/IIO.0000000000000587.

Angiogenesis Signaling in Retinoblastoma Prognosis and Therapy

Eleen Yang, BSc^{*}, Noa Odell, BS^{†,‡}, Helen Dimaras, PhD^{*,§}, Timothy W. Corson, PhD^{*,†}

^{*}University of Toronto, Toronto, ON, Canada

[†]Indiana University School of Medicine, Indianapolis, IN

[‡]Spelman College, Atlanta, GA

[§]Hospital for Sick Children, Toronto, ON, Canada

Abstract

Angiogenesis is a critical player in tumor metastasis that is involved in the pathophysiology of the pediatric ocular cancer retinoblastoma (RB). This review summarizes evidence linking angiogenesis to RB prognostication, response to treatment, and therapy. Vascular endothelial growth factor (VEGF), a major pro-angiogenic growth factor, has potential as a biomarker of therapy response to RB treatment. High VEGF correlates with poor chemotherapy response, subsequent local invasion, and lower patient survival. VEGF levels are also strongly correlated with choroidal invasion, poor differentiation, and an overall negative disease prognosis for RB patients. In contrast, decreasing VEGF levels can predict vitreous seed regression after intravitreal chemotherapy. Further investigation is needed to determine the accuracy and clinical value of using aqueous humor liquid biopsies to assess VEGF levels to predict prognosis or therapy response. Anti-angiogenic agents including approved drugs and experimental compounds have shown potential in RB models and may become potential therapeutics, adjuvants to current chemotherapies, or treatments for chemotherapy complications, although there is limited evidence that anti-angiogenic monotherapy may be sufficient for RB. Overall, future research aimed at integrating angiogenesis markers and therapies with existing RB strategies holds promise for improving patient outcomes and personalizing treatment approaches.

Introduction

Retinoblastoma (RB) is the most common form of eye cancer in children and is fatal if untreated. The malignancy arising in the retina can spread elsewhere in the central nervous system through the optic nerve or invade the sclera and orbit through the choroid.¹ The growing RB can deposit tumor cells below the retina known as subretinal seeds, or into the vitreous cavity known as vitreous seeds.¹ These seeds are a sign of advanced RB and are especially challenging to control, frequently leading to enucleation.² Angiogenesis is crucial in supporting the expanding RB tumor.^{3,4,5}

Angiogenesis is one of the first steps to metastasis and is triggered by tumor hypoxia causing neoplastic cells to release angiogenic growth factors, the most well-studied being vascular endothelial growth factor (VEGF).⁶ VEGF isoforms signal the formation of new blood vessels by binding to VEGF receptors (VEGFR) on the vascular endothelial cell surface. This leads to downstream signalling via multiple pathways, ultimately resulting in cell proliferation, cytoskeleton rearrangement, cell survival, and increased vascular permeability allowing for angiogenesis.⁷

It is well established that RB cells secrete VEGF in response to hypoxia.⁵ Furthermore, the progression and invasiveness of RB has been associated with increased levels of VEGF-A and VEGFR-2, the two most prominent isoforms of VEGF and VEGFR in angiogenesis signalling.⁴ This raises the possibility of RB treatments that target the VEGF angiogenic pathway and for VEGF levels to act as a prognostic factor.

Current treatments for RB include laser therapy, cryotherapy, enucleation, systemic chemotherapy, and intra-arterial or intravitreal chemotherapy. The most common systemic chemotherapeutic agents for RB are vincristine, etoposide, and carboplatin while melphalan and topotecan are used locally.^{8,9} However, chemotherapy treatments are still associated with vision loss, vitreous hemorrhage, persistent vitreous seeds, and a risk of relapse or secondary tumors.^{8,10} The development or addition of anti-angiogenic therapies have shown potential for treating RB and enhancing the efficacy of current chemotherapy. In addition, emerging research has shown the importance of angiogenesis signals as a biomarker for RB disease prognosis and therapy response. This paper will review the potential of angiogenic factors in the management of RB (Fig. 1).

VEGF as a Prognostic Factor for RB

Optic nerve and choroidal invasion are the two main factors indicating a poor prognosis for RB. However, this is usually determined only after enucleation provides a sample for histology analysis. Techniques such as liquid biopsy of the aqueous humor (AH) can provide an earlier prognosis and produce higher yield cell free DNA than peripheral blood samples.¹¹ VEGF can be found in the ocular fluids and shows potential for being a prognostic factor that can guide management prior to enucleation, although most studies have relied on enucleated eye tissue.¹¹

In 56 patients with advanced RB, intratumoral VEGF was observed on immunohistochemistry stains for tumors with choroidal invasion 77.1% of the time (Table 1).¹² In the same study, the c-Kit proto-oncogene was observed in cases of optic nerve invasion 73.1% of the time. c-Kit is a feature of aggressive RBs with a higher likelihood of combined pattern of tumor growth, optic nerve and choroidal invasion.¹² There was a significant positive correlation between c-kit and VEGF presence, suggesting that VEGF may also serve as a prognostic indicator of optic nerve invasion (Table 1).¹² Similarly, immunostaining of 12 enucleated eyes with RB showed increased expression of angiogenic factors VEGF-A, VEGFR-2, and CD105 (endoglin), which was associated with the expression of a stem cell marker Sox2 (Table 1).⁴ Stem cell markers in RB indicate poor differentiation, more aggressive progression, drug resistance, and a higher risk of recurrence.

