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CHILDREN'S ONCOLOGY GROUP

ARET12P1

A Multi-institutional Feasibility Study of Intra-Arterial Chemotherapy Given in the Ophthalmic Artery of Children with Retinoblastoma

A Limited Institution Pilot Study

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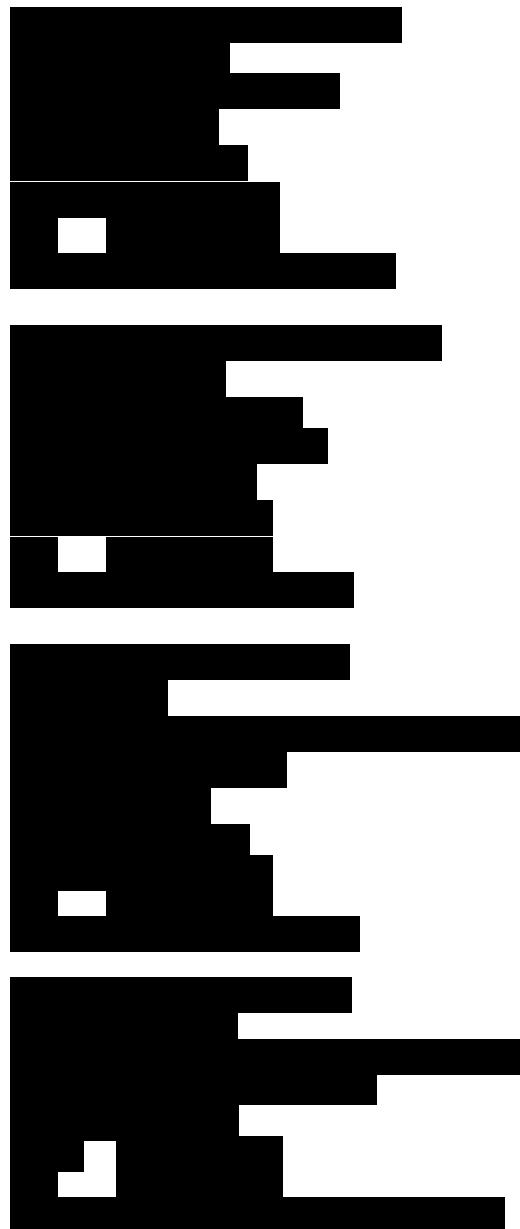
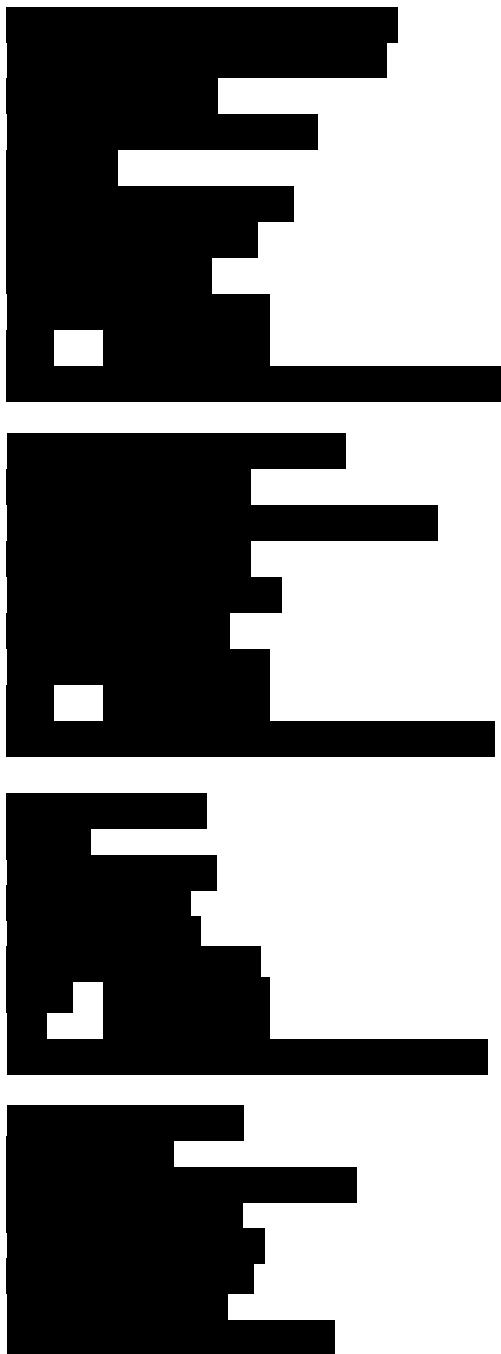
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AGENT	NSC#	AND	IND#'
Melphalan	008806		Exempt

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ABSTRACT

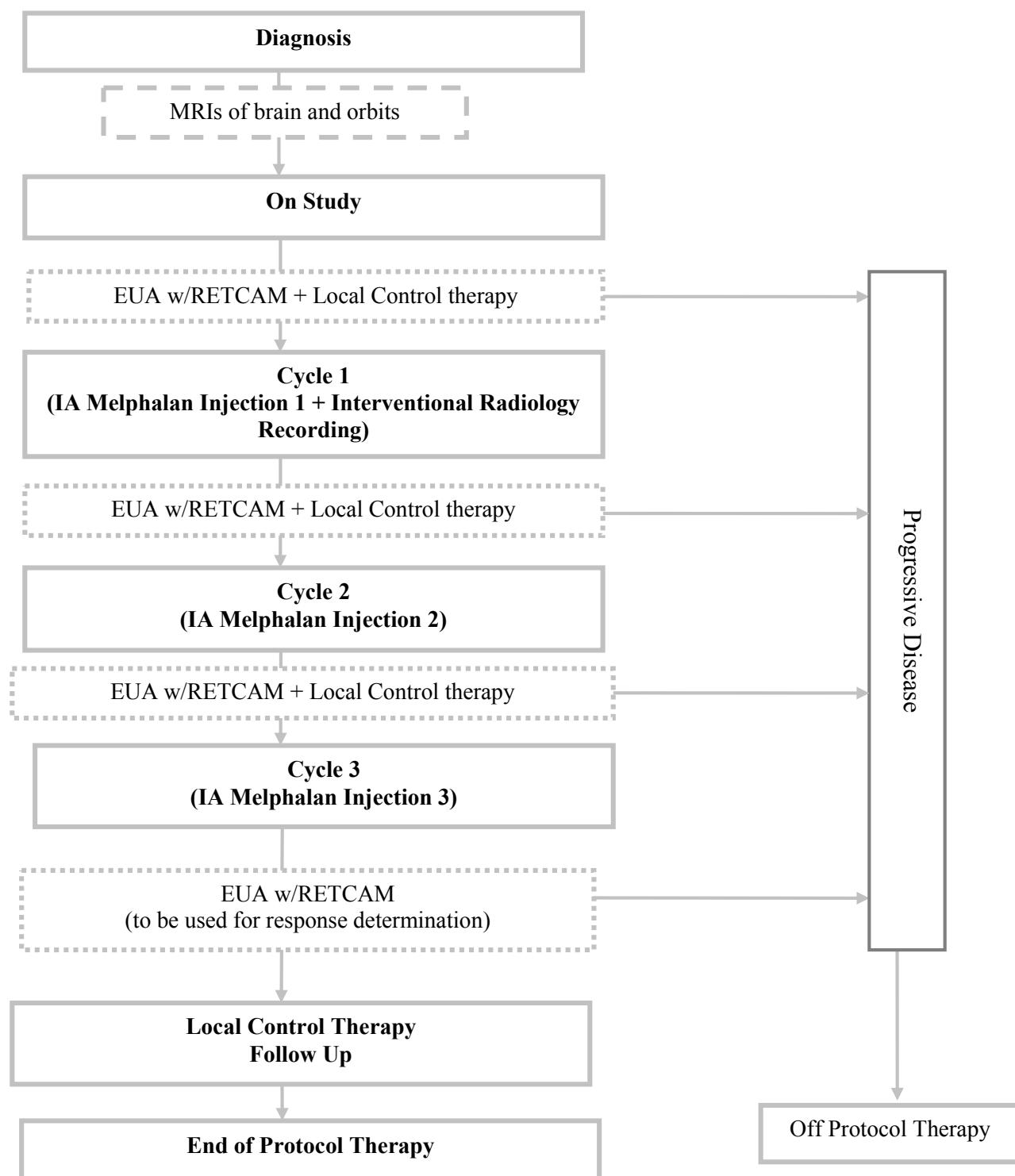
Retinoblastoma is the most common primary intra-ocular malignancy in childhood. Two-thirds of children diagnosed with retinoblastoma have disease in one eye only (unilateral disease). About 85% of the children with unilateral retinoblastoma have advanced disease at the time of diagnosis and the standard of care in these children is enucleation of the eye. Although this procedure is curative in over 90% of children, there is a desire among many families to preserve the eye. Furthermore, some of these eyes have useful vision that is lost with enucleation.

Systemic chemotherapy in combination with local ophthalmic therapy has been used with the goal of avoiding enucleation. Studies have shown that chemoreduction and local therapy do not provide effective control in patients with advanced unilateral disease and a high proportion of these patients eventually require either external beam radiation therapy or enucleation.

Localized delivery of chemotherapy directly into the ophthalmic artery may increase ocular salvage and result in less toxicity than systemic chemotherapy. However, this complex technique requires a high level of expertise that is currently available only at large pediatric centers with excellent infrastructure. Several institutions within the United States and in other countries are currently using this modality. As more centers engage in this treatment toxicities are being reported, including acute vitreous hemorrhage, chorioretinal atrophy, and ophthalmic artery stenosis. A properly designed prospective study to evaluate the feasibility and efficacy of this procedure in the context of a multi-institutional study is a critical next step towards the goal of establishing guidelines for the safe implementation of the intra-arterial technique across COG sites.

ARET12P1 is designed to test the feasibility of delivering melphalan directly into the ophthalmic artery in children with newly diagnosed unilateral retinoblastoma with Group D disease, by the International Classification System for Intraocular Retinoblastoma, who would otherwise be considered for enucleation. Response to therapy and toxicity of the regimens will also be assessed. This study will be the first attempt to perform a well-conducted clinical trial of intra-arterial therapy for unilateral retinoblastoma with emphasis on feasibility and efficacy in a multi-institutional trial.

EXPERIMENTAL DESIGN SCHEMA



1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Aims

- 1.1.1 To study the feasibility of delivering melphalan directly into the ophthalmic artery in children with newly diagnosed unilateral Group D retinoblastoma, who would otherwise be considered for enucleation.

1.2 Secondary Aims

- 1.2.1 To estimate the ocular salvage rate after treatment with intra-arterial melphalan in children with newly diagnosed unilateral retinoblastoma with Group D disease.
- 1.2.2 To evaluate the toxicities and adverse events associated with delivering multiple doses of intra-arterial chemotherapy.
- 1.2.3 To evaluate vision outcomes in children treated with intra-arterial chemotherapy.
- 1.2.4 To monitor the rate of the development of metastatic disease while on protocol therapy

1.3 Correlative Science Aims

- 1.3.1 To evaluate the effects of intra-arterial therapy on the histopathology of eyes enucleated for progression.

2.0 BACKGROUND

2.1 Introduction/Rationale for Development

Retinoblastoma is the most common primary intra-ocular malignancy in childhood. Two-thirds of children diagnosed with retinoblastoma have disease in one eye only (unilateral disease). About 85% of the children with unilateral retinoblastoma have advanced disease at the time of diagnosis and the standard of care in these children is enucleation of the eye. Although this procedure is curative in over 90% of children, there is a desire among many families to preserve the eye. Furthermore, some of these eyes have useful vision that is lost with enucleation.

Systemic chemotherapy in combination with one or more of other therapies such as cryotherapy and laser therapy have been used as alternative treatments for the prevention of metastatic disease and local recurrence and preservation of vision. External beam radiation or radioactive plaque therapy have also been used for effective orbital and intraocular disease control. Shields et al. have shown that chemoreduction alone in patients with advanced unilateral disease does not provide effective control of disease; in their study all such patients eventually required either external beam radiation therapy or enucleation.¹ Furthermore ocular oncologists strongly feel that to treat a patient with intraocular tumor(s) with systemic chemotherapy of several months duration with subsequent toxicities is not optimal.

In countries like Japan where there is particular emphasis by the families to preserve the globe, attempts were made by investigators to inject chemotherapy directly into the internal carotid artery with an inflated balloon above the origin of the ophthalmic artery.² The aim was to facilitate chemotherapy to enter the ophthalmic artery. The drug they chose for intra-arterial chemotherapy was melphalan based on in vitro studies.³ Using this technique Suzuki and Kaneko reported 563 injections in 187 patients with a low rate of complications. All of the eyes treated also received concomitant therapy with either hyperthermia, external beam radiation and/or, in 1 patient, intravitreal injections of melphalan. Investigators in the US have further developed the technique by performing selective cannulation of the

ophthalmic artery to deliver chemotherapy.⁴⁻⁷ In the most recent update, 95 eyes in 78 patients (30 unilateral, 48 bilateral) were treated with intra-arterial chemotherapy.⁷ The mean number of procedures was 3.2 per patient and 3.1 per eye. Chemotherapy included melphalan alone, or in combination with topotecan, and/or carboplatin. The ocular salvage rate was 81.7% for the 39 previously untreated eyes and 58.4% for the 56 R-E V eyes that had failed prior treatments. Catheterization was successful in 98.5% of patients. The ocular salvage rate was 81.7% for the 39 previously untreated eyes and 58.4% for the 56 R-E V eyes that had failed prior treatments. Catheterization was successful in 98.5% of patients. Neutropenia developed after 29 of 255 IA sessions (11.4%) and of these only 8 were grade 4; only one child required admission for fever and neutropenia. No child required blood products. All the children are alive including 2 who developed metastases, 7 and 9 months after enucleation.

Impressive as these results are, the heterogeneity of treatments administered as well as the lack of a proper study design to assess toxicity and outcomes in a prospective manner leave many questions unanswered. However several centers in the United States have started performing this procedure. Using this complex technique requires a high level of expertise that is only available at large pediatric centers with excellent infrastructure. Furthermore, as more centers are engaging in this treatment ocular toxicities are being reported, including acute vitreous hemorrhage, chorioretinal atrophy, and ophthalmic artery stenosis.^{8,9,10}

Therefore, a properly designed prospective study is felt to be critical. The COG Rare Tumors committee sponsored a meeting of ophthalmologists, interventional radiologists and pediatric oncologists from 10 large pediatric centers to design a study that could eventually establish guidelines for the safe implementation of the intra-arterial technique and to evaluate its feasibility and efficacy in the context of a multi-institutional study. It was agreed that patients with Group D disease, by the International Classification System for Intraocular Retinoblastoma, would be the appropriate treatment population and to avoid Group E eyes because of the possible increased incidence of high-risk factors for metastasis in these advanced tumors.^{11,12} On this study, there will be a rapid central review of RETCAM images at the time of enrollment and at the end of the 3rd cycle. Central review has worked very well within COG in the past. Of the 7 patients excluded from the study ARET0331 which was opened for patients with Group B disease, 6 had either Group C or D disease and therefore would have been significantly under-treated if they were enrolled on ARET0331.

