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Vitreous Disease in Retinoblastoma: Clinical Findings and Treatment

Jasmine H. Francis, MD^{1,2}, Larissa A. Habib, MD¹, and David H. Abramson, MD.^{1,2}

¹Memorial Sloan Kettering Cancer Center, New York, NY, United States

²Weill Cornell Medical College, New York Presbyterian Hospital, New York, NY, United States

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INTRODUCTION

Retinoblastoma is the most common primary cancer to occur in the eyes of children. It has a frequency of 1 per 15,000 births, and this rate is relatively equal and stable worldwide. Retinoblastoma can either be unilateral or bilateral, and in this latter case is typically hereditary and associated with a germline mutation in the RB1 gene though most children who have bilateral disease are the first in the family to be affected. Hereditary cases typical present at the age of one year, and non-hereditary cases present at two years of age. Diagnosis occurs most commonly after the caretaker notices a “white glint” in the pupil of the eye. The ophthalmologist confirms the presence of white tumor(s) on dilated fundus examination.

The tumors derive from the cone precursor cells and as such, tumors grow out from the retina towards the vitreous cavity. On occasions, these retinal tumors can generate little pieces of tumor that break off and float in the vitreous cavity, so called “vitreous seeds”. Five years ago, active vitreous disease would have been an ominous sign. Due to a lack of adequate treatment, vitreous disease would likely commit the eye to a destiny of enucleation. In recent years, however, due to developments in treatment, eyes with vitreous seeds can be saved. From these advancements, our understanding of vitreous disease and its treatment has also deepened immensely. This article will outline these latest advances.

Corresponding author: Jasmine H. Francis, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10065, Telephone: 212 744 1700, francij1@mskcc.org.

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VITREOUS DISEASE

In his comprehensive ophthalmic oncology textbook published in 1963 entitled, “Tumors of the Eye”, Algernon Reese sums up his entire description of vitreous seeds in four sentences. His fundus exam was restricted to a direct ophthalmoscope, making the larger context of the vitreous cavity difficult to fully appreciate. He called them “globules of disseminated tumor seeds of a rather dull, light gray color...” emanating ...” from the site where tumor first breaks through its confines”. Amemiya and colleagues wrote a descriptive account of vitreous seeds in 1979, titling their account, “Vitreous seeds in retinoblastoma, clinical significance and ultrastructure”(1). They explained that approximately a third of retinoblastoma eyes contain vitreous seeds, and they are rare in eyes where the tumor encompasses less than one-quarter of the retina(1). Histopathologically, they label seeds as being either necrotic tumor cells or “almost intact” tumor cells(1).

However, describing vitreous disease with the blanket statement “seeds”, does not explain the variability that is observed clinically. Therefore, our group differentiates vitreous seeds into three class based on morphological characteristics: dust (class 1), spheres, (class 2), and clouds (class 3) (2,3). This classification system is useful as a clinical descriptor and allows for comparisons between eyes in the literature. (Table 1) Furthermore, as has recently been discovered, the classification system can be associated with distinct patient and tumor characteristics along with response to treatment(2,4).

Dust

Dust appears like tumor powder sprinkled over the tumor surface. These small granules of vitreous opacities can often be appreciated as haze (figure 1). One belief is that they originate from tumor cell displacement into the vitreous(3). They typically occur in children of the youngest age (median 11 months) compared to spheres and clouds(4). They can originate in all locations of the fundus and do not have preponderance between unilateral and bilateral disease(4). Characteristically, they are found in a localized distribution, typically overlying a retinal tumor(4).

In response to intravitreal melphalan, they receive the lowest amount of cumulative drug, least mean dose of drug, and the fewest number of injections. They respond in approximately 2 weeks following the first weekly injection, which is the shortest amount of time compared to other seeds. Dust typically regresses into being ophthalmoscopically undetectable, so-called type 0 regression pattern.

Spheres

Spheres are spherically shaped and can be interspersed with dust or clouds. They may be homogeneous, or have an outer shell that appears distinct from the inner core (this can be translucent or transparent) (figure 2). Some posit that spheres are the result of clonal expansion of tumor cells that have translocated into the vitreous(3,5). They typically occur in children who are not as young as those with dust, and not as old as children with clouds(4). Spheres predominantly originate from the macula-equator region of the fundus

and equally between children with unilateral and bilateral disease(4). They can be found in a localized and diffuse distribution (4).