Thus, these angiogenic factors could be correlated with these high-risk features of RB indicating poor prognosis.⁴ These findings should spur further study on whether VEGF levels in AH have similar associations.

In 470 RB patients, VEGF overexpression correlated with optic nerve invasion and less differentiated RB cells (Table 1).¹³ In addition to VEGF, matrix metalloproteinases (MMPs) also facilitate malignant cell growth, invasion, and angiogenesis in RB cell lines.¹⁴ A meta-analysis of 725 eyes with RB revealed that increased expression of MMP-1, MMP-2, MMP-9, and VEGF were significantly associated with RB metastasis and poor histological differentiation indicating poor prognosis.¹⁴

VEGF as a Biomarker for Therapy Response

There remains a lack of sensitive and specific biomarkers for monitoring success of RB therapies, especially for invasive RB that is difficult to treat. A retrospective study of enucleated eyes from patients with RB found that the tumor's relative vascular area had a 100% sensitivity and 79% specificity at predicting dissemination after enucleation.¹⁵ VEGF levels closely correlate with tumor vascularity and several studies have shown that levels also correlate with therapy response.

In a study of 28 patients after neoadjuvant chemotherapy and enucleation the event-free survival and overall survival were significantly higher for patients with VEGF-negative RB cells (Table 1).¹⁶ This demonstrates that the presence of VEGF post-chemotherapy can predict a poor response and the potential for local invasion.¹⁶

In 11 patients with refractory RB, there was a significant reduction in VEGF levels extracted from the AH before and after intravitreal carboplatin + bevacizumab treatment.¹⁷ Patients who had reduced tumor size and vitreous seed regression all achieved post-treatment VEGF levels below 50 ng/L while patients who had poor control and required subsequent enucleation all had VEGF levels above 50 ng/L (Table 1).¹⁷ This suggests that high VEGF levels after treatment correlated with RB recurrence and need for enucleation. However, larger cohort studies are needed to investigate the threshold and whether it is consistent across different treatments.

In a rabbit xenograft model of vitreous seeds, we showed that VEGF-A levels in the AH decrease in response to intravitreal chemotherapy with melphalan, topotecan, or belinostat (Table 1).¹⁰ VEGF-A levels in the AH decreased with each serial injection. In contrast, VEGF-A levels continuously increased in non-treated xenografted eyes. The rabbit vitreous seeds expressed high levels of *VEGFA* mRNA (Table 1).¹⁰ In addition, VEGF-A levels in banked AH of patients who underwent intravitreal chemotherapy for vitreous seeds correlated with the amount of viable vitreous seeds present (Table 1).¹⁰ Vitreous seeds can persist in the eye even after they are nonviable, resulting in a delayed regression post-therapy when assessed clinically. This delay can result in excess injections of intravitreal chemotherapy even after vitreous seeds have necrosed. Since only viable vitreous seeds expressed VEGF-A, VEGF-A levels from the AH could determine vitreous seed viability and subsequent regression, acting as a predictive biomarker for treatment response. These

findings (Fig. 1C) are particularly exciting given the rise of AH biopsy as a tool for assessing retinoblastoma prognosis and response to therapy.¹¹

Anti-Angiogenic Drugs for RB Treatment

RB cells induce angiogenesis through stimulating vascular endothelial cell proliferation when co-cultured with human umbilical vein endothelial cells (HUVECs).¹⁸

Correspondingly, numerous anti-angiogenic drugs show potential for treating RB in preclinical work (Fig. 1A). Many of these drugs already exist for other indications and could be readily re-purposed for RB treatment.

Bevacizumab is an anti-VEGF monoclonal antibody indicated for several metastatic cancers including metastatic breast cancer, late-stage ovarian cancer, non-squamous non-small cell lung cancer and recurrent glioblastoma.¹⁹ In addition, intravitreal bevacizumab is used for ocular conditions such as neovascular age-related macular degeneration, retinopathy of prematurity, proliferative diabetic retinopathy and diabetic macular edema, and other retinal diseases associated with abnormal blood vessel growth.²⁰ In human RB cell xenografted mice, intraperitoneal injections of bevacizumab caused a 2-fold reduction in microvasculature density and a 75% reduction in RB growth (Table 2).¹⁹ Bevacizumab reduced tumor size in a dose-dependent manner and at doses that were safe and tolerable in mice.¹⁹ The tumors treated with bevacizumab also had lower vascularity.¹⁹ Bevacizumab reduced tumor size purely by decreasing VEGF levels and did not have any direct effect on the apoptosis of endothelial cells or RB cells.¹⁹

Aflibercept is an anti-VEGF recombinant antibody that is indicated for neovascular age-related macular degeneration, diabetic retinopathy, diabetic macular edema, and retinopathy of prematurity.¹⁸ In a subcutaneously xenografted mouse model, RB tumors treated with intraperitoneal aflibercept contained fewer blood vessels and increased apoptotic cells (Table 2).¹⁸ In addition, an intravitreally injected orthotopic mouse model showed that intraperitoneal aflibercept reduced subretinal invasion and improved vitreous haziness, indicating decreased RB cell proliferation.¹⁸ In vitro, aflibercept reduced RB-induced HUVEC proliferation.¹⁸

