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The Classification of Vitreous Seeds in Retinoblastoma and Response to Intravitreal Melphalan

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Purpose: To evaluate the clinical characteristics of the 3 classifications of vitreous seeds in retinoblastoma—dust (class 1), spheres (class 2), and clouds (class 3)—and their responses to intravitreal melphalan.

Design: Retrospective, bi-institutional cohort study.

Participants: A total of 87 patient eyes received 475 intravitreal injections of melphalan (median dose, 30 μg) given weekly, a median of 5 times (range, 1−12 times).

Methods: At presentation, the vitreous seeds were classified into 3 groups: dust, spheres, and clouds. Indirect ophthalmoscopy, fundus photography, ultrasonography, and ultrasonic biomicroscopy were used to evaluate clinical response to weekly intravitreal melphalan injections and time to regression of vitreous seeds. Kaplan−Meier estimates of time to regression and ocular survival, patient survival, and event-free survival (EFS) were calculated and then compared using the Mantel−Cox test of curve.

Main Outcome Measures: Time to regression of vitreous seeds, patient survival, ocular survival, and EFS.

Results: The difference in time to regression was significantly different for the 3 seed classes ($P < 0.0001$): the median time to regression was 0.6, 1.7, and 7.7 months for dust, spheres, and clouds, respectively. Eyes with dust received significantly fewer injections and a lower median and cumulative dose of melphalan, whereas eyes with clouds received significantly more injections and a higher median and cumulative dose of melphalan. Overall, the 2-year Kaplan−Meier estimates for ocular survival, patient survival, and EFS (related to target seeds) were 90.4% (95% confidence interval [CI], 79.7−95.6), 100%, and 98.5% (95% CI, 90−99.7), respectively.

Conclusions: The regression and response of vitreous seeds to intravitreal melphalan are different for each seed classification. The vitreous seed classification can be predictive of time to regression, number, median dose, and cumulative dose of intravitreal melphalan injections required. *Ophthalmology 2015;■:1−7 © 2015 by the American Academy of Ophthalmology.*

The current literature on retinoblastoma emphasizes vitreous seeding as the primary reason for treatment failure and loss of the eye.[1] In fact, vitreous seeds have been recognized as the defining feature for failure by the Reese and Ellsworth classification group (Vb)[2] and the International Classification of Retinoblastoma group (D).[3] With the increased adoption of intravitreal melphalan, salvage rates for eyes with vitreous seeds are surpassing all historical data.[4] However, describing vitreous disease with the blanket statement "seeds" does not capture the clinical heterogeneity that is seen; furthermore, we have noted that there is a spectrum of responses to intravitreal melphalan. Thus, our group believes differentiating vitreous seeds into 3 categories is a strong clinical tool. We have proposed a classification scheme[5] as a means of distinguishing among vitreous seeds to aid in the interpretation of disease and enhance reporting in the literature.

Our classification system is based on morphologic features of seeds and divides vitreous seeds into 3 groups: dust (class 1), spheres (class 2), and clouds (class 3).[5] In this study, we evaluate the clinical response of vitreous seeds to intravitreal melphalan to establish and define the clinical characteristics of each seed classification.

Methods

This institutional review board−approved study included all eyes that received intravitreal melphalan for vitreous disease at Jules-Gonin Hospital and Memorial Sloan-Kettering Cancer Center between September 2009 and April 2014. Informed consent was obtained for each patient from their guardian, caregiver, or parent. The study was Health Insurance Portability and Accountability Act compliant. Research adhered to the tenets of the Declaration of Helsinki.

After induction of anesthesia, the intraocular pressure was lowered with an anterior chamber paracentesis or by digital massage. Intravitreal melphalan (20−40 μg in 0.05 to 0.15 ml) was injected through the conjunctiva, sclera, and pars plana with a 32- or 33-gauge needle. Upon needle withdrawal, the injection site was sealed and sterilized with cryotherapy and the eye was shaken in all directions during cryo-application, as previously described.[4] In cases performed at Memorial Sloan-Kettering Cancer Center, the ocular surface was submerged in irrigating sterile water for 3 minutes.[6]

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Table 1. Summary of Vitreous Seed Classification and Clinical Findings