Spheres regress in response to intravitreal melphalan at approximately 6 to 8 weeks, which is a medium length of time compared to dust and spheres. They receive significantly fewer injections and less melphalan than clouds for regression to become apparent.

Spheres initially disperse (also described as pseudo-growth) and then assume one of three regression patterns: undetectable by ophthalmoscopy, calcific (regression type I) or amorphous/hyperreflectile (regression type II) or a combination of the latter two (regression type III).

Clouds

Clouds appear as their name would suggest: as a conglomeration of vitreous opacities that are often so dense, they can be difficult to distinguish from a retinal tumor. They can be identified as a sheet or globule of seed granules. They can have wispy edges the way a cloud may diffuse outward in the sky (figure 3). Dust and spheres can sometimes be found. One theory suggests that they result from a large transfer of tumor cells into the vitreous(3).

Clouds typically occur in the oldest children, with a median age approaching 3 years (4). They predominantly originate from the most anterior portion of the fundus defined as the equator-ora region. They have an obvious preponderance of occurring in children with unilateral disease(4). Characteristically, they distribute in a diffuse manner (4).

Clouds take the longest time to regress (7–8 months), receiving more injections and a higher cumulative and mean dose of melphalan. Typically, they initially regress to calcific or amorphous granules. Over the long course, they eventually become undetectable to ophthalmoscopy. Due to their prolonged regression, these eyes are sometimes incorrectly believed to still contain active disease and may undergo unnecessary additional treatment (such as enucleation).

TREATMENT OF VITREOUS DISEASE

History

Historical treatments for retinoblastoma, such as external beam radiation and systemic chemotherapy, do not have admirable efficacy in treating vitreous seeds. This is true of primary vitreous disease, and particularly of recurrent disease. In the past, this led investigators to seek an effective delivery system for the treatment of vitreous disease. The crusade began with Ericson et al. in 1961 with the use of thioTEPA, which is an alkylating agent from aziridine and thiophosphoryl chloride(6,7). Two separate groups used the same agent again, three and then four decades later(8–10). In the following years a number of drugs and agents were used in intraocular injections for retinoblastoma including thymidine kinase delivered via virus particles(6,11), bevacizumab(9,10), methotrexate(5,12), carboplatin(12) and even topotecan(5,13). (Table 2)

Concurrently ophthalmic artery chemosurgery (OAC) (an intraarterial delivery of chemotherapy to the ostium of the ophthalmic artery) grew in popularity and has since become the first line treatment of retinoblastoma in the majority of high volume retinoblastoma centers. Compared to previous treatments, OAC has very good efficacy against vitreous seeds. However it is highly dependent on a skilled coordinated team and, even then, it is not effective for all viable vitreous disease. Following the initial work of the Japanese, Francis Munier led a re-introduction to the use of intravitreal melphalan, which has proven to be a powerful treatment for vitreous seeds. (Table 3)

The extensive experience of Kaneko and his colleagues spearheaded the movement towards the use of intravitreal melphalan(14,15). Using *in-vitro* experiments, they had shown melphalan to be an effective drug against retinoblastoma(14,16), and it became what they would use for both their selective ophthalmic arterial injection therapy and intravitreal methods. Since Munier et al. published on this subject in 2012, it has increasingly been used in numerous retinoblastoma centers (figure 4). In fact, of the 52 papers indexed in medline with “retinoblastoma” and “vitreous” in their title 60% of these (31 papers) have been published since 2011, demonstrating the recent popularity of the topic in the past 5 years. The efficacy rates for intravitreal melphalan in the treatment of retinoblastoma have been estimated at 60–100%, which is a staggering response compared to prior modalities.

The drugs that are infused during ophthalmic artery chemosurgery would be an obvious choice for intravitreal injections. Besides melphalan, this includes carboplatin and topotecan. The world’s literature on intravitreal carboplatin in humans consists of two cases: one received a single 3mcg injection and the second received a 6mcg injection. Neither eye showed a response to treatment and both were enucleated. On the other hand, due to its mechanism of action, topotecan is typically given in combination with other chemotherapeutic agents. Likewise, some groups combine 20mcg injections of topotecan with intravitreal melphalan. Centers have described a response to vitreous seeds from intravitreal topotecan alone, although these results are not consistent across all centers.