Tigecycline is a tetracycline antibiotic that inhibits bacterial growth through suppressing protein synthesis.²¹ Pre-clinical studies have revealed tigecycline as a novel anti-cancer drug effective at killing leukemias, renal cancer cells, and liver cancer cells.²¹ In three RB cell lines, tigecycline reduced proliferation and induced apoptosis (Table 2).²¹ Tigecycline also inhibited capillary formation in human retinal microvascular endothelial cells (HRECs).²¹ Tigecycline inhibited mitochondrial protein translation, causing mitochondrial dysfunction and oxidative damage in RB cells and HRECs.²¹ Moreover, tigecycline affected normal retinal pigment epithelial cells to a lesser extent than RB cells, which may indicate a favorable toxicity profile. However, further investigations using animal models are needed to determine tolerability and efficacy.

Nicosamide is an anthelmintic drug indicated for treatment of tapeworms. It has anticancer effects on myeloid leukemia, ovarian, and breast cancer cells.²² In an in vitro study using

RB cells, niclosamide induced apoptosis in a dose dependent manner through inhibition of the Wnt/ β -catenin pathway, which is involved in angiogenesis and caspase-mediated apoptosis (Table 2).²² Niclosamide inhibited the migration, proliferation, and survival of HRECs, ultimately reducing capillary formation.²² These results suggest that niclosamide may be a potent angiogenesis inhibitor as well as direct anticancer drug against RB.

Celastrol is a triterpenoid from a traditional Chinese medicine with anti-inflammatory, anti-angiogenic, and anti-cancer properties.²³ Celastrol loaded into nano-micelles (CNM) was able to reduce hypoxia-induced endothelial cell migration, chemotactic motility, and proliferation through reducing VEGF-A and hypoxia-inducible factor-1 α (HIF-1 α) (Table 2).²³ In RB tumor xenografted mice, intraperitoneal CNM injections substantially suppressed tumor growth, with decreased tumor vascularization in the CNM treated tumors and no observable adverse effects.²³ In a further study, celastrol-loaded reduction-sensitive nanoparticles accelerated celastrol uptake in RB cells and increased RB cell apoptosis.²⁴

5-Aminoimidazole-4-carboxamide riboside (AICAR) is an adenosine monophosphate (AMP) analogue that stimulates AMP-activated protein kinase (AMPK), which acts as a sensor for metabolic stress including hypoxia and long-term starvation.²⁵ Excess AMPK activation through AICAR mimics a state of severe metabolic stress and inhibits proliferation and induces apoptosis in multiple myeloma, neuroblastoma, glioblastoma, acute lymphoblastic leukemia, colon cancer, breast cancer, and prostate cancer cell lines.²⁵ In RB cell lines, AICAR also inhibited proliferation through S phase arrest and inducing apoptosis (Table 2).²⁵ AICAR further showed tumor suppressing ability in subcutaneous RB xenografted mice. Both tumor weight and tumor volume were reduced by around 50% after 28 days of intraperitoneal AICAR injection (Table 2).²⁵ Immunofluorescence staining of the tumor capillaries revealed significantly reduced vessel density in AICAR-treated tumors.²⁵ These results suggest that AICAR may be a potential treatment for RB through inducing apoptosis and inhibiting angiogenesis. In addition, other components of the AMPK pathway, such as the upstream tumor suppressor LKB1, may be therapeutic targets for RB treatment.

Fosbretabulin (combretastatin A-4 phosphate) is an experimental vascular-targeting drug that binds to tubulin and destabilizes microtubule formation in endothelial cells.²⁶ In the TAg-RB transgenic RB mouse model, subconjunctival fosbretabulin caused dose-dependent reduction in tumor vessel density and surface area (Table 2).²⁶ There was no evidence of local toxicity on histology or systemic toxicity.

Anecortave acetate (AA) is an angiostatic steroid that inhibits angiogenesis via multiple pathways, originally developed for treatment of neovascular age-related macular degeneration.²⁷ AA has also shown efficacy in TAg-RB. In 12-week-old mice, subconjunctival injections of AA reduced tumor size to the same degree as carboplatin (Table 2).²⁷ However, AA showed significantly lower efficacy in 16-week-old mice.²⁷ Thus, this may suggest better efficacy at earlier timing for AA or other anti-angiogenic drugs for RB treatment but further studies are needed to validate this.

Rapamycin (sirolimus) is a mTOR inhibitor used as an immunosuppressant and to treat lymphangioleiomyomatosis.²⁸ mTOR is involved in angiogenesis; inhibition of mTOR

reduces VEGF and HIF-1 α .²⁸ Subconjunctival injections of rapamycin in TAg-RB mice decreased tumor burden (Table 2), correlating with percentage of tumor hypoxia.²⁸ In addition, rapamycin targeted small-caliber mature vessels preferentially since there was no significant reduction in neovessels or large-caliber vessels compared to control.²⁸

Anti-Angiogenic Drugs for Enhancing Chemotherapy

Perhaps more promising than single-agent use, anti-angiogenic drugs may be an effective adjuvant to existing chemotherapy for RB. Combination therapies have shown synergistic effects, which suggest that anti-angiogenic drugs could be a chemotherapy sparing agent and reduce adverse effects of chemotherapy. In addition, there is some evidence demonstrating the potential for anti-angiogenic drugs to treat chemotherapy complications.