Melphalan is an ideal drug for this technique. It does not need to be metabolized to an active agent and the half life of melphalan *oral* formulation is 90 minutes vs.75 minutes for the *intravenous* formulation. It is rapidly excreted mainly through the non-renal route. The dose used for intra-arterial injections for retinoblastoma is low and its efficacy in this setting has been proven by the above studies. In the largest and most recent report on IA therapy in children with retinoblastoma an initial dose finding phase was described where efficacy and toxicity were assessed during examination under anesthesia every 3-4 weeks after treatment.⁷ The recommended doses of melphalan from this study were 3 mg for patients between 6 and 12 months of age, 4 mg between the ages of 12 months and 24 months and 5 mg between 24 months and 36 months. Initial drug dosage was based on age which provides an estimate of the ocular size.^{13,14} However upon further review of the literature addressing growth in globe volume, we feel that consistent and uniform dosing across the ages will be obtained with 3 mg (6 to 11.99 months), 3.5 mg

(12-23.99 months), and 4 mg (24 months and older) doses (see figures 1, 2, and 3 below). These doses will also be consistent with the goal of not exceeding any single dose over 0.4 mg/kg which was recognized as the threshold for higher risk of developing Grade III/IV neutropenia (Ira Dunkel MD, personal communication 2013).

Ocular Volume vs Age

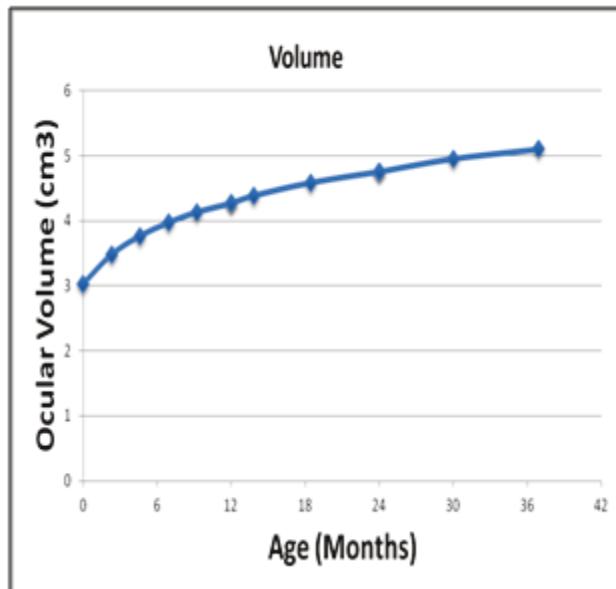


Fig. 1

Melphalan 3 – 4 – 5 mg dosing

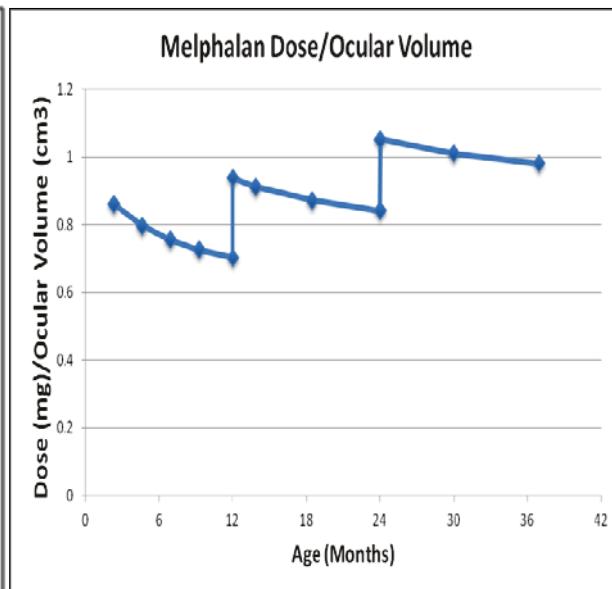


Fig. 2

Melphalan 3 – 3.5 – 4 mg dosing

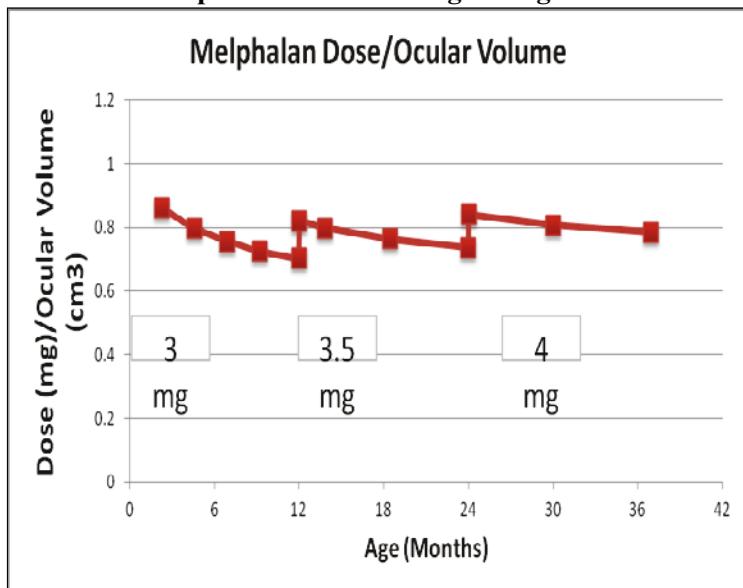


Fig. 3

2.2 Central Reviews for Eligibility, Assessment of Responses and IA Technique

Through previous COG protocols a central review mechanism was set up to review eligibility based on initial stage. In this study this mechanism will be used not only to confirm eligibility but also response at the end of the 3rd cycle. Three ophthalmologists with one alternate will be on the review panel to assess eligibility and three interventional radiologists with one alternate will review interventional radiology recordings of the first IA injection on every patient. The purpose of these reviews is to assess the technique of the individual interventional radiologist and the anatomy of the vasculature involving the ophthalmic artery. The reviewers may suggest a dose modification after studying the vasculature of the ophthalmic artery.

Several institutions within the United States and institutions in other countries (Italy, Switzerland, and Argentina) are using this modality. However this proposal will be the first attempt to perform a well-conducted clinical trial with emphasis on feasibility and efficacy in a multi-institutional trial.

There is an urgent need for the development of a prospective multi-institutional trial to test the feasibility of the technique in a cooperative group setting which may serve as a prelude to future studies with agents other than melphalan and more importantly help refine the delivery technique so the local toxicities can be minimized.¹⁵⁻¹⁷

2.3 Assessment of Vision

Historically and in a number of treating centers advanced unilateral retinoblastoma (Groups D/E) is managed with primary enucleation. As such any salvaged eye would have an improved visual acuity over controls.

Objective testing of vision in pre-verbal children at baseline is possible with methods such as Teller visual acuity. This approach requires a specialty trained technician monitoring the response of a cooperative child, as various striped cards are presented to the infant. Given the need to patch the normal eye in a newly diagnosed infant with unilateral disease many children will not be cooperative for this test and the measured acuity may be compromised. In some centers Teller vision assessment is not readily available and the quality of the technical support may vary. Requiring such a visual assessment at baseline may exclude some centers from participation or delay therapy from being initiated. Also the quality of data across institutions using this technique may render the results inconclusive.

Prior reports addressing vision in this cohort have looked at final visual acuity not the variance of vision from study entry to exit. In most cases the affected eye will present and remain amblyopic. The average age at study entrance will be 22-23 months but this will range on average from 6 months to 4 years. On this study, we will be assessing visual acuity once a year for 4 years, starting 1 year after completion of protocol therapy; most children should be between 3-5 years of age.

Options for testing visual acuity in the 3-5 year age range include: Snellen letters, Snellen numbers, Tumbling E, HOTV, and Allen figures. Each test is listed in decreasing order of cognitive difficulty. Given these criteria and age range we will be using the single surround HOTV – known as the amblyopia treatment study visual acuity protocol. The approach has been validated and has a high testability in 3-7 year olds. It is standardized and has been used in multi-center trials for amblyopia treatment.

We have consulted Dr M Repka a pediatric ophthalmologist with expertise in this field who has recommended the above testing method.

2.4 Histopathology of Eyes Enucleated for Progression

Eyes enucleated for progression will be processed and sent for central histopathology review by Dr Patricia Chevez-Barrios who is a leading expert in the pathology of Retinoblastoma. This will be the first prospective study of systematic assessment of histopathology features of eyes that are enucleated for progression following IA therapy.

A previous retrospective study of 8 eyes treated with intra-arterial chemotherapy for retinoblastoma has shown ocular complications that include thromboembolic events. In this study the eyes were enucleated for tumor viability, neovascular glaucoma, anaphylactic reaction from IAC, and persistent retinal detachment with poor visualization of the tumor.¹⁸ The tumor response ranged from minimal (n=1) to moderate (n=1) to extensive (n=4) to complete regression (n=2). Viable vitreous seeds (n=4 eyes), invasion into the optic nerve (n=3), reaching the lamina cribrosa in 2 cases, and invasion into the choroid (n=1) were observed. Histopathologic evidence of ischemic atrophy involving the outer retina and choroid was found in 4 eyes. One eye had extensive choroidal and outer retinal atrophy. This case showed orbital vascular occlusion and subendothelial smooth muscle hyperplasia. Intravascular birefringent foreign material was observed in 5 cases within occluded vessels, stimulating a granulomatous inflammatory response. The foreign material comprised cellulose fibers (n=3), synthetic fabric fibers (n=1), or unknown composition (n=2). Thrombosed blood vessels were identified in 5 eyes and involved ciliary arteries in the retrobulbar orbit (n=5), scleral emissarial canals (n=1), small choroidal vessels (n=1), and central retinal artery (n=1).¹⁸

3.0 STUDY ENROLLMENT PROCEDURES AND PATIENT ELIGIBILITY

3.1 Study Enrollment

3.1.1 Patient Registration

Prior to enrollment on this study, patients must be assigned a COG patient ID number.

This number is obtained via the COG Registry system once authorization for the release of protected health information (PHI) has been obtained. The COG patient ID number is used to identify the patient in all future interactions with COG. If you have problems with the registration, please refer to the online help.

In order for an institution to maintain COG membership requirements, every newly diagnosed patient needs to be offered participation in ACCRN07, *Protocol for the Enrollment on the Official COG Registry, The Childhood Cancer Research Network (CCRN)*, or APEC14B1, *Project:EveryChild A Registry, Eligibility Screening, Biology and Outcome Study*.

A Biopathology Center (BPC) number will be assigned as part of the registration process. Each patient will be assigned only one BPC number per COG Patient ID.

For additional information about the labeling of specimens please refer to the Pathology and/or Biology Guidelines in this protocol.

3.1.2 IRB Approval

Local IRB/REB approval of this study must be obtained by a site prior to enrolling patients. Sites must submit IRB/REB approvals to the NCI's Cancer Trials Support Unit (CTSU) Regulatory Office and allow 3 business days for processing. The submission must include a fax coversheet (or optional CTSU IRB Transmittal Sheet) and the IRB approval document(s). The CTSU IRB Certification Form may be submitted in lieu of the signed IRB approval letter. All CTSU forms can be located on the CTSU web page (<https://www.ctsu.org>). Any other regulatory documents needed for access to the study enrollment screens will be listed for the study on the CTSU Member's Website under the RSS Tab.

IRB/REB approval documents may be faxed (1-215-569-0206), E-mailed (CTSURegulatory@ctsu.coccg.org) or mailed to the CTSU Regulatory office.

When a site has a pending patient enrollment within the next 24 hours, this is considered a "Time of Need" registration. For Time of Need registrations, in addition to marking your submissions as 'URGENT' and faxing the regulatory documents, call the CTSU Regulatory Helpdesk at: 1-866-651-CTSU. For general (non-regulatory) questions call the CTSU General Helpdesk at: 1-888-823-5923.

3.1.3 Reservation Requirements

Patient enrollment for this study will be facilitated using the Slot-Reservation System in the Oncology Patient Enrollment Network (OPEN). Prior to discussing protocol entry with the patient, site staff must use the CTSU OPEN Slot Reservation System to ensure that a slot on the appropriate protocol stratum is available for the patient. Once a slot-reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study.

A reservation can be made by following the steps below:

- 1) Log in to <https://open.ctsu.org/open/> using your CTEP IAM user name and password.
- 2) In order to make a reservation, the patient must have an OPEN patient number. Click on the 'Slot Reservation' tab to create an OPEN patient number, under 'Patients'.
- 3) Using the OPEN patient number '**RESERVE**' a slot for that patient.
- 4) On the 'Create Slot Reservation' page, select the Protocol Number, enter the COG Patient ID, and choose the required stratum (if applicable) in order to obtain a reservation.

Refer to the 'SITE – Slot Reservation Quick Reference' guide posted under the 'Help' tab in OPEN for detailed instructions:

https://www.ctsu.org/readfile.aspx?fname=OPEN/OPEN_SlotReservation_QuickReference_SiteUserGuide_102612.pdf&ftype=PDF

Prior to obtaining informed consent and enrolling a patient, a reservation must be made following the steps above. Reservations may be obtained 24 hours a day through the OPEN system.

3.1.4 Study Enrollment

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). All site staff will use OPEN. OPEN is a web-based registration system available on a 24/7 basis. It is integrated with the CTSU Enterprise System for regulatory and roster data and, upon enrollment, initializes the patient position in the Rave database. The system can be accessed by entering credentials at <https://www.ctsu.org> and clicking on the OPEN tab, or by entering credentials at the OPEN portal URL <https://open.ctsu.org>.

Prior to accessing OPEN, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes. Site staff should use the registration forms provided on the group web site as a tool to verify eligibility.
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Access requirements for OPEN:

- Site staff will need to be registered with CTEP and have a valid and active CTEP-IAM account. This is the same account (user id and password) used for credentialing in the CTSU members' web site.
- To perform registrations, the site user must have been assigned the 'Registrar' role on the relevant Group or CTSU roster.