Class	Type	Description	Regression Characteristics	Median Time to Regression (wks)	Median No. of Injections	Median Melphalan Dose (μg)
Type I	Dust	Small granules of vitreous opacities Can be seen as a vitreous haze overlying tumor	Typically regress to type 0 (not visible)	2–3	3	20
Type 2	Spheres	Spherically shaped opacities within vitreous Dust may be present around spheres Can be homogenously opaque or have a translucent outer shell with relatively transparent or whitish center	Initially disperse (pseudo-growth) and then disappear, but can become calcific (type I), amorphous (type II), or a mixture of types I and II (type III)	6–7	5	30
Type 3	Cloud	Dense collection of punctate vitreous opacities Can appear as a sheet or globule of seed granules and often with wispy edge Dust and spheres are sometimes also visible	Initially disperse (pseudo-growth), become calcific, or disappear, but can remain calcific (type I) or amorphous (type II)	12–14	8	33

The clinical status was evaluated under anesthesia with indirect ophthalmoscopy, RetCam fundus photography (Clarity, Pleasanton, CA), B-scan ultrasonography (OTI Scan 2000; Ophthalmic Technologies, North York, Ontario, Canada), and ultrasonic biomicroscopy (OTI Scan 2000; Ophthalmic Technologies). At each subsequent examination, the burden of residual disease was reevaluated and intravitreal melphalan given every 7 to 10 days up to 12 injections.

Patient data included age, sex, laterality, age at start of injection course, eye status (salvaged or enucleated), life status (alive or dead), treatment status (naïve vs. prior treatment with systemic chemotherapy or external beam radiation), and follow-up time from beginning of injection course. Treatment data included time from initial injection to final regression, number of injections, cumulative/mean dose of melphalan, prior treatment with systemic chemotherapy, ophthalmic artery chemosurgery (OAC) or radiation (plaque brachytherapy or external beam), concomitant OAC defined as occurring within 1 month before initial injection or 1 month after final injection completion, concomitant focal treatment (laser or cryotherapy) performed at the time of injection but exclusive of the injection site cryotherapy, additional treatment *related* to the target vitreous disease for which the eye was receiving injections, and additional treatment *unrelated* to the target vitreous disease for which the eye was receiving injections (e.g., retinal tumor recurrence or a different focus of vitreous disease). Tumor data included the International Classification,[3] final seed regression pattern (type 0 = not visible, type 1 = calcific, type 2 = amorphous, type 3 = types 1 and 2),[5] seed classification at presentation (class 1 = dust, class 2 = spheres ± dust, or class 3 = clouds ± spheres or dust), and extent of disease (localized [≤1 quadrant] or diffuse [>1 quadrant]).

Outcome measurements were compared for all 3 seed classifications and included time to final regression, ocular survival, patient survival, and ocular event-free survival (EFS) *related* and *unrelated* to target vitreous seeds. Time to regression was calculated as the time from the initial injection to the first date of examination when regression was noted. For ocular EFS, an event was defined as recurrent (or new) disease that required additional focal treatment, OAC, radiation, intravitreal melphalan, or enucleation. Time to regression was compared for extent of disease, concomitant OAC, radiation and focal treatments, treatment status, number of injections (≤5 vs. >5), cumulative dose of melphalan (<160 vs. ≥160 μg), and mean dose of melphalan (<30 vs. ≥30 μg). Statistical analysis was performed using Prism (GraphPad Software, Inc, La Jolla, CA). Kaplan–Meier survival data with log-rank test was used to evaluate ocular and progression-free survival, and the Mantel–Cox test was used to compare curves.

Statistical analysis was performed with linear regression analysis, 2-tailed Student *t* test, and analysis of variance using GraphPad software (GraphPad Software Inc.) and NCSS software (NCSS, Kaysville, UT).

Results

A total of 35 patients had unilateral disease, and 52 patients had bilateral disease, of whom 30 were monocular. The median follow-up was 20.3 months (range, 2–56 months), and the median age at

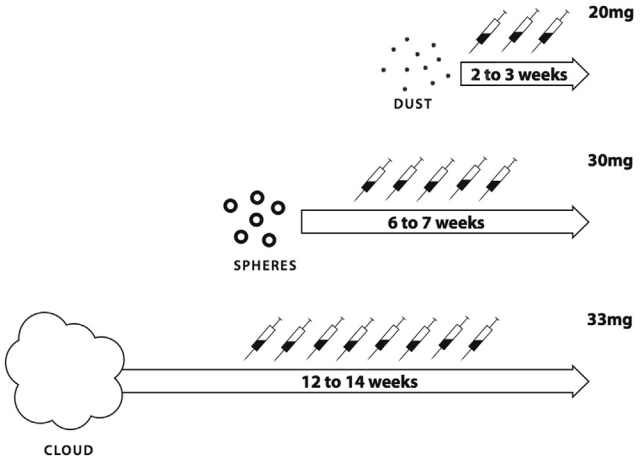

Figure 1. Illustration summarizing vitreous seed classification and response to intravitreal melphalan: number of injections received, time to response, and mean dose of melphalan per injection.