In RB xenograft mice, intraperitoneal carboplatin alone resulted in significant tumor volume reduction in 48% while carboplatin + bevacizumab resulted in 86%.²⁹ Bevacizumab also had a chemotherapy sparing effect by decreasing the dose of carboplatin needed to achieve similar tumor regression.²⁹ On a cellular level, carboplatin + bevacizumab increased apoptosis and inhibition of RB cell proliferation.³⁰ Intravitreal injections of carboplatin + bevacizumab were also used in a prospective study of 11 patients with refractory RB.¹⁷ Over 9 months, 7 patients achieved a reduction in vitreous seeds, tumor mass, and stabilized disease that did not need enucleation.¹⁷ Of the 4 patients that still required enucleation, histological examination showed no tumor invasion.¹⁷ Vitreous hemorrhage occurred in 1 patient while all other patients showed no complications.¹⁷ Future clinical studies should directly compare the effects of carboplatin + bevacizumab vs carboplatin alone.

Niclosamide also synergistically enhanced carboplatin inhibition of RB cells in vitro and in vivo. When niclosamide or carboplatin was administered to RB cells individually, both induced apoptosis in ~30% of RB cells. When administered together, apoptosis was induced in around ~100% of RB.²² In xenograft mice with subcutaneously implanted RB cells, the combination of niclosamide and carboplatin resulted in a tumor weight that was 1/3 of the weight when using both drugs alone.²²

Ranibizumab and aflibercept have also demonstrated potential for treating complications from systemic chemotherapy for RB. In a study of systemic chemotherapy complications, 94% of patients experienced retinal ischemia, 51% retinal detachment, 43% intravitreal hemorrhage, and 34% relapsed active tumor.³¹ Subsequent intravitreal injections of ranibizumab and aflibercept resulted in globe salvage of 51% of treated patients.³¹ However, further investigation is needed to validate these results and determine the mechanisms.

miRNAs as Angiogenic Targets for RB

Micro-ribonucleic acids (miRNAs) are small RNA transcripts not expressed as protein. They are known to regulate protein expression and abnormal levels of mRNA have been observed in numerous cancers, including RB.³² miRNAs may act as either oncogenes or tumor suppressors in RB, depending on the specific miRNA.³² Several miRNAs involved in the expression of angiogenesis factors have been shown to either promote or reduce tumor severity.

miRNA-106a showed potential for suppressing angiogenesis by downregulating HIF-1 α expression.³³ In RB cells, miRNA-106a overexpression decreased VEGF and HIF-1 α levels while miRNA-106a knockdown resulted in the opposite (Table 3).³³ Likewise, inhibition of miRNA-106a in RB cells lead to increased RB cell invasion and angiogenesis as assessed by tube formation assay.³³

In contrast, miRNA-92a-3p, miRNA-181b, and miRNA-224-3p promoted angiogenesis in RB through various mechanisms. Knocking down miRNA-92a-3p decreased proangiogenic factors VCAM1 and ICAM1 and reduced endothelial cell angiogenesis in HUVECs (Table 3).³⁴ Injecting exosomal miRNA-92a-3p into subcutaneous RB xenografted mice resulted in stronger blood flow, more endothelial cells, higher vascularity of the tumors, and ultimately larger tumor volume (Table 3).³⁴ miRNA-181b was upregulated in hypoxic RB cell cultures and stimulated capillary formation in HUVECs co-cultured with RB cells.³⁵ It downregulated the endothelial cell gene PDCD10 which suppresses angiogenesis.³⁵ miRNA-181b also repressed the GATA6 gene in endothelial cells which controls homeostasis of the ocular vasculature (Table 3).³⁵ In another study, miRNA-224-3p was found to be highly expressed in RB cell cultures and inhibited the expression of tumor suppressor gene LATS2.³⁶ LATS2 promotes apoptosis and inhibits angiogenesis in HUVECs through decreasing VEGF.³⁶ Correspondingly, miRNA-224-3p accelerated angiogenesis in HUVECs and repressed RB cell apoptosis through LATS2 inhibition (Table 3).³⁶ Thus, both downregulation of miRNA-224-3p and upregulation of LATS2 could be effective targets for angiogenesis-modulating RB treatment. Although promising, the understanding and evidence behind miRNAs as angiogenesis mediators in RB is still growing and further investigation is needed to clarify their therapeutic application.

Conclusions and Future Prospects

Angiogenesis is crucial for cancer progression and thus is likely a contributor to RB severity as well. There is promising evidence that angiogenesis signaling holds biomarkers and prognostic determinants for RB therapy response and progression. VEGF levels measured in AH may be the first high specificity and sensitivity assay to predict intravitreal chemotherapy response and RB prognosis. Since this biomarker can be measured before enucleation, there is considerable potential for VEGF levels to individualize treatment plans, ultimately reducing complications and bettering patient outcomes (Fig. 1). There are several existing anti-angiogenic drugs and experimental agents which may hold therapeutic value for RB patients. However, most of the literature in this area has not elicited further follow-up and there is limited evidence supporting the use of anti-angiogenic drugs as monotherapy for treating RB. Instead, there is perhaps greater potential for combination therapy with other chemotherapy agents. Further studies could determine whether adverse effects can be reduced by the chemotherapy sparing effect of anti-angiogenic drugs. Despite the long-known role for angiogenesis signaling in retinoblastoma, it may still yield new prognostic and therapeutic value.