The technique has shifted in recent years and may vary slightly between institutions. It is typically given via a pars plana approach, approximately 3 mm from the limbus (although this may change depending on the child’s age) (figure 5). Initially given at a weekly interval, some centers have prolonged the interval to monthly. Thirty mcg was the dose that most centers would give at the start, but some are now lowering it closer to 20mcg. Finally, some centers combine intravitreal melphalan with either periocular or intravitreal topotecan in the hopes of obtaining an enhanced effect. (Tables 4 and 5)

OCULAR TOXICITY OF INTRAVITREOUS MELPHALAN

Before the era of intravitreal melphalan, management options for eyes with active vitreous disease was limited, and in at least half the cases was restricted to enucleation. Intravitreal melphalan has dramatically improved salvage rates for eyes with vitreous seeds. Eyes that were once removed are now routinely being salvaged. With this striking advancement comes the inquiry about vision, and specifically, the influence of intravitreal melphalan on visual function and globe retention.

Part of visual potential hinges on retinal toxicity and this data comes from both clinical and pre-clinical data. Intravitreal melphalan at a dose of 50µg in a human eye results in substantial retinal toxicity including retinal necrosis, vascular occlusion, neovascularization, gliosis and hemorrhage(17). In preclinical models, electroretinogram (ERG) measurements in rabbits eyes showed normal responses when directly injected with a human equivalent of 23µg of melphalan (18). Abnormal responses were found when a human equivalent dose of 35µg melphalan was infused via vitrectomy(12,19). This would imply that a human eye has a toxic threshold for melphalan that is between 23 and 35µg.

Electroretinogram: clinical

Our group has taken a particular interest in understanding the toxic effects of intravitreal melphalan. Every injection that we administer is paired with electroretinogram recordings, and as such, we have the largest data compilation on electroretinography responses. It has previously been shown (and recently confirmed) that by linear regression analysis, every 30µg melphalan injection, results in an ERG degradation of approximately 5µV (20,21). This equates to about a 5% decrease in retinal response for each injection. The toxic effect is thought to happen soon after the injection and is measurable at one week. It does not continue to degrade (nor improve) thereafter. Other contributing factors for toxicity have been explored. Neither age at first injection, weight at first injection, new injection clock hour, concomitant topotecan, concomitant local therapy, nor interval between injections were significantly associated with worsening toxicity(21). However, there is evidence to suggest that retinal pigment epithelial changes (akin to salt-n-pepper retinopathy and a result of the injection), more deeply pigmented eyes, and concomitant ophthalmic artery chemosurgery within one week of the intravitreal injection are significantly associated with a worsening of ERG response.

Electroretinogram: animal

These clinical findings have been confirmed in a collaborative effort with our Argentinian colleagues using a rabbit model(20). Twelve rabbits were given a human equivalent dose of 30µg. Degradations in ERG responses were found in all eyes that were treated with 3 weekly injections of intravitreal melphalan(20). Histopathological evaluation of the eyes revealed a severely atrophic retina with loss of photoreceptors and outer nuclear layers. Toxicity was even observed at the level of the optic nerve (histocytic-like cells containing granular material and disorganized nerve fibers and glial cells with scant blood vessels). Fundus examination was consistent with retinal toxicity such that, in the melphalan treated eyes, both funduscopy and fluorescein angiography revealed retinal whitening and sclerotic vessels(20). In rabbits and humans, melphalan injections given weekly and at a dose of 30µg, may result in a nonprogressive ERG decrease. (Table 6)

Clinical signs of toxicity

The literature reports varying degrees of ocular toxicity from intravitreal melphalan, occurring both in the posterior and anterior segment of the eye. The toxic effects can also be divided between those induced by the drug and those by the technique itself, irrespective of the drug. Smith reviewed 10 published cases, amassing 295 eyes that they divided between those received standard melphalan dose (8–30µg), and those that received high-dose

melphalan (greater than 30µg). Side effects were more common in the group receiving higher doses of melphalan(22).

Posterior Segment Toxicity

In the posterior segment, the most common consequence observed is retinal pigment epithelial changes (or salt-n-pepper retinopathy). This is thought to be a form of “chemical burn” from close proximity of the drug to the retinal surface. This abnormality is typically found near the location of injection site, and in rare cases can track down to the macula. Vitreous, subretinal, and intraretinal hemorrhages have also been observed. At extremely high drug doses, phthisis has been described(23).