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

Further instructional information is provided on the CTSU members' web site OPEN tab or within the OPEN URL (<https://open.ctsu.org>). For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

3.1.5 Submission of Retcam Images and Retinal Drawing Prior to Study Entry

RetCam images and retinal drawings obtained from the diagnostic EUA **MUST** be submitted to IROC Rhode Island (QARC) within 7 days of the procedure for rapid central review. This is for confirmation of the diagnosis of Group D disease. The ARET12P1 Diagnostic EUA Eligibility Review Transmittal form must be included with the RetCam images and retinal drawings. This form can be found on the IROC Rhode Island (QARC) website, www.qarc.org. The central review committee will require a maximum of 7 days to return the review to the individual institution. Central review confirmation of Group D disease **MUST** be obtained before enrollment. See [Section 7.3.2](#).

3.1.6 Timing

Patients must be enrolled within 14 days of diagnostic EUA.

The date protocol therapy is projected to start must be no later than **five (5)** calendar days after the date of study enrollment.

All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated in the eligibility section below.

MRI of the brain and orbits must be performed within 14 days prior to enrollment.

3.2 **Patient Eligibility Criteria**

Important note: The eligibility criteria listed below are interpreted literally and cannot be waived (per COG policy posted 5/11/01). All clinical and laboratory data required for determining eligibility of a patient enrolled on this trial must be available in the patient's medical/research record which will serve as the source document for verification at the time of audit.

All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated. Laboratory values used to assess eligibility must be no older than seven (7) days at the start of therapy. Laboratory tests need not be repeated if therapy starts within seven (7) days of obtaining labs to assess eligibility. If a post-enrollment lab value is outside the limits of eligibility, or laboratory values are > 7 days old, then the following laboratory evaluations must be re-checked within 48 hours prior to initiating therapy: CBC with differential, bilirubin, ALT (SGPT) and serum creatinine. If the recheck is outside the limits of eligibility, the patient may not receive protocol therapy and will be considered off protocol therapy. Imaging studies, if applicable, must be obtained within 2 weeks prior to enrollment (repeat the tumor imaging if necessary).

See [Section 7.1](#) for required studies to be obtained prior to starting protocol therapy.

INCLUSION CRITERIA

3.2.1 Age

6 months or greater at time of study enrollment.

3.2.2 Diagnosis

Newly diagnosed patients with Unilateral Group D Retinoblastoma (see [Appendix I](#): Classification System for Intraocular Retinoblastoma.)

3.2.3 Evaluations required to determine eligibility

- MRI (or CT If MRI is not available) of the brain must be performed within 14 days prior to study entry
- Diagnostic EUA must be performed within 14 days prior to study entry.
- Rapid central review confirmation of Group D disease based on RetCam images from diagnostic EUA must be obtained before starting treatment.

3.2.4 Performance Level

Patients must have a performance status corresponding to ECOG scores of 0, 1 or 2. See https://members.childrensoncologygroup.org/prot/reference_materials.asp under Standard Sections for Protocols.

3.2.5 Life Expectancy

Patients must have a life expectancy of \geq 8 weeks.

3.2.6 Organ Function Requirements

3.2.6.1 Patients must have adequate renal function, defined as:

- Creatinine clearance or radioisotope GFR \geq 70 mL/min/1.73 m² or
- A serum creatinine based on age/gender as follows:

Age	Maximum Serum Creatinine (mg/dL)	
	Male	Female
1 month to < 6 months	0.4	0.4
6 months to < 1 year	0.5	0.5
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
\geq 16 years	1.7	1.4

The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR (Schwartz et al. J. Peds, 106:522, 1985) utilizing child length and stature data published by the CDC.

3.2.6.2 Patients must have adequate liver function, defined as:

- Total bilirubin \leq 1.5 x upper limit of normal (ULN) for age, and
- SGPT (ALT) $<$ 2.5 x upper limit of normal (ULN) for age.

3.2.7 Exclusion Criteria

- 3.2.7.1 Patients with bilateral disease
- 3.2.7.2 Unilateral retinoblastoma with Group A, B, C, or E eyes
- 3.2.7.3 Prior chemotherapy or radiation therapy for this disease (laser and cryotherapy are allowed and are not considered exclusion criteria)
- 3.2.7.4 Clinical or neuroimaging evidence of extraocular disease or orbital optic nerve involvement

3.2.8 Regulatory Requirements

- 3.2.8.1 All patients and/or their parents or legal guardians must sign a written informed consent.
- 3.2.8.2 All institutional, FDA, and NCI requirements for human studies must be met.

4.0 TREATMENT PROGRAM

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable per COG administrative Policy 5.14 (except where explicitly prohibited within the protocol).

4.1 Overview of Treatment Plan

4.1.1 Treatment

Eligible patients will receive 1 intra-arterial injection (IA) of melphalan every 21-30 days. Injections may be repeated every 21-30 days (up to a maximum of 3 cycles) assuming the patients meets the criteria to begin the next cycle.

4.1.2 Criteria to start subsequent cycles

All Cycles: ANC \geq 1000/ μ L, platelets \geq 100,000/ μ L, Hemoglobin \geq 8 g/dL, SGPT (ALT) $<$ 2.5 x upper limit of normal (ULN) for age.

Cycle 2: Central review comments based on the interventional radiology recording from Cycle 1 must be obtained for each patient before starting Cycle 2 treatment.

4.1.3 Interventional Radiologist Criteria

Site requirements for the interventional radiologist participating in the study (if you use a backup interventional radiologist he/she must meet the same requirements)

1. At least 2 years post fellowship
2. Prior experience in performing at least 20 procedures above the skull base in children < 6 years
3. Experience with flow-directed catheters
4. New participant must submit reports of the 20 procedures for review by the central INR committee
 - a. Submit reports to Dr. Darren Orbach at Darren.Orbach@childrens.harvard.edu
5. The designated interventional radiologist who meets the above criteria should be the one to perform all interventional radiology procedures for intra-arterial chemotherapy.
6. The designated institutional interventional radiologist from each institution is strongly encouraged to visit Dr. Pierre Gobin to observe the technique prior to enrolling patients on the protocol.

4.1.4 Interventional Radiology Recording

Interventional radiology recordings of the first IA melphalan injection must be taken during the procedure and sent to IROC Rhode Island (QARC) for central review within 14 days of the procedure. If this recording is not submitted within the 14 day window, the subject will remain on study for feasibility, but will be considered inevaluable for response and salvage.

4.1.5 EUA

An examination under anesthesia (EUA) must be performed at the following time points:

1. Up to 14 days prior to enrollment (diagnostic EUA). EUA does not have to be repeated prior to the first IA injection.
2. Up to 7 days prior to each subsequent IA injection.
3. 14-28 days after the final IA injection.
4. As clinically indicated.

Patients with evidence of disease progression (see [Section 10.3](#) for definitions) at the time of EUA will be removed from protocol therapy at specified time points. RETCAM images and retinal drawings of the two (2) most recent EUAs should be sent for central review within 7 days of the EUA showing disease progression.

4.1.6 Local Control Therapy Guidelines

Local control (cryotherapy, laser therapy, or plaque therapy) will be performed as needed per ophthalmologist's discretion at the time of EUA. See [Section 15.0](#) and [Section 16.0](#) for local control therapy guidelines. Once the 3 doses of IA melphalan have been administered, patients will continue to receive local control (laser, cryotherapy, or plaque) as needed per ophthalmologist's discretion. Radiation exposure during the interventional radiology procedures will be recorded. Variations in the timing or technique of delivering local therapies will not be considered a violation of the protocol.

4.1.7 Supportive Care Guidelines

Appropriate antibiotics, blood products, anti-emetics, fluids, electrolytes and general supportive care are to be used as needed per institutional guidelines.

For COG Supportive Care Guidelines see:

https://members.childrensoncologygroup.org/prot/reference_materials.asp under Standard Sections for Protocols.

4.1.8 Instructions for intra-arterial injection of melphalan

The injection must be completed within 60 minutes of product reconstitution.

Cerebral angiography in infants poses unique technical challenge, with potential risk of severe morbidity to both access and target vessels, as well as risk of possible severe neurological morbidity, arising quickly.

Fastidious technique to reduce patient radiation exposure is particularly critical when treating infants with retinoblastoma, as the infant brain typically absorbs 50% of the incident skin radiation dose. Digital subtraction angiography exposes patients to a much higher radiation dose than does live fluoroscopy. As such, formal angiographic runs should be minimized as much as possible, to a degree that is still consistent with safely performing the procedure and ensuring normal runoff to the brain and eye in all arterial branches. Roadmaps and live fluoroscopy are to be generally preferred.

For a unilateral treatment, a total skin dose of under 100 mGy (frontal and lateral planes summed) can be expected.

The procedure is performed under general anesthesia with endotracheal intubation.

Oxymetazoline 0.05% (two sprays) is sprayed in the nostril on the treatment side, to reduce the blush from the nasal mucosa and risk of epistaxis.

Phenylephrine hydrochloride 10% ophthalmic solution (two drops) may be spread over the skin of the forehead above the eye on the treatment side, to vasoconstrict the skin and reduce the risk of skin erythema. (optional)

Heparin is administered intravenously at 70 International Units/Kg.

Heparin is administered after femoral artery puncture at the time of microcatheter manipulation inside the arteries

One femoral artery (alternatively right/left) is punctured with a 3F micropuncture system and a 3F femoral sheath is placed.

A straight microcatheter such as a Marathon (EV3 Irvine, CA) or a Magic 1.5 (Balt, Montmorency, France) is advanced over a micro-guide wire such as the Mirage (EV3 Irvine, CA) into the internal carotid artery. A 4F guide catheter is usually not necessary. A cerebral angiogram is performed.

The microcatheter is placed at the origin of the ophthalmic artery, without catheterizing it. Then, imaging of the ophthalmic artery is performed to visualize the angioanatomy, the choroid blush, and that there is no reflux of contrast into the internal carotid. To minimize radiation exposure, this last imaging is performed by subtracted fluoroscopy rather than angiography.

If the ophthalmic artery is not appropriate for selective catheterization, one may catheterize the middle meningeal artery and perform a selective angiogram to find if the orbital branch is large enough for catheterization and intra-arterial injection.

The chemotherapy drug should be prepared according to [Section 6.1](#) prior to delivery of the medication to the angio suite under sterile conditions. The drug is then injected manually by repeated small bolus –pulsatile injection- at a rate of 1 mL/minute.

After completion of administration of the drug flush the catheter while still in place with 0.5 ml normal saline.

All of the drug is expected to reach the ophthalmic artery.

Operators should perform intermittent contrast or fluoroscopy to ensure that the catheter has not moved, or that spasm has not occurred.

After drug delivery, another substracted fluoroscopy of the ophthalmic artery is performed; the microcatheter is pulled into the internal carotid artery and another internal carotid angiogram performed.

The microcatheter is removed, an ACT is performed and if < 2.5 baseline the sheath is removed and hemostasis of the femoral artery obtained with manual compression for 10-20 minutes.

After awakening, the child should be monitored for at least 6 hours post procedure before being discharged home for observation.

4.2 Cycles 1, 2, and 3 (each cycle lasts 28 days)**Melphalan: IA**

Day 1

Dose: Melphalan dose will be adjusted to age:

- Patients = 6 - 11.99 months will receive 3 mg
- Patients = 12 - 23.99 months will receive 3.5 mg
- Patients \geq 24 months will receive 4 mg.

See [Section 4.1.8](#) for injection instructions. See [Section 5.0](#) for Dose Modifications based on Toxicities.

Local control therapy may be administered during EUAs. See [Section 4.1.6](#) for details.

Following completion of each cycle, the next cycle starts on Day 29 if criteria to start next cycle have been met (see [Section 4.1.2](#)).

Interventional Radiology Recording of First Injection

Prior to the second injection, the central review of the interventional radiology recording taken after the first intra-arterial injection must be completed and sent for central review. Review comments will be communicated to the PI and CRA of the institution. This is important, as the review may affect dose modifications for the second and third injections (see [Section 5.0](#)).

The therapy delivery map (TDMs) for all three cycles are on the next page.

4.2.1 Intra-arterial Injection of Melphalan (All Cycles)

This therapy delivery map covers all cycles and is on one (1) page. Each cycle lasts 28 days (21-30 days). Details of therapy are in [Section 4.0](#). This therapy delivery map is to be used for each one of the 3 cycles.

CIRCLE ONE: RIGHT EYE LEFT EYE

Begin therapy when ANC \geq 1000/ μ L, platelets \geq 100,000/ μ L, Hemoglobin \geq 8 g/dL, SGPT (ALT) $<$ 2.5 x upper limit of normal (ULN) for age.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES	OBSERVATIONS
Melphalan	Intra-arterial	Age Based Dosing: = 6 - 11.99 months - 3 mg = 12 - 23.99 months - 3.5 mg ≥ 24 months - 4 mg	1		<ul style="list-style-type: none"> a. History, Ht, Wt, BSA b. Performance Status c. Physical exam d. CBC (differential/platelets) e. Electrolytes (incl. Ca⁺⁺, Po₄, Mg⁺⁺), creatinine, SGPT, bilirubin f. EUA with RETCAM images g. MRI of brain and orbits <p>See Section 7.1 for details on follow-up observations.</p> <p>OBTAI OTHER STUDIES AS REQUIRED FOR GOOD PATIENT CARE</p>

Enter Cycle #: _____ Ht _____ cm Wt _____ kg BSA _____ m²

Date Due	Date Given	Day	melphalan ____ mg	Studies	Comments (Include any held doses, or dose modifications)	
Enter calculated dose above and actual dose administered below						
		1	____ mg	(a,b,c,d,e)* g [#] , f [%]		
		7		c ¹ ,d,		
		14		c ¹ ,d,g ²		
		21-30		b, c ¹ ,d, f ²		
			<i>Local Control Therapy may be performed during EUAs. See Section 16.0 for details. Start next cycle when ANC\geq1000/μL; platelets\geq100,000/μL; Hgb\geq8 g/dL.</i>			

See [Section 5.0](#) for Dose Modifications for Toxicities and the COG website posted materials for Supportive Care Guidelines.

*Baseline observations must be performed \leq 7 days prior to starting treatment

To be performed \leq 14 days prior to enrollment only.

% To be performed \leq 14 days prior to enrollment and \leq 7 days prior to each subsequent IA injection. Local Control Therapy may be performed during EUAs.

¹ Cycle 1 only

² Cycle 3 only. To be performed 14-28 days post 3rd IA melphalan injection.