Conflicts of interest and source of funding

The authors declare no conflicts of interest relevant to this manuscript. Related work in the laboratory of TWC is supported by NIH/NEI R01EY025641, R01EY031939, R01EY035159, NSERC RGPIN-2025-04563, the Retina

Research Foundation, the Canada Foundation for Innovation, and Research to Prevent Blindness. NO was supported by the Indiana University Melvin and Bren Simon Cancer Center and NIH/NIGMS 5R25GM060566.

References

1. Dimaras H, Corson TW. Retinoblastoma, the visible CNS tumor: A review. *J Neurosci Res.* 2019;97(1):29–44. doi:10.1002/jnr.24213 [PubMed: 29314142]
2. Kaliki S, Vempuluru VS, Fabian ID. Retinoblastoma with and without extraocular tumor extension: A global comparative study of 3435 patients. *Ophthalmol Sci.* 2025;5(2):100637. doi:10.1016/j.xops.2024.100637 [PubMed: 39758129]
3. Apte RS, Harbour JW. Inhibiting angiogenesis in retinoblastoma. *Ophthalmic Res.* 2007;39(4):188–190. doi:10.1159/000103578 [PubMed: 17556838]
4. Garcia JR, Gombos DS, Prospero CM, et al. Expression of angiogenic factors in invasive retinoblastoma tumors is associated with increase in tumor cells expressing stem cell marker SOX2. *Arch Pathol Lab Med.* 2015;139(12):1531–1538. doi:10.5858/arpa.2014-0262-OA [PubMed: 26619025]
5. Pe'er J, Neufeld M, Baras M, et al. Rubeosis iridis in retinoblastoma: Histologic findings and the possible role of vascular endothelial growth factor in its induction. *Ophthalmology.* 1997;104(8):1251–1258. doi:10.1016/s0161-6420(97)30150-x [PubMed: 9261311]
6. Ghalebandi S, Yuzugulen J, Pranjol MZI, Pourgholami MH. The role of VEGF in cancer-induced angiogenesis and research progress of drugs targeting VEGF. *Eur J Pharmacol.* 2023;949:175586. doi:10.1016/j.ejphar.2023.175586 [PubMed: 36906141]
7. Simons M, Gordon E, Claesson-Welsh L. Mechanisms and regulation of endothelial VEGF receptor signalling. *Nat Rev Mol Cell Biol.* 2016;17:611–625. doi:10.1038/nrm.2016.87 [PubMed: 27461391]
8. Dimaras H, Corson TW, Cobrinik D, et al. Retinoblastoma. *Nat Rev Dis Primers.* 2015;1:15021. doi:10.1038/nrdp.2015.21 [PubMed: 27189421]
9. Pekacka A, Figus M. The role of intraarterial chemotherapy in the management of retinoblastoma. *J Ophthalmol.* 2020;2020:1–16. doi:10.1155/2020/3638410
10. Daniels AB, Sishla KL, Bogan CM, et al. Aqueous VEGF-A levels as a liquid biopsy biomarker of retinoblastoma vitreous seed response to therapy. *Invest Ophthalmol Vis Sci.* 2024;65(6):18. doi:10.1167/iovs.65.6.18
11. Muniyandi A, Jensen NR, Devanathan N, Dimaras H, Corson TW. The potential of aqueous humor sampling in diagnosis, prognosis, and treatment of retinoblastoma. *Invest Ophthalmol Vis Sci.* 2024;65(1):18. doi:10.1167/iovs.65.1.18
12. Youssef NS, Said AM. Immunohistochemical expression of CD117 and vascular endothelial growth factor in retinoblastoma: Possible targets of new therapies. *Int J Clin Exp Pathol.* 2014;7(9):5725–5737. [PubMed: 25337214]
13. Wu Q, Sun X, Zheng G. VEGF overexpression is associated with optic nerve involvement and differentiation of retinoblastoma: A PRISMA-compliant meta-analysis. *Medicine (Baltimore).* 2018;97(51):e13753. doi:10.1097/MD.00000000000013753 [PubMed: 30572521]
14. Zhu J, Zhang X, Ai L, Yuan R, Ye J. Clinicohistopathological implications of MMP/VEGF expression in retinoblastoma: A combined meta-analysis and bioinformatics analysis. *J Transl Med.* 2019;17(1):226. doi:10.1186/s12967-019-1975-3 [PubMed: 31311559]
15. Marback EF, Arias VEA, Paranhos A, et al. Tumour angiogenesis as a prognostic factor for disease dissemination in retinoblastoma. *Br J Ophthalmol.* 2003;87(10):1224–1228. doi:10.1136/bjo.87.10.1224 [PubMed: 14507753]
16. Radhakrishnan V, Kashyap S, Singh L, Bakhshi S. VEGF expression in residual tumor cells in orbital retinoblastoma (IRSS stage III) treated with NACT: A prospective study. *Pediatr Blood Cancer.* 2012;59(3):567–569. doi:10.1002/pbc.24009 [PubMed: 22086846]
17. Hou X, Cheng Y, Zhang Q, Liang J, Li X. [Efficacy of intravitreal carboplatin plus bevacizumab in refractory retinoblastoma]. *Zhonghua Yan Ke Za Zhi.* 2015;51(2):126–129. [PubMed: 25908004]