Anterior Segment Toxicity

Consequences to the anterior segment have been described and include cataract, iris depigmentation and thinning, iris recession, a filtering conjunctival bleb, and focal scleromalacia with localized pigmentation(24). Like the posterior segment changes, these toxic effects are more common in the meridian of the injection where the drug concentration is highest. Finally, fluctuations in intraocular pressure have been described, from hypotony to elevation(6,7,9,23,25).

SYSTEMIC TOXICITY OF INTRAVITREOUS MELPHALAN

Information on the systemic toxicity of intravitreal melphalan is restricted to data from a single paper. In this study, 46 blood specimens were assessed for complete blood counts(20). There were no cases of grade 3 or 4 neutropenia, and this even included a group of patients that received concomitant ophthalmic artery chemosurgery. Further analysis showed that cumulative weekly injections were not associated with changes in absolute neutrophil count levels. This is what would have been predicted with a 30µg injection into the vitreous: this dose is less than the 0.4mg/Kg threshold for neutropenia that has been measured in patients treated with ophthalmic artery chemosurgery(26).

SAFETY OF INTRAVITREOUS INJECTIONS

Enhancing safety

Concerns about externalization of retinoblastoma with therapeutic intravitreal injections have been mitigated by the use of safety-enhanced techniques. Published literature report a low risk of extraocular extension: in a review of 1304 reported injections, only 2 cases had tumor along the needle tract that were attributable to the injection(27). From this, Smith et al. calculated the proportion of eyes with extraocular disease from intravitreal injections to be 0.007(27). The low risk may be the result of various techniques that have been implemented to improve safety, and which are described below.

Decreasing volume of drug and needle size

As Boyle’s law would predict, introducing volume into the closed space of the globe would increase pressure. From here, there are concerns that this would heighten the risk of effluxing intraocular contents outwards. As a result, there has been an initiative to use

increasingly small volumes of intravitreal drug (Table 1). For example, in the 1960's and 1990's, fluid volumes of 0.3 to 0.5mL were injected into retinoblastoma eyes(7,9). Since then, volumes of drug have been reduced to 0.1mL and even as low as 0.05mL(5,11,28). In a similar manner to drug volume, the size of the needle diameter has also progressively got smaller. It is believed a smaller puncture site results in less space for cells to escape. For example, the needles used in the 1960's measured 0.3mm in diameter(7). In the past five years, centers have been using either 30 or 32 gauge needles, the latter of which has an outer diameter measuring 0.24mm. Our group uses 33 gauge needles, which have an outer diameter of 0.21mm(11) (figure 6).

Lowering intraocular pressure

Using the same principle of Boyle's Law, there has been an effort to lower intraocular pressure in retinoblastoma eyes immediately before being injected. It is well recognized that intravitreal injections temporarily increase intraocular pressure. This was also recognized in the early work on injections for retinoblastoma. In the 1960's, Ericson et al. wrote, "an increase in pressure arises, disappearing after 15–20 minutes"(7). Chevez-Barrios et al. was the first to propose an anterior chamber paracentesis in 2005, as a way of lowering intraocular pressure in these retinoblastoma eyes(6). This was subsequently used in 2011 by Kivela and colleagues(5). Munier and colleagues reincorporated this technique and proclaimed the benefits of generating a transient hypotony to prevent efflux(8). Our group employs a less invasive technique by using digital massage to lower the intraocular pressure to lower than 10mmHg: this can be achieved typically within 1 minute, is performed immediately prior to sterilization, and can be conveniently confirmed with a handheld pressure-measuring device(11). It has the benefit of lowering intraocular pressure without the passage of an additional needle into a tumor-laden eye.

Injection site precautions

Sterilizing the needle injection site and even tract, is thought to help reduce the risk of extraocular tumor. Chevez-Barrios et al. utilized "a transcorneal approach" instead of the more widely used pars plana location, "to minimize the risk of tumor spread during the injection"(6). This technique obliged the needle to be inserted through the iris and zonules while avoiding the lens(6).

Another approach involves using supplementary techniques to sterilize the injection site area. For instance, Kivela et al. write about placing a swab at the injection site "to wipe away any vitreous from the needle when retracting it"(5). As an alternative, and as was first reported by Chevez-Barrios et al.(6), cryotherapy can be given at the needle site. Every group uses a slightly different method to deliver the cryotherapy. The Swiss group performs the freezing over the injection site in a triple-freeze-thaw fashion, after the needle is removed and without indenting the eye(8). Our group uses a modified technique: a single-freeze-thaw cycle is used to create an ice ball, which encompasses the needle insertion site prior to needle withdrawal, thereby sealing and sterilizing the puncture site and needle (figure 7)(20,21).