5.0 DOSE MODIFICATIONS FOR TOXICITIES

5.1 Dose of melphalan can be increased by 0.5 mg in case of large extraorbital branches of the ophthalmic artery (meningeal, ethmoid arteries) for the second and third cycle not to exceed the maximum allowed dose in Section 5.1.1. This increase in dose will be based on central review of the interventional radiology recording of the first injection. This central review will be performed within 14 days of receiving the data from the institution.

5.1.1 Age based minimum and maximum allowed doses:

= 6 - 11.99 month:	2.5 - 3.5 mg
= 12 - 23.99 months:	3.0 - 4.0 mg
≥ 24 months:	3.5 - 4.5 mg

5.2 The standard dose should be decreased by 0.5 mg if there are signs of poor tolerance of previous intra-arterial treatment, not to fall below the minimum allowed dose in Section 5.1.1. This includes any Grade 4 inflammation of the eyelids and conjunctiva (see grading scale below.)

5.2.1 Grading scale for inflammation of the eyelids and conjunctiva:

- Grade 1. Mild erythema of eyelids, orbit or forehead
- Grade 2. Erythema and swelling over eyelids, orbit or forehead
- Grade 3. Pain in the eye or orbit with erythema and/or swelling
- Grade 4. Swelling and/or pain lasting more than 7 days

Patients who develop post-injection central artery occlusion that does not recanalize prior to the subsequent injection will be taken off protocol therapy.

6.0 DRUG INFORMATION

See the consent document for toxicities.

6.1 MELPHALAN - INTRA-ARTERIAL (08/28/13)

(L-phenylalanine mustard, phenylalanine mustard, L-PAM, L-sarcolysin, Alkeran®) NSC #008806

Source and Pharmacology:

Melphalan, a phenylalanine derivative of nitrogen mustard, is a bifunctional alkylating agent. Melphalan forms covalent cross-links with DNA or DNA protein complexes thereby resulting in cytotoxic, mutagenic, and carcinogenic effects. The end result of the alkylation process results in the misreading of the DNA code and the inhibition of DNA, RNA, and protein synthesis in rapidly proliferating tumor cells. It is cell cycle non-specific. After IV administration, melphalan plasma concentrations decline rapidly in a bi-exponential manner with distribution phase and terminal elimination phase half-lives of approximately 10 and 75 minutes, respectively. Plasma melphalan levels are highly variable after oral dosing, both with respect to the time of the first appearance of melphalan in plasma (range approximately 0 to 6 hours) and to the peak plasma concentration achieved. These results may be due to incomplete intestinal absorption, a variable "first pass" hepatic metabolism, or to rapid hydrolysis. The oral dose averages $61\% \pm 26\%$ of that following IV administration. The terminal elimination plasma half-life of oral melphalan is 1.5 ± 0.83 hours. The steady-state volume of distribution of melphalan is 0.5 L/kg. The extent of melphalan binding to plasma proteins ranges from 60-90%. Melphalan is eliminated from plasma primarily by chemical hydrolysis to monohydroxymelphalan and dihydroxymelphalan. The 24-hour urinary excretion of parent drug is approximately 10% suggesting that renal clearance is not a major route of elimination of parent drug. Penetration into CSF is low. Despite the fact that the contribution of renal elimination to melphalan clearance appears to be low, one pharmacokinetic study suggests dosage may need to be reduced in patients with renal impairment.

Toxicity:

Only limited data exist on the toxicity of intra-arterial melphalan. The anticipated toxicities for intra-arterial melphalan are listed in the first table. Toxicities of intravenous melphalan are provided in the second table for completeness. It is not known if these toxicities will occur in a patient receiving intra-arterial melphalan for retinoblastoma.

The table below lists the anticipated toxicity profile of melphalan (intra-arterial for retinoblastoma):

Incidence	Toxicities
Common (>20% of patients)	Retinal vascular disorder, retinopathy, eye disorders – other: loss of vision, blurred vision, cataract, eye disorders – other: thinning or loss of eyelashes, eye disorders – other: periocular edema
Occasional (5-20% of patients)	Neutrophil count decreased, fever, bronchospasm
Rare (<5% of patients)	Injection site reaction, reproductive system and breast disorders – other: amenorrhea, azoospermia, reproductive system and breast disorders – other: sterility or male infertility, anaphylaxis, allergic reaction, pneumonitis, pulmonary fibrosis, treatment related secondary malignancy, febrile neutropenia
Pregnancy & Lactation	Pregnancy Category D Melphalan was embryolethal and teratogenic in rats following oral (6 to 18 mg/m ² /day for 10 days) and intraperitoneal (18 mg/m ²) administration. Malformations resulting from melphalan included alterations of the brain

	(underdevelopment, deformation, meningocele, and encephalocele) and eye (anophthalmia and microphthalmos), reduction of the mandible and tail, as well as hepatomegaly (exomphaly). It is unknown whether the drug is excreted in breast milk.
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The table below lists the toxicity profile of melphalan IV:

Incidence	Toxicities
Common (>20% of patients)	Endocrine disorders – other: syndrome of inappropriate antidiuretic hormone (SIADH), anemia, neutrophil count decreased, white blood cells decreased, platelet count decreased, alopecia, diarrhea, nausea, vomiting, mucositis oral
Occasional (5-20% of patients)	Reproductive system and breast disorders – other: amenorrhea, azoospermia, reproductive system and breast disorders – other: sterility or male infertility, injection site reaction, alanine aminotransferase increased, aspartate aminotransferase increased, blood bilirubin increased
Rare (<5% of patients)	Cardiac arrest, atrial fibrillation, vasculitis, rash maculo-papular, skin ulceration, seizures, anaphylaxis, allergic reaction, hepatobiliary disorders – other: hepatitis, hepatobiliary disorders – other: jaundice, hepatobiliary disorders – other: sinusoidal obstruction syndrome, blood and lymphatic system disorders, other: hemolytic anemia, pneumonitis, pulmonary fibrosis, treatment related secondary malignancy
Pregnancy & Lactation	Pregnancy Category D Melphalan was embryolethal and teratogenic in rats following oral (6 to 18 mg/m ² /day for 10 days) and intraperitoneal (18 mg/m ²) administration. Malformations resulting from melphalan included alterations of the brain (underdevelopment, deformation, meningocele, and encephalocele) and eye (anophthalmia and microphthalmos), reduction of the mandible and tail, as well as hepatomegaly (exomphaly). It is unknown whether the drug is excreted in breast milk.

Formulation and Stability:

Melphalan for Injection is supplied as a sterile, nonpyrogenic, freeze-dried powder. Each single-use vial contains melphalan hydrochloride equivalent to 50 mg melphalan and 20 mg povidone. Melphalan for Injection is reconstituted using the sterile diluent provided. Each vial of sterile diluent contains sodium citrate 0.2 g, propylene glycol 6.0 mL, ethanol (96%) 0.52 mL, and SWFI to a total of 10 mL. Store at controlled room temperature 15°-30°C (59°-86°F) and protect from light.

Intra-arterial Preparation:

- Using appropriate aseptic technique, reconstitute to a concentration of 5 mg/mL by rapidly injecting 10 mL of the supplied diluent directly into the vial of lyophilized powder using a sterile needle (20-gauge or larger needle diameter) and syringe. Immediately shake vial vigorously until a clear solution is obtained. Rapid addition of the diluent followed by immediate vigorous shaking is important for proper dissolution.
- Immediately dilute the dose to be administered in up to 20 mL of NS; the final volume of the product should be 20 mL. The concentration of the final drug product should be ≤ 0.45 mg/mL.
- The final drug product should be filtered (e.g., with a 0.22 micron filter) prior to being dispensed.
- A precipitate forms if the reconstituted solution is stored at 5°C. Do not refrigerate the reconstituted product.

(The time between reconstitution/dilution and administration of melphalan should be kept to a minimum because reconstituted and diluted solutions of melphalan are unstable. Over as short a time as 30 minutes, a citrate derivative of melphalan has been detected in reconstituted material from the reaction of melphalan with the sterile diluent for melphalan. Upon further dilution with saline, nearly 1% label strength of melphalan hydrolyzes every 10 minutes.)

Guidelines for Administration: See [Treatment](#) (Section 4.0) and [Dose Modifications](#) (Section 5.0) sections of the protocol.

Patients should receive oxymetazoline 0.05% (e.g., Afrin) two puffs into the nares on the side of the procedure—prior to the procedure to reduce blushing.

The drug is then injected manually by repeated small bolus –pulsatile injection- at a rate of **1 mL/minute**. **The injection must be completed within 60 minutes of product reconstitution.**

Supplier: Commercially available. See package insert for further information.

7.0 EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable per COG administrative Policy 5.14 (except where explicitly prohibited within the protocol).

7.1 Required Clinical, Laboratory and Disease Evaluations

All baseline studies must be performed prior to starting protocol therapy unless otherwise indicated below. **Obtain other studies prior to start of phase unless otherwise indicated.**

STUDIES TO BE OBTAINED	Baseline	Cycle 1	Subsequent Cycles	End of Therapy	During Follow-up
REQUIRED STUDIES					
History	X	X	X	X	X ³
Physical Exam with VS	X	Weekly	X	X	X ³
Ht, Wt, BSA	X	X	X		
CBC, differential, platelets	X	Weekly	Weekly	X	
Performance Status	X	X	X	X	
Electrolytes including Ca ⁺⁺ , PO ₄ , Mg ⁺⁺	X	X	X	X	
Creatinine, SGPT, bilirubin	X	X	X	X	
Interventional radiology recording		X			
EUA [#]	X	X%	X	X	
MRI or CT of the brain and orbits with and without contrast	X			X ⁶	X ⁴
Visual Assessment Test					X ²
OPTIONAL STUDIES					
Histopathology of enucleated eyes					X ¹
EUA					X ⁵

[#] Please see [7.3](#) below for collection of required RETCAM images and Retinal Drawings with EUA.

¹ Enucleation (if required). See [Section 13.2](#) for details.

²To be administered once a year for 4 years, starting 1 year after completion of protocol therapy. Only for patients who receive all 3 courses of IA melphalan. See [Section 13.1](#) for details.

³To be performed every 3 months for the first year, every 4 months for the second year, and every six months for the third year
⁴To be performed every 6 months for two years.

⁵**Recommended** follow up EUA: every 6 weeks x2, followed by every 8 weeks x3 or more frequently as clinically indicated post protocol therapy.

⁶ To be performed 14-28 days post 3rd IA melphalan injection.

⁷ Use diagnostic EUA prior to cycle 1.

This table only includes evaluations necessary to answer the primary and secondary aims. Obtain other studies as indicated for good clinical care.

7.2 Follow-up

See COG Late Effects Guidelines for recommended post treatment follow-up:
<http://www.survivorshipguidelines.org/>

Note: Follow-up data are expected to be submitted per the Case Report Forms (CRFs) schedule.

7.3 Collection of RetCam images, Retinal Drawings, Interventional Radiology Recording, and QA Documentation

All central review should include:

1. Retcam photo
2. Retinal drawing
3. Some statement about the presence and location of vitreous or subretinal seeds (preferred but not mandatory)

Item #3 can be written on the retinal drawing

7.3.1 RetCam Images and Retinal Drawings

RetCam images and retinal drawings from EUA's are required to be submitted electronically to IROC Rhode Island (QARC) at the following time points:

- a. Diagnostic EUA (performed within 14 days prior to enrollment)-to be submitted within 7 days
- b. End of therapy (performed 14-28 days post injection #3) to be submitted within 35 days of 3rd injection.
- c. In the event of disease progression: RETCAM images and retinal drawings of the last 2 EUAs should be sent for central review within 7 days of the EUA showing disease progression.

7.3.2 Rapid Central Review

Electronic copies of the RetCam images from each of the required time periods in Section 7.3.1 must be sent to IROC Rhode Island (QARC). DICOM files of these images are the preferred format. These files can be submitted via sFTP. Information for obtaining an sFTP account and submission instructions can be found at www.QARC.org. Follow the link labeled digital data. Alternatively, if sFTP is not feasible, the imaging may be burned to a CD and mailed to IROC Rhode Island (QARC). Sites using Dicommunicator may submit imaging via that application. Contact IROC Rhode Island (QARC) with questions or for additional information. BMP files are discouraged but will be accepted if DICOM files are not available. The BMP files can be e-mailed to IROC Rhode Island (QARC) at DataSubmission@QARC.org or can be submitted via sFTP. **The RetCam images taken at diagnostic EUA must be submitted to IROC Rhode Island (QARC) within 7 days of the procedure for a rapid review of eligibility. Central review of diagnostic EUA will be done prior to study entry to confirm International Classification System for Intraocular Retinoblastoma Group D.** The ARET12P1 Diagnostic EUA Eligibility Review Transmittal form must be included with the RetCam images and retinal drawings. This form can be found on

the IROC Rhode Island (QARC) website, www.qarc.org. Central review for disease progression will occur within 2 weeks. If disease progression is confirmed by central review, the patient will be removed from protocol treatment and treated in a standard fashion at investigator discretion.

7.3.3 Required Submission of Interventional Radiology Recording of 1st IA injection

Electronic copies of the interventional radiology recording from the first IA melphalan injection must be sent to IROC Rhode Island (QARC). DICOM files of these images are the preferred format. These files can be submitted via sFTP. Information for obtaining an sFTP account and submission instructions can be found at www.QARC.org. Follow the link labeled digital data. Alternatively, if sFTP is not feasible, the imaging may be burned to a CD and mailed to IROC Rhode Island (QARC) (see address below.) Sites using Dicommunicator may submit imaging via that application. Contact IROC Rhode Island (QARC) with questions or for additional information. BMP files are discouraged but will be accepted if DICOM files are not available. The BMP files can be e-mailed to IROC Rhode Island (QARC) at DataSubmission@QARC.org or can be submitted via sFTP. These file can also be submitted (via SFTP only) as common movie file types such as; .avi, .mpg, or .wmv. **The interventional radiology recording taken during the first IA melphalan injection must be submitted to IROC Rhode Island (QARC) within 14 days of procedure.**

IROC Rhode Island (QARC)
Building B, Suite 201
640 George Washington Highway
Lincoln, RI 02865-4207
Phone: (401) 753-7600
Fax: (401) 753-7601

7.3.4 Required Submission of Retinal Drawings

Required Submission of Retinal Drawings Retinal drawings corresponding to each RetCam study should be submitted to IROC Rhode Island (QARC) when the RetCam images are submitted. The retinal drawings can be scanned and e-mailed to DataSubmission@QARC.org or can be submitted via sFTP. The treating ophthalmologist at each institution must submit a drawing of the eye based on the EUA from each of the required time periods in [Section 7.3.1](#). The drawing may be done in whatever format is used at the local institution. Tumors should be numbered. Each tumor should be clearly labeled as abnormal without depending on color coding since form will be copied in black and white. This drawing will be utilized by the central reviewers to assist in confirmation of staging of the eye(s). The drawing should be labeled with the patient's COG identification number and the study number (ARET12P1).