18. Kim DY, Choi JA, Koh J-Y, Yoon YH. Efficacy and safety of aflibercept in in vitro and in vivo models of retinoblastoma. *J Exp Clin Cancer Res.* 2016;35(1):1–10. doi:10.1186/s13046-016-0451-7 [PubMed: 26728266]
19. Lee SY, Kim D-K, Cho JH, Koh J-Y, Yoon YH. Inhibitory effect of bevacizumab on the angiogenesis and growth of retinoblastoma. *Arch Ophthalmol.* 2008;126(7):953–958. doi:10.1001/archophth.126.7.953 [PubMed: 18625942]
20. Zong Y, Miyagaki M, Yang M, et al. Ophthalmic use of targeted biologics in the management of intraocular diseases: Current and emerging therapies. *Antibodies (Basel).* 2024;13(4):86. doi:10.3390/antib13040086 [PubMed: 39449328]
21. Xiong Y, Liu W, Huang Q, et al. Tigecycline as a dual inhibitor of retinoblastoma and angiogenesis via inducing mitochondrial dysfunctions and oxidative damage. *Sci Rep.* 2018;8(1):11747. doi:10.1038/s41598-018-29938-x [PubMed: 30082885]
22. Li Z, Li Q, Wang G, et al. Inhibition of Wnt/ β -catenin by anthelmintic drug niclosamide effectively targets growth, survival, and angiogenesis of retinoblastoma. *Am J Transl Res.* 2017;9(8):3776–3786. [PubMed: 28861168]
23. Li Z, Guo Z, Chu D, et al. Effectively suppressed angiogenesis-mediated retinoblastoma growth using celastrol nanomicelles. *Drug Deliv.* 2020;27(1):358–366. doi:10.1080/10717544.2020.1730522 [PubMed: 32091275]
24. Guo Z, Shi L, Feng H, et al. Reduction-sensitive nanomicelles: Delivery celastrol for retinoblastoma cells effective apoptosis. *Chin Chem Lett.* 2021;32(3):1046–1050. doi:10.1016/j.ccllet.2020.03.066
25. Theodoropoulou S, Brodowska K, Kayama M, et al. Aminoimidazole carboxamide ribonucleotide (AICAR) inhibits the growth of retinoblastoma in vivo by decreasing angiogenesis and inducing apoptosis. *PLoS One.* 2013;8(1):e52852. doi:10.1371/journal.pone.0052852 [PubMed: 23300996]
26. Escalona-Benz E, Jockovich ME, Murray TG, et al. Combretastatin A-4 prodrug in the treatment of a murine model of retinoblastoma. *Invest Ophthalmol Vis Sci.* 2005;46(1):8–11. doi:10.1167/iovs.04-0751 [PubMed: 15623747]
27. Boutrid H, Pina Y, Cebulla CM, et al. Increased hypoxia following vessel targeting in a murine model of retinoblastoma. *Invest Ophthalmol Vis Sci.* 2009;50(12):5537–5543. doi:10.1167/iovs.09-3702 [PubMed: 19578014]
28. Piña Y, Decatur C, Murray T, et al. Advanced retinoblastoma treatment: targeting hypoxia by inhibition of the mammalian target of rapamycin (mTOR) in LHBETATAG retinal tumors. *Clin Ophthalmol.* 2011;5:337–343. doi:10.2147/OPHTH.S16172 [PubMed: 21468343]
29. Assayag F, Nicolas A, Vacher S, et al. Combination of carboplatin and bevacizumab is an efficient therapeutic approach in retinoblastoma patient-derived xenografts. *Invest Ophthalmol Vis Sci.* 2016;57(11):4916–4926. doi:10.1167/iovs.15-18725 [PubMed: 27654418]
30. Zhang Q, Cheng Y, Huang L, et al. Inhibitory effect of carboplatin in combination with bevacizumab on human retinoblastoma in an in vitro and in vivo model. *Oncol Lett.* 2017;14(5):5326–5332. doi:10.3892/ol.2017.6827 [PubMed: 29098028]
31. Stathopoulos C, Gaillard M-C, Moulin A, et al. Intravitreal anti-vascular endothelial growth factor for the management of neovascularization in retinoblastoma after intravenous and/or intraarterial chemotherapy: Long-term outcomes in a series of 35 eyes. *Retina.* 2019;39(12):2273–2282. doi:10.1097/iae.0000000000002339 [PubMed: 30312257]
32. Chai P, Jia R, Li Y, et al. Regulation of epigenetic homeostasis in uveal melanoma and retinoblastoma. *Prog Retin Eye Res.* 2022;89:101030. doi:10.1016/j.preteyeres.2021.101030 [PubMed: 34861419]
33. Liu Y, Xin Z, Zhang K, Jin X, Wang D. LncRNA NEAT1 promotes angiogenesis of retinoblastoma cells through regulation of the miR-106a/HIF-1 α axis. *Heliyon.* 2024;10(6):e27653. doi:10.1016/j.heliyon.2024.e27653 [PubMed: 38524558]
34. Chen S, Chen X, Luo Q, et al. Retinoblastoma cell-derived exosomes promote angiogenesis of human vesicle endothelial cells through microRNA-92a-3p. *Cell Death Dis.* 2021;12(7):695. doi:10.1038/s41419-021-03986-0 [PubMed: 34257272]