Another means of needle site sterilization involves depositing chemotherapy in the subconjunctival or subtenon space. Seregard et al. reported this after intravitreal thiotepea in which, “a small amount was left subconjunctivally at the injection site”(9). Smith et al. describe a couple of cases in which “subtenon” carboplatin was deposited to “mitigate the risk of orbital tumor seeding”(12). With their ample experience, the Japanese group report injecting a small amount of melphalan in the subconjunctival space as the needle is removed from the eye, with the intent of sterilizing this space.

Injection site

It has been recognized that appropriate selection of eyes and injection sites is an important strategy in reducing the risk of extraocular extension of tumor. For instance, Kivela et al. select an injection site “where the conjunctiva adhered to the sclera” in an area with some distance from a tumor focus(5). Munier et al. have used clinical exam and ultrasonic biomicroscopy to highlight a number of injection site exclusion criteria(14). Any location involving the following would be an inappropriate injection site: the presence of vitreous seeds, tumor, retinal detachment, an anterior hyaloid detachment, or invasion of the anterior or posterior chamber(14). Injecting into a site with one of these findings would be thought to increase the risk of escaped tumor cells or damage to the eye.

Water submersion/irrigation

Following the injection, some groups may take an additional precaution by “washing” the needle tract area with irrigating fluid. This was reportedly done copiously by Chevez-Barrios and colleagues(6). There is published data to highlight the necessity of choosing an optimal irrigating solution. One experiment used an in-vitro set-up to submerge retinoblastoma cells in two fluids. Deionized, distilled water resulted in cell non-viability by three minutes. By contrast, normal saline did not have the same tumoricidal effect, leaving a host of cells still alive at three minutes (29). This principle has been applied clinically, so that following the intravitreal injection, the eye is submerged in sterile water for three minutes. This step is thought to kill any escaped tumor cells. Following this, the ocular surface effluent may be collected for further analysis.

Ocular and needle washings

There is evidence to suggest that the escape of tumor cells after an intravitreal injection is a rare and unlikely event. In a study that analyzed the ocular surface washings of 200 injection and the needle washings of 202 injections, there were no tumor cells recovered(30). As would be expected, there were a few instances of squamous cells in the effluent specimens. One limitation with this analyzing technique is the possible inability of capturing effluxed cells that may have trapped in the subconjunctival space. Despite this, it is encouraging to find no escaped tumor cells with this method, and confirms published data showing a low risk of extraocular extension following intravitreal injections.

Other consideration

Even though there is evidence to show tumor cells rarely escape the eye, a couple of cases show that the intraocular cells and the adjacent vitreous may course closely to the needle

puncture site (31). Specifically, there are two cases where the vitreous seed became tethered to the internal injection site(31). In both cases, the seed regressed with additional intravitreal melphalan (31). One could speculate on the mechanism of seed tethering: perhaps the negative pressure generated by needle withdrawal results in the intraocular contents moving towards the injection site(31). It becomes clear that performing a 360-degree exam of the peripheral fundus is an important task in identifying and appropriately monitoring inadvertent seed tethering.

FUTURE INVESTIGATIONS

It is evident that intravitreal melphalan is currently the most effective treatment for vitreous seeds. Its wide adoption has still further increased our efforts at salvaging retinoblastoma eyes. However, it is equally apparent that intravitreal melphalan can be toxic to the retina in as little as a single injected dose. The future will ponder these two findings, and like much of cancer treatment, the future will attempt to find a drug and delivery method that is equally (if not more) effective than intravitreal melphalan, while less toxic to the eye and for vision.

Questions will arise not only on the optimal drug, but also on the delivery method. Is a sustained release option more advantageous: would it be as effective, less toxic, and safer, since it could also reduce the number of injections and thus penetrations into the eye? Perhaps future advancements will teach us more about the toxicity of the drug, and how to limit visual consequences.

Also important is our growing understanding of vitreous seeds. It would be of value to have in depth histopathological correlations of the vitreous seed classes for both naïve and treated and primary and recurrent seeds. Taking it one step further, the genetic analysis of the vitreous seed classes may prove invaluable in understanding pathology of vitreous disease and perhaps even guide future targeted treatment options.