8.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

8.1 Criteria for Removal from Protocol Therapy

- a) Progressive disease - Unable to control disease

- b) Any non-protocol chemotherapy or external beam radiation therapy is given
- c) Intraocular progression of disease is documented by the institutional assessment. **Note:** RETCAM images of the last 2 EUAs should be sent for central review within 7 days of the EUA showing disease progression
- d) Positive disease found in the contralateral eye after receiving protocol treatment
- e) Occurrence of metastases
- f) Massive/Profound intra-vitreal hemorrhage
- g) Enucleation occurred prior to any Melphalan injection
- h) Refusal of further protocol therapy by patient/parent/guardian
- i) Completion of planned protocol therapy
- j) Physician determines it is in patient's best interest
- k) Development of a second malignancy
- l) Repeat eligibility studies (if required) are outside the parameters required for eligibility
- m) Central artery occlusion after an injection which does not recanalize prior to the subsequent injection

Patients who are off protocol therapy are to be followed until they meet the criteria for Off Study (see below). Follow-up data will be required unless patient is taken off study.

8.2 Off Study Criteria

- a) Death
- b) Lost to follow-up
- c) Patient enrollment onto another COG study with tumor therapeutic intent (eg, at recurrence)
- d) Withdrawal of consent for any further data submission.
- e) The fifth anniversary of the date the patient was enrolled on this study

9.0 STATISTICAL CONSIDERATIONS

9.1 Sample Size and Duration

A survey of potential enrollment at the 11 primary institutions participating in this trial indicates we can expect at least 24 patients per year will be enrolled with disease characteristics and organ function that meet the eligibility criteria for the protocol. Thirty-one (31) eligible and feasibility-evaluable patients will be required in order to apply the statistical rules described below. Accounting for the possibility that 10% of patients will be either inevaluable or ineligible, we will enroll up to 34 patients. At the time of the amendment of the study design, 10 patients were enrolled; none were identified as inevaluable for the primary endpoint according to the definition in the amended protocol. We estimate enrollment can be concluded within 18 months of implementation of the modified design. Twelve months of accrual will be required and up to six months will be required to determine the feasibility status of all patients of patients enrolled in the first stage of the Study Design.

9.2 Study Design

The primary goal of this trial is to determine the feasibility of delivering melphalan directly into the ophthalmic artery.

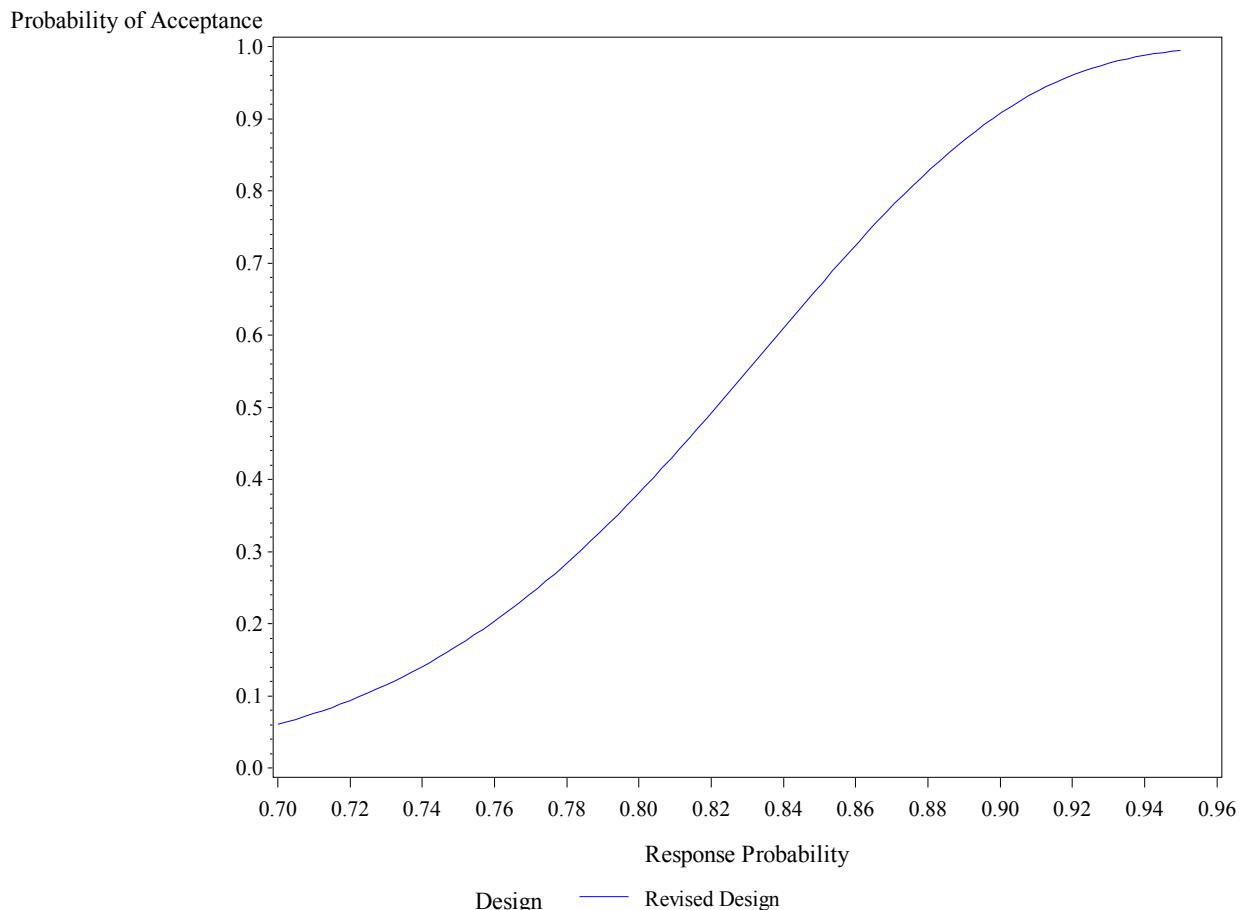
Feasibility Outcome: Intra-arterial (IA) feasibility will be assessed over the 3 injections into the ophthalmic artery. A patient will be considered to have experienced IA therapy feasibility failure if: a) the interventional radiologist is not able to access the ophthalmic artery for chemotherapy administrations during the first three cycles of therapy; b) patient develops central retinal artery occlusion after the first or second cycle that does not reopen by the time the next injection is due; or c) the patient cannot receive all three treatments with IA therapy because of CTC AE complications Grade III or IV that are considered possibly, probably or likely related treatment.

Evaluability for Feasibility Outcome: Any patient who does not experience IA feasibility failure event and is removed from protocol therapy prior to the third IA injection because: (1) the physician stops protocol therapy in the best interests of the patient; (2) the parent or guardian refuses further therapy; (3) disease is found in the contralateral eye; or (4) the patient does not qualify because repeat eligibility studies are outside enrollment requirements will be considered inevaluable for the primary outcome measure and replaced for evaluation of the primary goal. All other patients will be considered evaluable for the primary endpoint. Each patient will require, at most, 4 months to complete the feasibility evaluation. Any feasibility evaluable patient who does not experience feasibility failure will be considered a feasibility success.

Design for the Primary Outcome Measure: Feasibility will be evaluated using a two-stage design.

	Cumulative Number of Feasibility Successes	Decision
Stage 1: Enter 12 feasibility evaluable patients	8 or fewer	Terminate the trial because the therapy is considered not feasible to deliver.
	9 or more	Proceed to Stage 2.
Stage 2: Enter 19 additional feasibility evaluable patients	25 or fewer	Terminate the trial with the conclusion the therapy is considered not feasible to deliver.
	26 or more	Terminate the trial because the therapy is considered feasible to deliver.

Power Characteristics



If the true feasibility failure rate is 28%, the regimen will be identified as not feasible to deliver with probability 91%. If the true feasibility failure rate is 10%, the regimen will be identified as feasible to deliver with probability 90%.

Assessment of Ocular Salvage

Ocular Salvage: Ocular salvage will be assessed over the 2 years following start of protocol therapy. A patient will be considered an ocular-salvage success if enucleation because of disease progression or toxicity is not required during the 2 years following enrollment onto ARET12P1. Any patient who is lost to follow-up or for whom follow-up is terminated electively prior to 2 years by patient or parent preference will be considered inevaluable for the ocular salvage endpoint.

The primary analytic endpoint of this trial will be feasibility of delivery of IA therapy. The accrual target for this study will not be adjusted for the number of patients considered not evaluable for the ocular salvage endpoint.

Design for Ocular Salvage Assessment: Interim assessment of this aim will not be done since the salvage endpoint could require 2 years of follow-up after enrollment to ascertain.

The probability of ocular salvage will be assessed after 31 feasibility -evaluable patients have been enrolled. If 11 or fewer patients have ocular salvage (observed salvage rate 32%), we will consider the therapy to have an insufficient ocular salvage probability; otherwise, the data will be considered of sufficient interest

with respect to ocular salvage for further investigation. If the true probability of ocular salvage is 0.50, the therapy will be considered of sufficient interest for further study with probability 93%. If the true probability of ocular salvage is 0.25, the therapy will be considered not of sufficient interest for further study with probability 94%. If, at any time, 21 or more patients experience ocular salvage failure, enrollment will be suspended with the conclusion the therapy does not have a sufficient salvage rate for further consideration.

If fewer or greater than 31 patients are evaluable for the ocular salvage endpoint, the cutoff for the number of patients who have ocular salvage success will be adjusted to preserve the probability of identifying the therapy as not of sufficient interest for further examination to be at least 90% when the true probability of ocular salvage is only 25%. Examples of such adjustments are provided in the following table.

Number of Patients Evaluable for Ocular Salvage	Number of Ocular Patients with Ocular Salvage Success That Implies the Therapy is not of Interest for Further Investigation	Probability The Therapy is Considered Not of Interest if The True Ocular Salvage Rate is 25%	Probability The Therapy is Considered of Interest if The True Ocular Salvage Rate is 45%
34	12 or fewer	0.94	0.94
28	10 or fewer	0.93	0.91
26	9 or fewer	0.91	0.92

Assessment of the Need for Systemic Chemotherapy

Systemic Chemotherapy Administration: The need to administer systemic chemotherapy because of progressive disease in the eye considered for IA melphalan feasibility will be assessed over the 2 years following start of protocol therapy. A patient will be considered as requiring systemic chemotherapy if systemic cytotoxic chemotherapy of any kind is required because of disease progression in the affected eye at any time during the 2 years following enrollment onto ARET12P1. Any patient who is lost to follow-up or for whom follow-up is terminated electively prior to 2 years by patient or parent preference will be considered inevaluable for the evaluation of the need for systemic chemotherapy to control progressive disease in the affected eye.

Design for Assessing the Need for Systemic Chemotherapy: Interim assessment of this aim will not be done since the salvage endpoint could require 2 years of follow-up after enrollment to ascertain.

The probability of the need for systemic chemotherapy will be assessed after 31 systemic chemotherapy-evaluable patients have been enrolled. If all 31 such patients are evaluable for ocular salvage, the following design will be employed. If 19 or more patients require systemic chemotherapy (observed frequency 61%), we will consider the therapy to have an insufficient freedom from systemic chemotherapy probability; otherwise, the data will be considered of sufficient interest with respect to freedom from systemic chemotherapy for further investigation. If the true probability of freedom from chemotherapy is 0.50, the therapy will be considered of sufficient interest with respect to this endpoint with probability 93%. If the true probability of the need for systemic chemotherapy is 0.75, the therapy will be considered not of sufficient interest for this endpoint with probability 94%. If, at any time, 19 require chemotherapy to control progressive disease in the affected eye, enrollment will be suspended with the conclusion the therapy does not meet the criteria for a treatment sufficient interest for this endpoint.

If fewer or greater than 31 patients are evaluable for the need for chemotherapy endpoint, the cutoff for the number of patients who require systemic chemotherapy will be adjusted to preserve the probability of identifying the therapy as not of sufficient interest for further examination to be at least 90% when the true

probability of the need for chemotherapy is at least 75%. Examples of such adjustments are provided in the following table.

Number of Patients Evaluable for Ocular Salvage	Number of Ocular Patients that do not Require Systemic Chemotherapy	Probability The Therapy is Considered Not of Interest if The True Probability of Requiring Chemotherapy is 75%	Probability The Therapy is Considered of Interest if The True Probability of Requiring Systemic Chemotherapy is 50%
34	12 or fewer	0.94	0.94
28	10 or fewer	0.93	0.91
26	9 or fewer	0.91	0.92

Assessment of Rate of Metastases of Retinoblastoma

Metastatic Disease Failure: Patients will be considered for the evaluation of metastatic disease provided the individual receives at least 1 dose of IA therapy and is followed until the patient experiences disease progression or relapse, is diagnosed with a second malignant neoplasm, dies or completes two years of follow-up without any of the aforementioned events occurring, whichever occurs first. The new finding of extraocular disease after the initiation of protocol therapy will be considered metastatic disease failure. Appearance of intraocular tumor in the other eye either during or after protocol therapy will not be considered metastatic disease. Appearance of an isolated pineal tumor during therapy and follow up will not be considered as metastatic recurrence especially in the context of a young patient with a genetic predisposition to developing bilateral/trilateral retinoblastoma.

Design for Assessing Rate of Metastatic Disease Incidence: The primary analytic endpoint of this trial will be feasibility of delivery of IA therapy. The accrual target for this study will not be adjusted for the number of patients considered not evaluable for the metastatic disease endpoint.

The probability of metastatic disease will be assessed after 31 feasibility-evaluable patients have been enrolled. If all 31 such patients are evaluable for metastatic disease incidence, the following design will be employed. If 1 or fewer patients experience metastatic disease, (observed rate of metastatic disease incidence: 3.2%), we will consider the therapy to have a sufficiently low rate of metastatic disease to be considered for further evaluation; otherwise, the data will be considered indicative of an excessive rate of metastatic disease. If the true rate of metastases is 0.10, the therapy will be considered of to have an excessive rate of metastases probability 83%. If the true rate of metastases is 0.03, the therapy will be considered have a sufficiently low rate of metastases with probability 76%. If, at any time, 2 or more patients experience metastatic disease as defined above, the COG DSMC will be notified and CTEP consulted regarding the next steps to take with respect to the protocol.