35. Xu X, Ge S, Jia R, et al. Hypoxia-induced miR-181b enhances angiogenesis of retinoblastoma cells by targeting PDCD10 and GATA6. *Oncol Rep.* 2015;33(6):2789–2796. doi:10.3892/or.2015.3900 [PubMed: 25872572]
36. Song L, Huang Y, Zhang X, et al. Downregulation of microRNA-224–3p hampers retinoblastoma progression via activation of the Hippo-YAP signaling pathway by increasing LATS2. *Invest Ophthalmol Vis Sci.* 2020;61(3):32. doi:10.1167/iovs.61.3.32

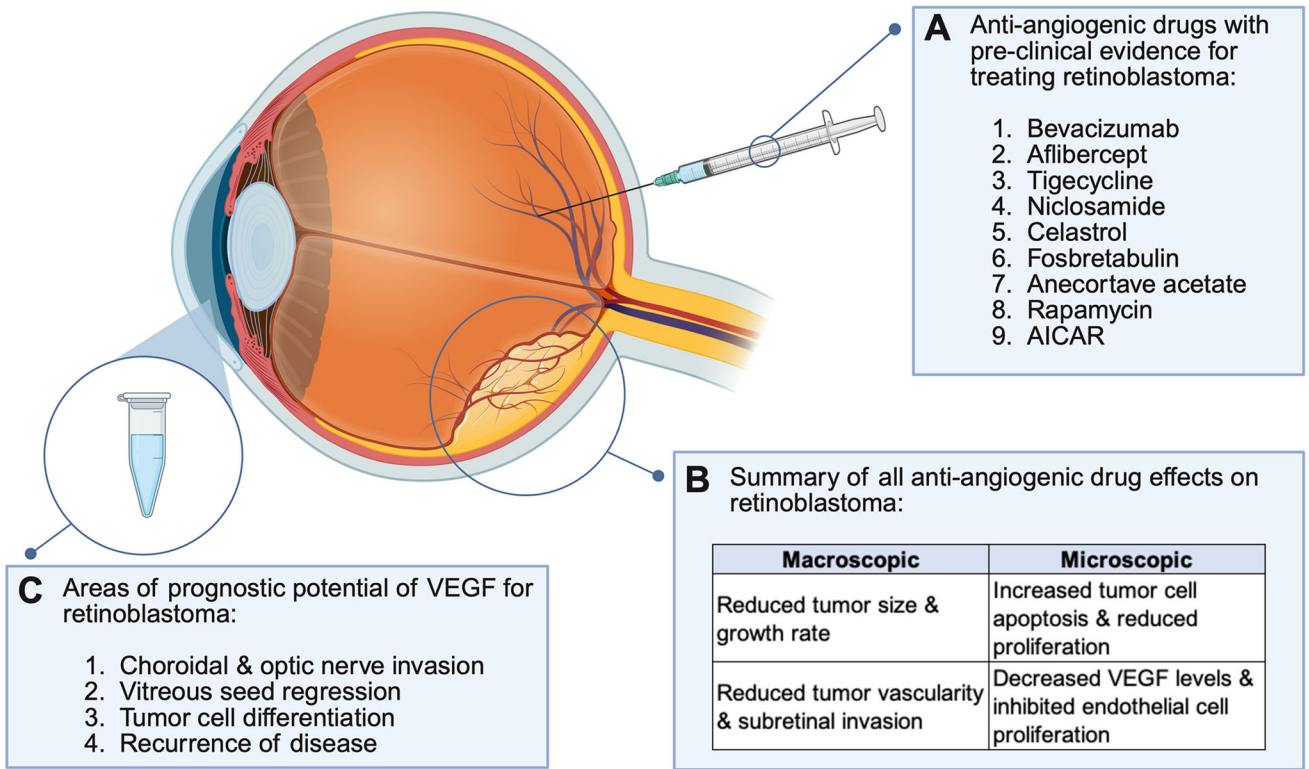


Fig. 1. Summary of anti-angiogenic drugs, effects, and prognostic potential of VEGF for retinoblastoma.

A) Existing anti-angiogenic agents that show pre-clinical efficacy for retinoblastoma. B) Pre-clinical effects demonstrated by anti-angiogenic drugs on retinoblastoma, both at the tumor level (macroscopic) and molecular level (microscopic). C) Prognostic variables that correlate with VEGF levels in retinoblastoma, with potential for assessment in aqueous humor.