CONCLUSION

Over the course of one hundred and fifty years, the patient survival rate for retinoblastoma increased from a dismal 5% to greater than 95%. Over the past decade, the ocular survival rate for retinoblastoma has made an equally staggering improvement from 5% to near 95% for advanced unilateral disease(32). This is in large part due to the ophthalmic artery technique, and also to the efficacy of intravitreal melphalan in successfully treating vitreous disease. Eyes that were once lost are now being saved. Now that vitreous seeds have stretched beyond being an ominous sign and a signal for salvaging efforts or enucleation, so too has our knowledge of them expanded. We now recognize three distinct classes of vitreous seeds. They can be distinguished based on morphologic characteristics, and these classes are also associated with particular tumor and patient characteristics, and with response to intravitreal melphalan. We are equipped with knowledge that we did not have 5 years ago: this is knowledge that can influence treatment plans, and that we can share with the parents of children with retinoblastoma to help guide them through the treatment course. Even though we have gained a deeper understanding of vitreous seeds and their treatment,

we are at the frontier of future investigations deepening and broadening this comprehension even further.

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Keypoints

- Vitreous seeds can be classified into three categories based on morphologic characteristics.
- Vitreous seed classes are associated with response to intravitreal melphalan, including amount of drug received and time to regression.
- Intravitreal melphalan is relatively safe with the adoption of safety techniques, but can be toxic to the retina as demonstrated in pre-clinical and clinical studies.
- The toxicity of intravitreal melphalan may be influenced by the extent of ocular pigmentation, and when given within 1 week of concomitant ophthalmic artery chemosurgery.

Figure 1.
An eye with class 1, dust, vitreous seeds

Figure 2.
An eye with class 2, spheres, vitreous seeds

Figure 3.
An eye with class 3, cloud, vitreous seeds

Figure 4.
A representative eye that has been prepped and draped prior to an injection of intravitreal melphalan

Figure 5.
A representative case where calipers are used to measure out 3mm: the distance from the limbus where the injection will be given

Figure 6.
A representative intravitreous melphalan injection using a 33-gauge needle

Figure 7.
A representative intravitreal melphalan injection where cryotherapy creates an ice formation that encompasses the needle site prior to withdrawal

Table 1

Classification of Vitreous Seeds

Class	Type	Description	Response to Melphalan	Median Age (mths)	Location	Distribution
Type 1	Dust	-Small granules of vitreous opacities	2–3 weeks to regress. Requires least drug/injections	11	All	Localized
Type 2	Spheres	-Vitreous haze overlying tumor -Spherically shaped in vitreous -Dust may be present around spheres -Opaque or with opaque outer shell	6–7 weeks to regress. Requires medium amount of drugs/injections	15.5	Macula-equator	Localized/diffuse
Type 3	Cloud	-Dense collection punctate opacities -Sheet or globule of seed granules; often wispy edge -Dust and spheres may also be visible	30–32 weeks to regress. Requires most amount drugs/injections	32	Equator/ora	Diffuse

Table 2

Historical Intravitreal Injections

Author	Year	Injections (no.)	Needle	Drug	Dose	Volume
Ericson et al.	1961	6	0.3mm	Thio-tepa	NR	0.3 (mL)
Seregard et al.	1995	3	NR	Thio-tepa	2mg	0.5 (mL)
Lee et al.	2003	1	NR	Thio-tepa	NR	NR
Chevez- Barrios et al.	2005	21	30g	Thymidine kinase	10 ⁶ 11 vp	NR
Wells and Hubbard	2011	1	NR	Bevacizumab	NR	NR
Kivela et al.	2011	51	30 g	MTX	400µg	0.1 (mL)

Table 3

Intraarterial Chemotherapy for Vitreous Seeds

Author	Year	Primary or secondary	Number of eyes	Mean Number intra-arterial chemotherapy sessions	Drug	Percent control
Shields et al.	2014	Primary	39	3	Melphalan, +/- topotecan, +/- carboplatin	87
Francis et al.	2015	Secondary	17	2	Melphalan, +/- topotecan, +/- carboplatin	73.90
Abramson et al.	2012	Primary	76			64