Because of the definition of evaluability for feasibility, the number of patients evaluable for the occurrence of metastatic disease may differ from 31. In such cases the cutoff for the number of patients who are diagnosed with metastatic disease as defined above will be adjusted to preserve the probability of identifying the therapy as having an excessive metastatic disease probability to be at least 80% when the true probability of metastatic disease is 10%. Examples of such adjustments are provided in the following table.

Number of Patients Evaluable for Metastatic Disease	Number of Ocular Patients with Metastatic Disease That Implies the Therapy is May not be of Interest for Further Investigation	Probability The Therapy is Considered Not of Interest if The True Metastatic Disease Rate is 10%	Probability The Therapy is Considered of Interest if The True Metastatic Disease Rate is 3%
34	2	0.87	0.73
28	1	0.95	0.43

Evaluation of the toxicities and adverse events associated with delivering multiple doses of intra-arterial chemotherapy: The occurrence of each Grade 3 or higher CTC AE adverse experience will be recorded in each patient-cycle. The percentage of patients with each toxicity will be tabulated per cycle according to the methodology in place for the study progress report at the time.

The study will be temporarily suspended for evaluation if there is any episode of stroke or death and the study will be terminated if it is determined that the IA procedure was a significant cause.

Evaluation of vision outcomes in children treated with intra-arterial chemotherapy: All patients who retain the affected eye 1 year after completion of therapy will be evaluated for visual acuity according to the Amblyopia Treatment Study Visual Acuity Testing Protocol. The mean visual acuity that will be obtained 1 year after protocol therapy will be estimated by the average visual acuity amongst patients evaluated¹⁹ the expected width of the 95% confidence interval for the visual acuity score (logMAR) is presented below as a function of the number of patients who are evaluated at the one-year-post-treatment endpoint. The expected standard error of logMAR is reported to be 0.064

Number of Patients Evaluated	Expected Width of the 95% Confidence Interval
10	0.040
30	0.023

Evaluation of the effects of intra-arterial therapy on the histopathology of eyes enucleated for progression: The monitoring rule for ocular salvage will limit the number of eyes available for evaluation. We project a maximum of 25 eyes will be available for evaluation. As noted in [Section 2.0](#), various characteristics such as viable vitreous seeds and invasion into the optic nerve were observed. In previous examinations there has also been evidence of ischemic atrophy involving the outer retina and choroid as well as extensive choroidal and outer retinal atrophy. The proportion of enucleated eyes with each of these various characteristics will be calculated, as well as the 95% confidence intervals. The expected size of the confidence intervals, as a function of the number of eyes available and relative frequency of the particular characteristic.

Number of Eyes Examined	True Proportion with the Particular Histopathological Characteristic	Expected 95% Confidence Interval
10	0.1	0.011-0.42
10	0.25	0.058-0.59
10	0.5	0.20-0.80
25	0.1	0.023-0.28
25	0.25	0.10-0.46
25	0.5	0.30-0.70

Monitoring for complications of catheter insertion:

The analytic unit for this outcome is the patient-catheter insertion. Each time a catheter insertion procedure is performed, the patient will be monitored for the subsequent 48 hours for the occurrence of any of the following complications: (1) thrombosis of the femoral artery; (2) dissection of any artery; (3) hematoma at the site of insertion of 3 centimeters or more in diameter; (4) emboli cerebral; or (5) any embolus in the lower extremity that results in vascular insufficiency. If any of these 5 events a catheter event will be considered to have occurred. Otherwise, the patient will be considered free of catheter events for that particular insertion.

A Bayesian monitoring rule will be used to assess the consistency of the data with a catheter event rate that does not exceed 20% with high probability. Each analytic unit will be considered independent of all other analytic units. A beta prior with $\alpha=0.2$ and $\beta=0.8$ to represent that the maximum complication rate considered acceptable as 20% with a substantial standard deviation to quantify the uncertainty regarding the complication rate. At each review of the study data where 10 or more patient-catheter insertion instance have occurred, the posterior probability of an excessive complication rate will be calculated as:

$$\Pr(p_{\text{Excessive Complication Rate}} > 0.20 | \text{Data}) = \frac{\int_{0.20}^1 \binom{n}{x} p^x (1-p)^{n-x} \frac{\Gamma(1)}{\Gamma(0.2)\Gamma(0.8)} p^{-0.8} (1-p)^{-0.2} dp}{\int_0^1 \binom{n}{x} u^x (1-u)^{n-x} \frac{\Gamma(1)}{\Gamma(0.2)\Gamma(0.8)} u^{-0.8} (1-u)^{-0.2} du}$$

Where n is the number of patient-catheter insertions and x is the number of those analytic units during which a catheter event is observed. If this probability exceeds 80%, we will identify the regimen to the relevant COG DSMC, the rare tumor leadership and CTEP as associated with catheter event rate that is excessive. Examples of situations in which the rule will indicate excessive toxicity are presented below.

Number of Patient-Catheter Insertion Instances	Number of Analytic Units Where a Catheter Event Occurs	$\Pr(p_{\text{Excessive Complication Rate}} > 0.20 \text{Data})$
10	4	0.90
15	5	0.86
20	6	0.83
25	7	0.81

9.3 Gender and Minority Accrual Estimates

The gender and minority distribution of the study population is expected to be:

Ethnic Category	Accrual Targets			
	Sex/Gender		Total	
	Females	Males		
Hispanic or Latino	7	+ 5	= 12	
Not Hispanic or Latino	9	+ 13	= 22	
Ethnic Category: Total of all subjects	16 (A1)	+ 18 (B1)	= 34 (C1)	
Racial Category				
American Indian or Alaskan Native	0	+ 0	= 0	
Asian	0	+ 4	= 4	
Black or African American	7	+ 6	= 13	
Native Hawaiian or other Pacific Islander	0	+ 0	= 0	
White	9	+ 8	= 17	
Racial Category: Total of all subjects	16 (A2)	+ 18 (B2)	= 34 (C2)	

10.0 EVALUATION CRITERIA

10.1 Common Terminology Criteria for Adverse Events (CTCAE)

This study will utilize the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. The descriptions and grading scales found in the revised CTCAE version 4.0 will be utilized for reporting beginning October 1st, 2010. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0, which can be downloaded from the CTEP web site (<http://ctep.cancer.gov>).

10.2 Response Criteria

There are no established response criteria for retinoblastoma that describe the response of the entire eye inclusive of all retinal lesions and vitreous disease. The response criteria defined below will NOT be used as study endpoints, but will be used to gather preliminary information on overall response and patterns of failure of eyes treated on this protocol therapy that may be useful in designing response criteria for future protocols. RetCam images will be submitted centrally to correlate with reported response from treating site. The study endpoint will be defined only by [Section 10.3](#) Definition of Disease Progression for an individual eye.

10.2.1 Type I or IV Response

Complete Type I regression pattern with calcification or Type IV with flat chorioretinal scars, or avascular, linear, white gliosis regression of all retinal tumors documented by at least two EUA's minimum of three weeks apart. No evidence of active vitreous tumor. No evidence of new lesions. Complete response is dated from the time all lesions have complete regression.

10.2.2 Type II or III Response

Reduction in size with Type II or III regression pattern in some lesions (i.e. not all lesions have Type I and/or Type IV regression pattern). No evidence of active vitreous tumor. Partial response is dated from the time of first observation. In addition, there can be no appearance of new lesions or progression of any lesion.

10.2.3 Stable Disease (SD)

No change in size/regression of retinal tumors and/or persistence of any active vitreous tumor. No evidence of new lesions.

10.2.4 Local Recurrence

Tumor regrowth at prior sites and/or new sites of retinal tumors that can be controlled by local therapy and/or plaque.

10.2.5 Retinal Recurrence

Tumor regrowth at prior sites and/or new sites of retinal tumors that canNOT be controlled by local therapy and/or plaque.

10.2.6 Subretinal Recurrence

Tumor regrowth involving expansion of new onset of subretinal seeding.

10.2.7 Vitreal Recurrence

Tumor regrowth involving expansion or new onset of vitreous seeding.

10.2.8 Extraocular Recurrence

Tumor regrowth involving optic nerve, and/or orbit.

10.2.9 Metastatic Systemic Recurrence

Tumor metastases to bone, bone marrow, lymph node, brain and/or other distant organ outside eye.

10.3 Definition of Disease Progression for an Individual Eye

Any tumor regrowth requiring enucleation and/or external beam radiation, and/or further chemotherapy beyond that given per protocol. At the time of disease progression, the pattern of recurrence will be specified using the Response Criteria (10.2.4-10.2.9) by choosing all sites of recurrence that apply. Any extraocular recurrence and/or metastatic systemic recurrence will be considered disease progression and the patient will be considered treatment failure.

11.0 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Purpose

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents.

11.2 Determination of Reporting Requirements

Reporting requirements may include the following considerations: 1) the characteristics of the adverse event including the *grade* (severity); 2) the *relationship to the study therapy* (attribution); and 3) the *prior experience* (expectedness) of the adverse event.

Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. In some cases an agent obtained commercially may be used for indications not included in the package label. In addition, NCI may on some occasions distribute commercial supplies for a trial. Even in these cases, the agent is still considered to be a commercial agent and the procedures described below should be followed.

Determine the prior experience Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered *unexpected*, for reporting purposes only, when either the type of event or the severity of the event is not listed in:

- *The current known toxicities for each commercial agent as provided in the Drug Information for Commercial Agents Used by the Children's Oncology Group posted on the COG website; or*
- *The drug package insert*

Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (eg, treatment with investigational agent/intervention, radiation or chemotherapy). A metastasis of the initial neoplasm is not considered a secondary malignancy.

All secondary malignancies that occur following treatment need to be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy.
- Myelodysplastic syndrome
- Treatment related secondary malignancy

11.3 Reporting of Adverse Events for Commercial Agents - CTEP-AERS abbreviated pathway

Commercial reporting requirements are provided in Table B. The commercial agent(s) used in this study are listed in the front of this protocol immediately following the Study Committee roster.

- COG requires the CTEP-AERS report to be submitted **within 7 calendar days** of learning of the event.
- Use the NCI protocol number and the protocol-specific patient ID provided

during trial registration on all reports.

CTCAE term (AE description) and grade: The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting and are located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/etc.htm. All appropriate treatment areas should have access to a copy of the CTCAE.

Table B

Reporting requirements for adverse events experienced by patients on study who have NOT received any doses of an investigational agent on this study.

CTEP-AERS Reporting Requirements for Adverse Events That Occur During Therapy With a Commercial Agent or Within 30 Days¹

Attribution	Grade 4		Grade 5
	Unexpected	Expected	
Unrelated or Unlikely			CTEP-AERS
Possible, Probable, Definite	CTEP-AERS		CTEP-AERS

¹This includes all deaths within 30 days of the last dose of treatment with a commercial agent, regardless of attribution. Any death that occurs more than 30 days after the last dose of treatment with a commercial agent which can be attributed (possibly, probably, or definitely) to the agent and is not due to cancer recurrence must be reported via CTEP-AERS.

11.4 Routine Adverse Event Reporting

Note: The guidelines below are for routine reporting of study specific adverse events on the COG case report forms and do not affect the requirements for CTEP-AERS reporting.

The NCI defines both routine and expedited AE reporting. Routine reporting is accomplished via the Adverse Event (AE) Case Report Form (CRF) within the study database. For this study, routine reporting will include all toxicities reported via CTEP-AERS and all Grade 3 and higher Adverse Events.

12.0 STUDY REPORTING AND MONITORING

The Case Report Forms and the submission schedule are posted on the COG web site with each protocol under “*Data Collection/Specimens*”. A submission schedule is included.

12.1 CDUS

This study will be monitored by the Clinical Data Update System (CDUS). Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31. This is not a responsibility of institutions participating in this trial.

12.2 Data and Safety Monitoring Committee

To protect the interests of patients and the scientific integrity for all clinical trial research by the Children's Oncology Group, the COG Data and Safety Monitoring Committee (DSMC) reviews reports of interim analyses of study toxicity and outcomes prepared by the study statistician, in conjunction with the study chair's report. The DSMC may recommend the study be modified or terminated based on these analyses.

Toxicity monitoring is also the responsibility of the study committee and any unexpected frequency of serious events on the trial is to be brought to the attention of the DSMC. The study statistician is responsible for the monitoring of the interim results and is expected to request DSMC review of any protocol issues s/he feels require special review. Any COG member may bring specific study concerns to the attention of the DSMC.

The DSMC approves major study modifications proposed by the study committee prior to implementation (e.g., termination, dropping an arm based on toxicity results or other trials reported, increasing target sample size, etc.). The DSMC determines whether and to whom outcome results may be released prior to the release of study results at the time specified in the protocol document.

13.0 SPECIAL STUDIES SPECIMEN REQUIREMENTS

13.1 Visual Assessment Study

This study will test the visual acuity of the affected eye using a single surround HOTV, also known as the amblyopia treatment study visual acuity protocol. The test will be performed by either an ophthalmologist or their appropriately trained designate – an optometrist, ophthalmic technician or orthoptist.

This study is required for all patients who complete all three courses of IA melphalan treatment and retain the affected eye one year after the completion of therapy. Most children on this study will be between 3-5 years of age

On this study visual acuity will be tested once a year for 4 years, starting 1 year after completion of protocol therapy.

13.2 Histopathology of Eyes Enucleated for Disease Progression

This optional study is open to patients who are removed from protocol therapy due to disease progression or due to parent's request or physician recommendation and subsequently undergo enucleation. This study is designed carefully to assess eyes enucleated due to retinoblastoma for the presence certain histopathologic features that are known or suspected to affect prognosis adversely and to evaluate the changes associated to intra-arterial chemotherapy. See [Appendix II](#) for tissue handling guidelines.

13.2.1 Summary of Tissue Segments to be Submitted

- 1-2 H&E stained slides: Main A-P P-O segment
- **P-O Sections containing complete optic nerve head are mandatory** – at least 1 good representative level is recommended; if possible 2 levels are preferred.
- 2 H&E stained slides: Segments of breadloafed calottes (2 cassettes)

- 1 H&E stained slides: Transverse section of optic nerve from region of true surgical margin (remove and place in separate container of fixative prior to harvesting fresh tumor tissue).
- 1 set of unstained slides of same cassettes as H&E stained slides submitted

13.2.2 Labeling

All slides must be labeled with the study number (ARET12P1), the patient's COG patient I.D. number, the patient's surgical pathology number, block number and the collection date.

Data should be recorded on the Specimen Transmittal Form. A print out of this form along with the institutional pathology report with the patient's name removed must accompany the sample(s).