Table 1: Summary of preclinical evidence on prognostic value of VEGF for retinoblastoma (RB).

| Prognostic Correlations | Pre-clinical evidence | Clinical evidence | Authors, year [reference] |
|--------------------------|--|--|--|
| Choroidal invasion | N/A | VEGF presence in human RB tumors correlated with choroidal invasion [12] | Youssef et al., 2014 [12] |
| Optic nerve invasion | N/A | Positive correlation between VEGF presence and c-Kit in human RB [12]; VEGF overexpression in human RB cells correlated with optic nerve invasion [13] | Youssef et al., 2014 [12] Wu et al., 2018 [13] |
| Vitreous seed regression | Reduced VEGF levels in aqueous humor (AH) of WERT-Rb1 xenograft rabbits after intravitreal melphalan, topotecan, or belinostat [10]; high <i>VEGFA</i> mRNA expression in vitreous seeds of xenograft rabbits [10] | High VEGF-A levels in AH of RB patients [17]; positive correlation between VEGF-A levels in AH and amount of viable vitreous seeds present in human RB [10] | Daniels et al., 2024 [10] Hou et al., 2015 [17] |
| Poor differentiation | N/A | Positive correlation between VEGF-A levels and stem cell marker Sox2 in human RB; VEGF overexpression in human RB cells correlated with less differentiation [4] | Garcia et al., 2015 [4] |
| Recurrence of disease | N/A | VEGF-negative human RB cells correlate with longer event free survival after chemotherapy and enucleation; post-combination therapy VEGF levels over 50 ng/L in AH correlated with disease recurrence [16] | Radhakrishnan et al., 2012 [16] |

Table 2: Summary of anti-angiogenic drugs in preclinical studies for retinoblastoma (RB).

| Drug Name | Drug Class | In vitro evidence | In vivo evidence | Authors, year [reference] |
|--------------------|--------------------------------|--|---|----------------------------------|
| Bevacizumab | anti-VEGF monoclonal antibody | Decreased VEGF levels in endothelial cells, no direct apoptosis of endothelial or Y-79 RB cells [19] | Reduced tumor size and vascularity in Y-79 RB xenografted mice [19] | Lee et al., 2008 [19] |
| Aflibercept | anti-VEGF recombinant antibody | Increased Y-79 RB cell apoptosis, reduced RB-induced endothelial cell proliferation [18] | Reduced subretinal invasion in Y-79 intravitreally injected orthotopic mice [18]; Reduced tumor vascularity and vitreous haziness in Y-79 subcutaneously injected xenografted mice [18] | Kim et al., 2016 [18] |
| Tigecycline | Tetracycline antibiotic | Reduced proliferation and increased apoptosis in Y-79, WERI-Rb-1, and RB116 cells [21]; inhibited endothelial cell proliferation [21] | N/A | Xiong et al., 2018 [21] |
| Niclosamide | Anthelmintic | Increased apoptosis in Y-79, WERI-Rb-1, and RB116 cells [22]; inhibited endothelial cell proliferation, migration, and survival [22] | N/A | Li et al., 2017 [22] |
| Celastrol | Cytotoxic triterpenoid | Increased apoptosis in SO-Rb 50 cells [23]; decreased VEGF and HIF-1 α levels, inhibited endothelial cell migration, chemotactic motility, proliferation [23] | Suppressed tumor growth, decreased tumor weight and vascularization in SO-Rb 50 subcutaneously injected xenografted mice [23] | Li et al., 2020 [23] |
| AICAR | AMP analogue | Induced S phase arrest and apoptosis in Y-79 RB cells [35] | Reduced tumor weight, volume, and vessel density in Y-79 subcutaneously injected xenografted mice [35] | Theodoropoulou et al., 2013 [25] |
| Fosbretabulin | Microtubule destabilizer | N/A | Reduced tumor size and vascularity in simian virus (SV) 40 large T antigen (TAG-RB) transgenic mice [26] | Escalona-Benz, 2005 [26] |
| Anecortave acetate | Angiostatic cortisene | N/A | Reduced tumor size in TAG-RB transgenic mice [27] | Boutrid et al., 2009 [27] |
| Rapamycin | mTOR inhibitor | N/A | Reduced number of small-caliber mature vessels and tumor burden in TAG-RB transgenic mice [28] | Piña et al., 2011 [28] |

Table 3:

Summary of miRNAs influencing retinoblastoma angiogenesis.

| | In vitro anti-angiogenic and anti-retinoblastoma (RB) evidence | Authors, year [reference] |
|---------------------|---|----------------------------------|
| miRNA-106a | Decreased VEGF and HIF-1 α levels in Y-79, SO-RB 50, and HXO-RB44 Cells compared to retinal pigment epithelial cells [33]; reduced Y-79 RB cell invasion and angiogenesis [33] | Liu et al., 2024 [33] |
| miRNA-92a-3p | Knockout decreased VCAM1 and ICAM1 in WERI-Rb1 cells [34]; reduced endothelial cell angiogenesis, increased blood flow, vascularity, and volume of tumors in WERI-Rb1 subcutaneously injected xenograft mice [34] | Chen et al., 2021 [34] |
| miRNA-181b | Downregulated PDCD10 and GATA6 gene and promoted angiogenesis in human RB cells [35] | Xu et al., 2015 [35] |
| miRNA-224-3p | Downregulated LATS2, promoted angiogenesis, decreased apoptosis in human RB cells [36] | Song et al., 2020 [36] |