Table 4

Current Intravitreal Injections

Author	Year	injections (no.)	Needle	Drug #1	Dose #1	Drug #2	Dose #2	Volume (mL)
Munier et al.	2012	135	32 g	Melphalan	8–30µg			0.1
Smith et al.	2012	2	32g	Carboplatin	3–6µg			0.05–0.1
Ghassemi et al.	2012	33	30 g	Melphalan	8–50µg			0.05
Sun et al.	2013	6	30/32g	Melphalan	20mg			0.05
Francis et al.	2013	107	33g	Melphalan	30µg			0.07
Shields et al.	2014	55	31g	Melphalan	20µg			0.5
Ghassemi et al.	2014	40	30g	Melphalan	40µg	Topotecan	8–20µg	0.08
Tuncer et al.	2015	14	30g	Melphalan	20µg			0.1
Suzuki et al.	2015	1067	27/30/32g	Melphalan	8,16,24µg			0.2,0.1,0.05
Shields et al.	2016	192	32g	Melphalan	20–30µg	Topotecan	20µg	0.5
Kiratli	2016	70	30g	Melphalan	20–40µg			0.1
Ji et al.	2016	123	30g	Melphalan	20µg			0.1
Francis et al.	2017	630	33g	Melphalan	25–30µg	Topotecan	20µg	0.05–0.072

Table 5**Efficacy of Intravitreal Injections**

Author	Year	Injections (no.)	eyes (no.)	Drug	Dose	Result	Mean length of follow up
Ericson et al.	1961	6	6	Thio-tepa	NR	83% temporary regression, 50% globe salvage	10.5 months
Seregard et al.	1995	3	3	Thio-tepa	2mg	66% regression after vitrectomy, 67% globe salvage	53.7 months
Lee et al.	2003	1		Thio-tepa	NR	Enucleation	NA
Chevez-Barrios et al.	2005	21	8	Thymidine kinase	10 ¹¹ vp	87.5% resolution vit seeds, 100% enucleated for progression retinal tumor	13.3 months
Wells and Hubbard	2011	1	1	Bevacizumab	NR	progression	NA
Kivela et al.	2011	51	2	MTX	400µg	Complete response at 2 months; 1 enucleation for choroidal relapse	40 months
Munier et al.	2012	122	23	Melphalan	8–30µg	Complete resolution 91.3%; globe salvage 87%	22 months
Smith et al.	2012	2	2	Carboplatin	3–6µg	Enucleation	37 months
Ghassemi et al.	2012	33	12	Melphalan	8–50µg	60% complete control of vitreous seeds at >6 months	37 months
Sun et al.	2013	6	1	Melphalan	20mg	Complete resolution vitreous seeds	12 months
Francis et al.	2013	107	16	Melphalan	30µg	Complete resolution in 92%	5 months
Shields et al.	2014	55	11	Melphalan	20µg	Complete resolution in 100%	9 months
Ghassemi et al.	2014	40	9	Melphalan/Topotecan	40µg/8–20µg	Complete resolution in 100%, globe salvage in 67%	15 months
Tuncer et al.	2015	14	7	Melphalan	20µg	Complete resolution in 100%, globe salvage in 86%	20 months
Suzuki et al.	2015	1067	264	Melphalan	8,16,24µg	Complete control in 68%	125 months
Shields et al.	2016	192	40	Melphalan	20–30µg	Complete resolution vitreous seeds in 100%, enucleation in 12.5% for subretinal seeds	36 months
Kiradi et al.	2016	70	39	Melphalan	20–40µg	Complete resolution in 69.2%	6–38 months
Ji et al.	2016	123	19	Melphalan	20µg	Complete control in 84.16%, globe salvage in 73.6%	27 months
Francis	2017	630	130	Melphalan/Topotecan	25–30µg/	Disease free survival 86.2%, globe salvage 94.2%	24 months

Table 6

ERG Responses to Intravitreal Injections

Author	Year	Subject	Method	Medication	Dose (Human eq)	ERG change
Ueda	1995	Rabbit	in vitro	Melphalan	23µg	No effect
				Melphalan	46µg	Moderate change
				Melphalan	210µg	Greatly deteriorated
Shimoda	2002	Rabbit	PPV	Melphalan	17.5µg/ml	No effect
				Melphalan	35µg/ml	a- and b-wave amp dec
				Melphalan	70µg/ml	a- and b-wave amp dec
Francis	2014	Human/Rabbit	Intravitreal	Melphalan	30µg	–5.8 µV/decrease in a and b-wave amplitude
Francis	2016	Human	Intravitreal	Melphalan/Topotecan	25–30µg/20µg	–5.3µV