13.2.3 Specimen Shipping

For courier account information, please refer to the courier account usage guidelines found at: <https://members.childrensoncologygroup.org/prot/biology.asp>

Slides, pathology reports and transmittal forms should be sent to the Biopathology Center.

COG Biopathology Center – ARET12P1
Nationwide Children's Hospital
700 Children's Drive, WA1340**
Columbus, OH 43205
Phone: 614-722-2865
Fax: 614-722-2897

**Be sure to include the room number. Packages received without the room number may be returned to the sender.

14.0 IMAGING STUDIES REQUIRED AND GUIDELINES FOR OBTAINING

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable per COG administrative Policy 5.14 (except where explicitly prohibited within the protocol).

14.1 Imaging Guidelines

CT and MRI guidelines are available on the COG Member site at:

https://members.childrensoncologygroup.org/_files/reference/RefMaterial/DiagnosticImagingGuidelines_MRI&CT.pdf.

See also [Section 10](#) for overview of response assessment and preferred imaging studies.

Patients should have the traditional imaging/staging studies performed at their treating hospital for diagnosis (see [Table 7.1.1](#)).

MRI with and without gadolinium of head and orbits is required 2 weeks prior to study entry, at end of protocol therapy and every 6 months for 2 years during follow-up for all patients. If MRI is not available, CT with and without contrast may be substituted.

MRI may have advantages over CT, especially for intraocular disease, and is thus recommended, but not required. It should not be used in place of ophthalmologic exams. If MRI is used, it is recommended that both post- gadolinium T1-weighted images be obtained for surveillance of intracranial disease, including trilateral retinoblastoma and orbital T2-weighted MRI of the eye with fast spin echo (FSE) and surface coils (thin section 3-D FSE T2-weighted sequence) be obtained for surveillance of intraocular disease.^{[4.6.20](#)}

Head MRI's (or CT) done at study entry and for surveillance are NOT required to be submitted for central review. There will be central review of RetCam images and imaging studies that are required for the evaluation of the radiation therapy.

15.0 GUIDELINES FOR RADIOACTIVE PLAQUE TREATMENT

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable per COG administrative Policy 5.14 (except where explicitly prohibited within the protocol).

Radiation therapy (RT) for patients on COG protocols can only be delivered at approved COG RT facilities (per COG administrative policy 3.9).

No external beam radiation therapy including intensity modulated radiotherapy (IMRT), stereotactic radiotherapy, stereotactic radiosurgery, or proton beam therapy is permitted on this protocol. Plaque brachytherapy is the only radiotherapy permitted by protocol.

For eyes that fail protocol therapy by definitions in [Section 10.3](#), the further therapy will be determined at the discretion of the treating physician and will not be mandated by this protocol.

15.1 Timing

There must be at least 6 weeks between the last local ophthalmic therapy (any included in [Section 16](#)) or systemic chemotherapy (whichever was later) and the start of plaque radiotherapy

15.2 Isotope

Iodine-125 or Ruthenium-106 can be utilized for plaques.

15.3 Plaque Size

The plaque size is chosen so that the tumor base is covered entirely by the plaque with a tumor free margin of at least 1 – 2 mm on all sides. (For example, a tumor with a basal diameter of 10 mm would be plaqued with a plaque of at least 12-14 mm in diameter.)

An exception is permissible if the tumor is ≤ 2 mm of the optic nerve. In this case, the plaque may be trimmed (or “notched”) around the optic nerve so that the posterior edge of the plaque lies between the optic nerve and the posterior edge of the tumor.

15.4 Radiation Dose

The tumor dose is prescribed at the apex of the tumor. The dose rate at the prescription point must be ≥ 0.4 Gy/h but ≤ 0.8 Gy/h.

Three dimensional (3D) treatment planning is encouraged. International Commission on Radiation Units and Measurements (ICRU) Reports 50, 62, and 72 (www.icru.org) define prescription methods and nomenclature that will be utilized for this study. Imaging studies for this protocol must have slice thicknesses of 1 mm or less. Treatment planning will be based on the following definitions:

Gross target volume (GTV) is the volume of disease seen on planning imaging studies.

Clinical target volume (CTV) is equal to the GTV

Planning target volume (PTV) is equal to CTV.

If two dimensional (2D) treatment planning is employed, the thickness of the tumor is defined as the distance from the interior surface of the sclera to the apex of the tumor. The sclera is assumed to be 1 mm thick. Thus, if a tumor is said to be 3 mm thick, the point of dose prescription is 4 mm from the surface of the radioactive plaque, i.e. 3 mm thick tumor + 1 mm thick sclera = 4 mm. If the plaque is placed over one of the ocular muscles, the plaque must be considered to be 1 mm away from the external surface of the sclera, i.e. 3 mm thick tumor + 1 mm thick sclera + 1 mm muscle thickness = 5 mm. If overlying vitreous seeds are present, the dose is designed to include those seeds.

The prescription dose to the tumor apex is 36 Gy and will serve as the prescription dose for this protocol for both 2D and 3D treatment planning.

This dose, with allowance for minor deviations of up to 10% or up to 39.6 Gy, has supportive data for safety in salvaged eyes in multiple series.^{21,22}

15.5 Normal tissue dosimetry

The organs at risk guidelines in this section are recommendations. If the recommended doses to the organs at risk are exceeded because of target volume coverage requirements or other conditions, and explanation should be included in the quality assurance documentation.

Table 15.5: Organs at risk (OAR) and Planning organ at risk (PRV) dose guidelines for 3D treatment planning

Organ	Volume (%)	Dose (Gy)
Lens	100%	18 Gy
Optic Nerve	100%	54 Gy
Lacrimal Gland	100%	30 Gy

15.5.1 For 3D treatment planning the following defines required normal tissue DVH data:

Sclera

Defined as a 1 mm thick shell making up the radiographic “edge” of the eye. For the purposes of this study the cornea will be included in this volume for simplicity. No scleral necrosis was seen in the salvage series from C. L. Shields, et al. with tumor base dose of 120 Gy and maximal tumor base dose of 358 Gy with 5-year follow-up.^{21,22}

Optic nerve

The optic nerve will generally be shielded by the lip of the plaque and exposure minimized by careful plaque positioning.

Lacrimal Gland

Lens

15.5.2 For 2D treatment planning the following define required
Sclera

The dose to the sclera is estimated and reported at a point on the central axis of the plaque 1 mm from the surface of the plaque. This point is approximately coincident with the internal surface of the sclera near the center of the tumor.

Optic nerve

The dose to the center of the optic disc is calculated and reported.

Contralateral Retina (same eye)

The dose to the retina opposite the tumor is calculated and reported. The dose is calculated 16 mm from the scleral surface at the base of the tumor measured along a diameter of the globe passing through the apex of the tumor for infants \leq 6 months of age and 20 mm for patients $>$ 6 months of age.

Lens

The dose to the center of the lens is calculated and reported.

15.6 QA Documentation

No on-treatment review will be required for this protocol. It is recognized that the dose distribution from brachytherapy implants is inherently non-uniform.

15.6.1 **Institutions are required to submit the treatment plan in digital format.**
Submission of treatment plans in digital format (either DICOM RT or RTOG format) is required. Digital data must include CT scans, structures, plan, and dose files. Submission may be by either sFTP or CD. Instructions for data submission are on the IROC Rhode Island (QARC) web site at www.qarc.org under "Digital Data." Any items on the list below that are not part of the digital submission may be included with the transmission of the digital RT data via sFTP or submitted separately. Screen captures are preferred to hard copy for items that are not part of the digital plan.

15.6.2 **Supportive Data**

- All diagnostic imaging used to plan the target volume. Digital format is required (DICOM).
- Radiotherapy record (treatment chart) including prescription and daily and cumulative doses to all required areas and organs at risk. This included copies of the procedure notes related to the placement and the removal of the plaque.
- If the recommended doses to the organs at risk are exceeded, an explanation should be included for review by IROC Rhode Island (QARC) and the radiation oncology reviewers.

15.6.3 The Brachytherapy Physics Reporting Form available at www.QARC.org should be submitted for all patients treated with radioactive plaque treatment. The completed form should be scanned and e-mailed to DataSubmission@QARC.org or faxed to IROC Rhode Island (QARC).

IROC Rhode Island (QARC)

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15.7 Definition of Deviation in Protocol Performance

Table 15.7 Radiation Therapy Deviation Definitions

	DEVIATION	
	Minor	Major
Prescription Dose		
	Difference in prescribed or computed dose is 6-10% of protocol specified dose	Difference in prescribed or computed dose is > 10% of protocol specified dose
Volume		
	3D: GTV does not encompass MR-visible tumor (gap is less than 2 mm) 2D: Measured tumor thickness is less than 2mm different on imaging from prescription depth	3D: GTV does not encompass MR-visible tumor (gap is more than 2 mm) 2D: Measured tumor thickness is more than 2mm different on imaging from prescription depth
Organs at Risk		
	Dose to any OAR exceeds dose listed in table 15.5 by 6-10%.	Dose to any OAR exceeds dose listed in table 15.5 by > 10%.

16.0 OPHTHALMOLOGIC GUIDELINES

16.1 Local Therapy

Local therapy is used to eradicate local disease after reduction of the tumor volume by chemotherapy and may include cryotherapy, green laser, infrared laser and/or radioactive plaque. See [Section 15.0](#) for Plaque Guidelines. The goal of local therapy is to achieve Type I regression pattern with calcification or Type IV with flat chorioretinal scars, or avascular, linear, white gliosis.

16.1.1 Cryoablation

Cryotherapy can be utilized following the completion of the first cycle of IA melphalan, for ablating tumor remnants/recurrences after protocol therapy up to 3 mm in height that are located at or anterior to the equator. Tumors will be treated with 3 freeze-thaw cycles at each session. The ice ball will be allowed to thaw completely between freezes. Tumor, including any overlying vitreous seeds, must be completely enclosed by ice to be considered treated. If the lesion has an extensive base, part of the lesion (up to 4 sites) may be frozen in one session and then the additional part of the lesion at a later session with next EUA. It is recommended that no more than 4 different sites be frozen in one eye at a single session. The only acceptable regression patterns following cryotherapy are either flat chorioretinal scars or chorioretinal scars with a central calcific deposit; achieving this may require multiple treatments. There is less likelihood of creating vitreous seeds with cryotherapy if a tumor has been previously treated with chemotherapy. Extensive cryotherapy has been associated with significant persistent retinal detachment, particularly if the retina was originally detached prior to chemotherapy. Retinal breaks can be caused by cryotherapy. Including calcified Type I regression in the ice ball should be avoided if possible. The site of transition between the retina and the calcific mass is at risk to develop a retinal break following cryotherapy.

16.1.2 Green Laser Photoablation

After cycle one of IA melphalan, green laser (argon or 532 nm) can be used to ablate tumors up to 8 mm in thickness, especially posterior to the equator following chemotherapy. The shorter wave-length 532 nm laser is selected when there is a minimal amount of underlying pigmentation to absorb the energy. Through a widely dilated pupil, a 500 μ spot size is delivered directly to the tumor by indirect ophthalmoscopy (the 28D indirect lens gives a larger laser spot on the lesion than the 20D lens), at a low energy output, increasing the power by 50 mW increments until "blanching" or gentle "whitening" of the tumor occurs. This end-point is established at the edge of the tumor with a portion of the spot on the normal retina. Begin with a power of 200-300 mW and do not exceed 700 mW or 1000 msec.

The tumor is outlined with burns half on and half off the retina. There should be 30% spot overlap. After outlining the tumor, the entire tumor should be covered with 30 % overlapping spots. The "take" may not be obvious in thicker parts of the tumor. Complete coverage is considered "1 laser treatment". Each numbered lesion should receive a minimum of 3 complete "laser treatments" with only one "complete laser treatment" given at each EUA.

The response to laser energy depends to a large extent on the amount of pigmentation underlying the area being treated. In patients with significant pigmentation, relatively little energy is needed to achieve end-point. Within pigmented scars, the uptake of energy varies with the amount of pigment and the exposure has to be adjusted accordingly. In lightly pigmented fundi, more energy is required to achieve end point. Small hemorrhages may occur within tumors.

The immediate response to the green laser is whitening of the retina and tumor. Reduced volume and increased translucency of the tumor is considered a good outcome response, but a flat scar with pigment epithelial proliferation is preferred. Several laser sessions may be required to achieve this. The green laser used with excessive power might cause hemorrhage or breaks in Bruch's membrane. Laser-induced hemorrhage has been associated with vitreous seeding. Inadequate dilation of the pupil can cause laser burns to the iris.

16.1.3 Infrared Laser Photablation

Infrared laser can be selected to treat RB tumors up to 8 mm thickness that have an intact retinal pigment epithelium following IA melphalan. Either the 810 nm diode or 1064 nm continuous wave YAG laser apparently has a similar effect on RB tumors. Using the operating microscope or indirect ophthalmoscope delivery system, a spot size \geq 1000-2000 μ is applied directly to the tumor. Starting with a low power at 1-second durations, the power or time is gradually increased until a slight whitening is observed, which is then applied to the entire surface of the tumor. Do not exceed 800 MW in power. Although very little response may be seen acutely with the infrared laser, subsequent observations will show a decrease in tumor volume with good response. Complete response often results in a residual flat mass, with a white, gliotic appearance, devoid of tumor vessels. This response may be very difficult to interpret and the activity can only be judged by repeated observations. The use of excessive power may result in hemorrhage or vitreous dissemination of tumor, but starting with low power and gradually increasing avoids these problems.

16.1.4 Plaque Radiotherapy

Plaque radiotherapy may be used to treat local recurrences up to 8 mm in thickness and 15 mm in base. See [Section 15.0](#) for guidelines. Proliferative retinopathy secondary to plaque will be monitored on this study, with an early stopping rule if incidence is $> 15\%$ (See [Statistical Section 9.0](#)).

16.1.5 Frequency of Local Ablative Therapy

An EUA will then be done within 1 week prior to each cycle of IA melphalan and within 4 weeks of completion of the third cycle of IA Melphalan. After completion of protocol therapy, an EUA is recommended every 1-4 months until age 3 years, then every 4-6 months until age 5 years, then 6-12 months. Patients should be transitioned to an office exam without anesthesia when old enough to cooperate in an effort to ensure that anterior recurrences are not missed, B-scan ultrasonography can be used as an adjunct to the office indirect ophthalmoscopy when scleral depression is not possible.

APPENDIX I: CLASSIFICATION SYSTEM FOR INTRAOCULAR RETINOBLASTOMA**INTERNATIONAL CLASSIFICATION SYSTEMS FOR INTRAOCULAR RETINOBLASTOMA****Group A***Small intraretinal tumors away from foveola and disc*

- All tumors are 3 mm or smaller in greatest dimension, confined to the retina *and*
- All tumors are located further than 3 mm from the foveola **and** 1.5 mm from the optic disc

Group B*All remaining discrete tumors confined to the retina*

- All other tumors confined to the retina not in Group A
- Tumor-associated subretinal fluid less than 3 mm from the tumor with no subretinal seeding

Group C*Discrete Local disease with minimal subretinal or vitreous seeding*

- Tumor(s) are discrete
- Subretinal fluid, present or past, without seeding involving up to 1/4 retina
- Local fine vitreous seeding may be present close to discrete tumor
- Local subretinal seeding less than 3 mm (2 DD) from the tumor

Group D*Diffuse disease with significant vitreous or subretinal seeding*

- Tumor(s) may be massive or diffuse
- Subretinal fluid present or past without seeding, involving up to total retinal detachment
- Diffuse or massive vitreous disease may include “greasy” seeds or avascular tumor masses
- Diffuse subretinal seeding may include subretinal plaques or tumor nodules

Group E*Presence of any one or more of these poor prognosis features*

- Tumor touching the lens
- Tumor anterior to anterior vitreous face involving ciliary body or anterior segment
- Diffuse infiltrating retinoblastoma
- Neovascular glaucoma
- Opaque media from hemorrhage
- Tumor necrosis with aseptic orbital cellulitis
- Phthisis bulbi

APPENDIX II: GUIDELINES FOR TISSUE HANDLING

Summary of Tissue Segments to be submitted plus one set of UNSTAINED slides of same cassettes.

1-2 H&E slides: Main A-P P-O segment (Figure 1 P.O.)

P-O Sections through optic nerve head are mandatory- at least 1 good representative level is recommended; if possible 2 levels are preferred. Please refer to "Basic goals of histopathologic evaluation" below for comprehensive information.

2 H&E slides: Segments of breadloafed calottes (2 cassettes) (Figure 1- S.C and I.C.)

1 H&E slide: Transverse section of optic nerve from region of true surgical margin (remove and place in separate container of fixative prior to harvesting fresh tumor tissue). (Figure 1 O.N.)

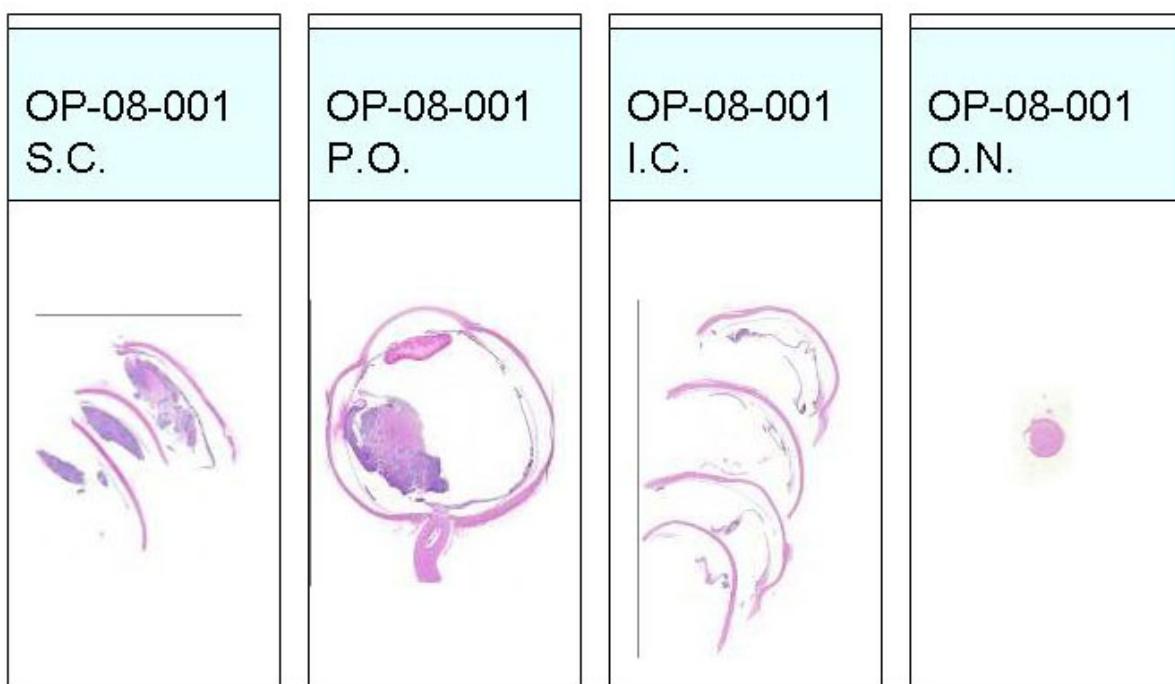


FIGURE 1.

FIXATION, GROSSING AND SUBMISSION FOR HISTOLOGIC EXAMINATION:

Fixation

The specimen should be fixed immediately after enucleation. Since the eye has failed therapy harvesting of fresh tumor is not recommended. The eye should be immersed in a container of 50-100 mL of 10% Formalin. At least 48 hours of fixation are recommended. Do not open cornea or sclera or inject formalin.

Basic Goals of Histopathologic Evaluation

The histopathology part of this study is designed carefully to assess eyes enucleated for retinoblastoma for the presence certain histopathologic features that are known or suspected to affect prognosis adversely and to evaluate the changes associated to intra-arterial chemotherapy.

These include:

- The presence or absence of tumor in the **optic nerve surgical margin**.
- The presence and depth of **optic nerve invasion**, especially the presence of retrolaminar nerve invasion
- The presence and degree of **uveal stromal invasion** i.e. infiltration of the stroma of the choroid, ciliary body and/or iris, including the presence of grossly visible or massive invasion. For the purpose of this study massive invasion is defined as follows
 - I: Posterior uveal invasion absent
 - II: Posterior uveal invasion present
 - IIA: Largest dimension of intrachoroidal tumor on slide less than 1 mm
 - IIB: Largest dimension between 1-3 mm
 - IIC: Largest dimension greater than 3 mm (*MASSIVE*)
 - IID: Posterior uveal tumor noted grossly (*MASSIVE*)
- The presence of **extraocular extension** and **orbital invasion**

Treatment Effect

- Other histologic features with potential prognostic significance include invasion of the anterior chamber and trabecular meshwork, and invasion of the scleral and scleral emissarial canals.
- Changes in the tumor (calcification, giant cells, macrophages, necrosis)
- Changes in retina and choroid (vascularization, necrosis, atrophy, ischemic changes, inflammation)
- Changes in optic nerve vasculature and cellularity
- Foreign body reaction in any structure of the eye
- Anterior chamber changes (cataract, neovascularization of iris)
- Vascular changes

Grossing Eyes: General Principles and Technique**The P-O Segment**

Routinely, histologic sections of eyes are cut from a paraffin block that includes a short cylindrical anteroposterior segment of the globe called the **P-O** or pupil-optic nerve segment. The **P-O** segment includes most of the cornea, iris and pupil anteriorly and the optic nerve posteriorly (Figure 1 & 3).

The Calottes

The dome-shaped caps of tissue that are removed when the P-O segment is prepared are called **calottes** (French for visor-less cap). The first calotte should include about one-fourth or one-fifth of the peripheral anterior chamber. The first calotte should also include the scleral window if one has been performed (Figure 1 & 3).

Standard Measurements

Before the globe is opened, a standard series of measurements are made using a caliper or a millimeter rule. **The A-P** (anteroposterior including the cornea) and the **horizontal and vertical dimensions** (viewed from the front) **are measured and recorded in that order**. The length of the attached segment of optic nerve is also recorded. **The horizontal and vertical diameter of the cornea and the diameter of the pupil** also are recorded (Figure 2). The iris color is also noted.

Globe: AP, Horizontal, Vertical, length of optic nerve.

Cornea- horizontal and vertical

Diameter of pupil

Other Lesions of interest- (if any, approximate dimension of intraocular tumor.)

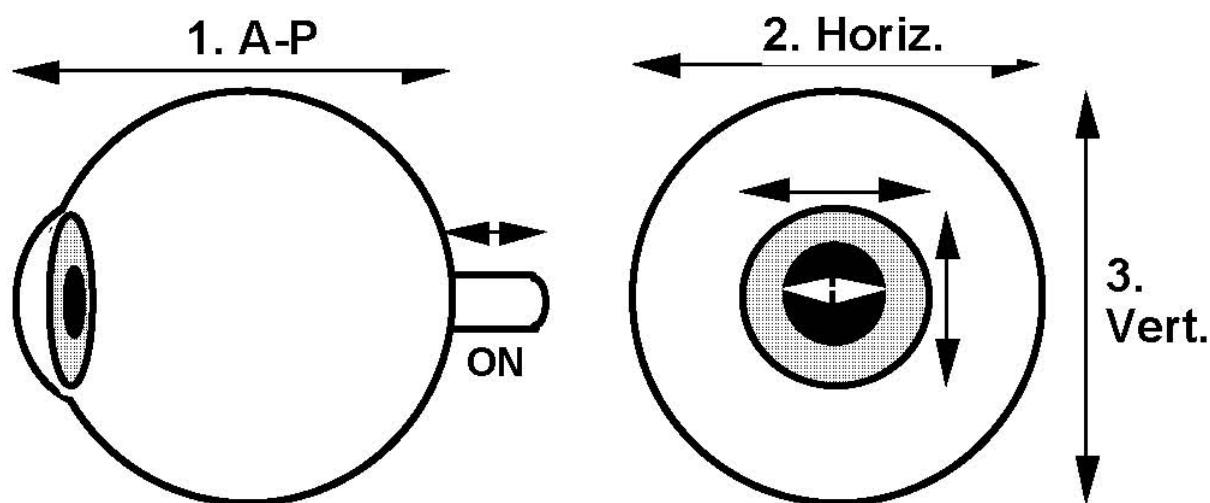


FIGURE 2

Preparing the main P-O Segment

Eyes should be carefully sectioned using a standard double-edge razor blade or half a blade, which has been snapped in half within its protective wrapper. The two ends of the blade are held between the apposed surfaces of the thumb and forefinger. The blade is oriented perpendicular to the dissector's chest and a gentle sawing motion is used. A scalpel should never be used to open an eye because it tends to indent and compress the eye too forcefully (Figure 3).

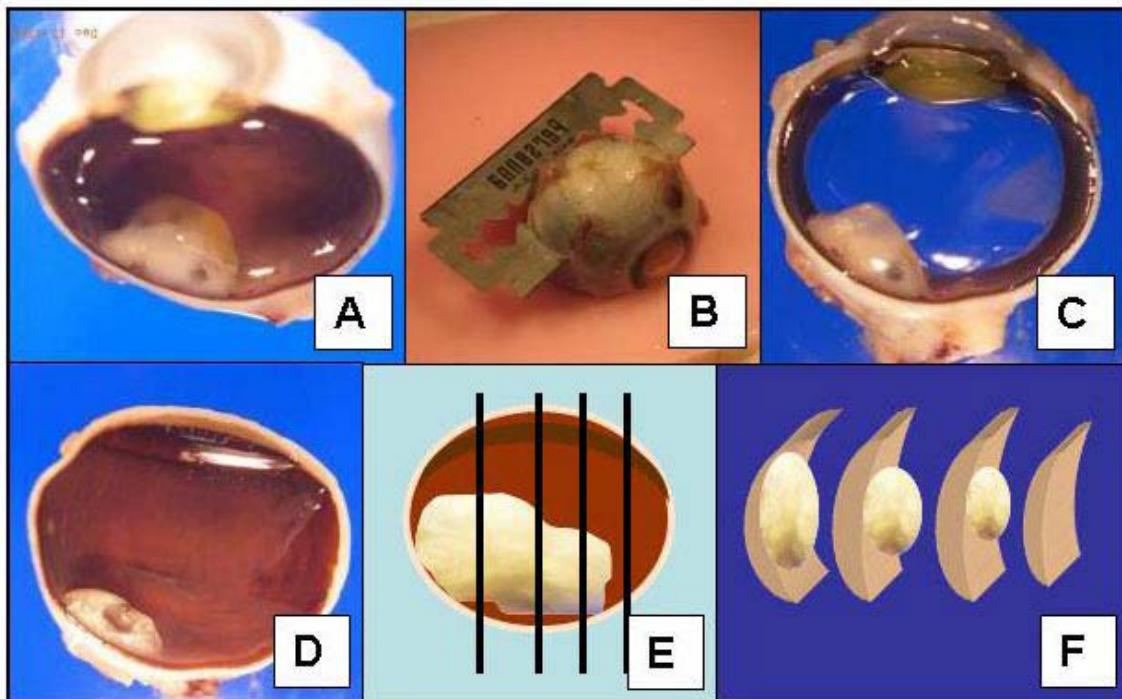


FIGURE 3

Removing the First Calotte

During the initial cut (removal of the first calotte), the eye is placed corneal side-down on the cutting block and steadied with the non-dominant hand. Guidelines drawn on the eye with colored pencil facilitate sectioning and help to assure that the eye is oriented properly prior to sectioning. The incision is started posteriorly and extended anteriorly. At the start of the incision, the blade is positioned close to the optic nerve, but external to its dural sheath. A gentle sawing motion is used. Try not to bend the blade during sectioning. In addition, care must be taken to avoid compressing the eye too forcefully, as this can collapse the eye and express the tumor and intraocular contents. The cut should enter the periphery of the anterior chamber anteriorly. The first calotte should include about one-fifth to one-fourth of the peripheral anterior chamber (Figure 3A).

Stereomicroscopic Examination

After the first calotte is removed, the eye should be examined with a dissecting microscope. If uveal invasion by tumor is noted or suspected grossly it should be documented.

Removing the Second Calotte

The second calotte is then removed. This is most easily done by placing the eye cut-surface down on the cutting block, and making a second parallel cut (Figure 3B-D).

The main PO segment is submitted. At least two levels comprising 10 slides each that include the central portion of the optic nerve head should be cut.

Submission of Additional Tissue Segments from the Calottes to Exclude Uveal Invasion.

It is extremely important to examine the residual tissue in the calottes to exclude uveal stromal invasion that may not be evident grossly.

Both calottes should be sectioned in a “breadloaf” fashion and submitted entirely (Figure 3E-F). If a significant amount of tumor is present in a calotte, one may choose to prepare an addition A-P segment of the entire globe prior to preparing the breadloafed segments.

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