DRCR Retina Network

Single-Arm Study Assessing the Effects of Pneumatic Vitreolysis on Macular Hole (Protocol AH)

IDE Sponsor: Jaeb Center for Health Research (JCHR)

Version 3.0

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Signature Page

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LIST OF ABBREVIATIONS

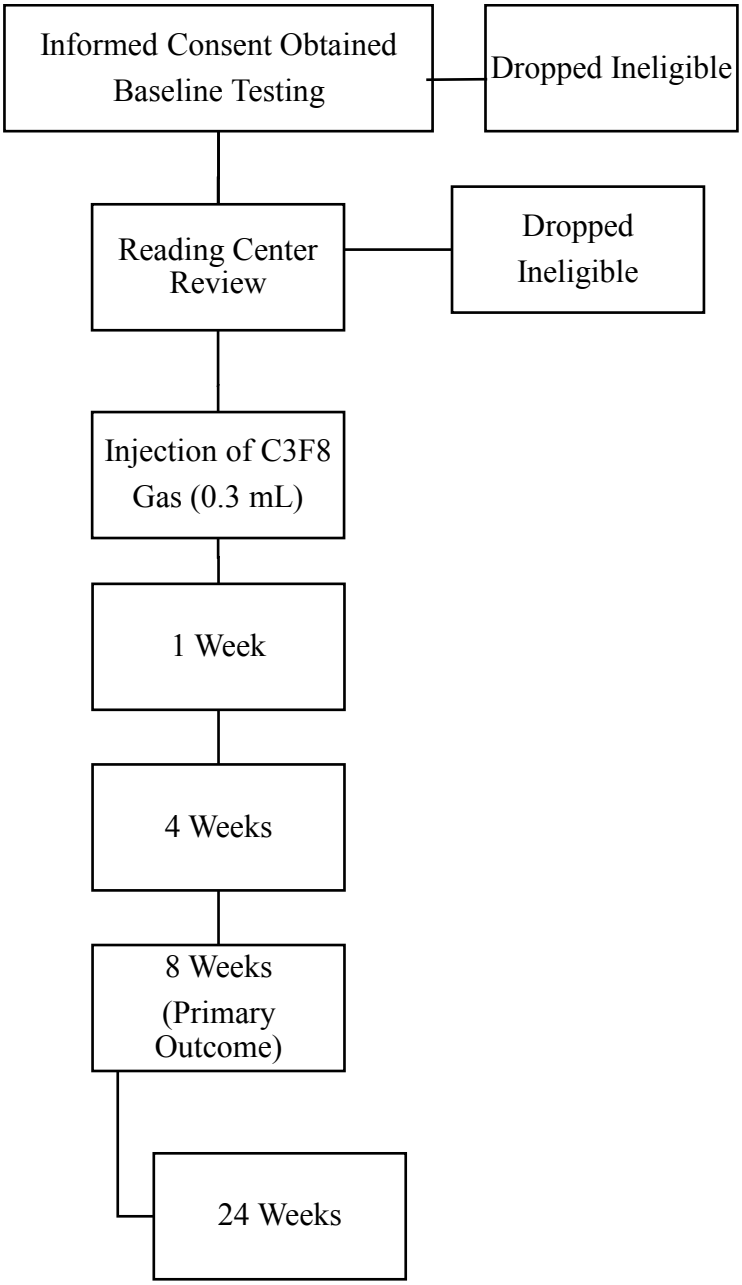
ABBREVIATION	DEFINITION
AUC	Area Under the Curve
CI	Confidence Interval
CRF	Case Report Form
DSMC	Data and Safety Monitoring Committee
E-ETDRS	Electronic-Early Treatment Diabetic Retinopathy Study
ERM	Epiretinal Membrane
ETDRS	Early Treatment Diabetic Retinopathy Study
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
ID	Identification
IDE	Investigational Device Exemption
IOP	Intraocular Pressure
IRB	Institutional Review Board
ITT	Intention-To-Treat
JCHR	Jaeb Center for Health Research
MH	Macular Hole
OCT	Optical Coherence Tomography
PVD	Posterior Vitreous Detachment
PVL	Pneumatic Vitreolysis
SADE	Serious Adverse Device Event
SAE	Serious Adverse Event
SD	Standard Deviation
UADE	Unanticipated Adverse Device Effect
VMT	Vitreomacular Traction
VMA	Vitreomacular Adhesion

PROTOCOL SUMMARY

PARTICIPANT AREA	DESCRIPTION
Title	Single-Arm Study Assessing the Effects of Pneumatic Vitreolysis on Macular Hole
Précis	Eyes with vitreomacular traction (VMT) and full-thickness macular hole (MH) will be enrolled into a non-randomized cohort treated with PVL to determine the proportion with VMT release and MH closure and to assess factors associated with success.
Investigational Device	Intraocular gas (C_3F_8) injection
Objectives	To obtain estimates of the proportion of eyes with MH closure of the inner retinal layers for eyes with VMT and full-thickness MHs treated with PVL.
Rationale	Understanding the rates of VMT release and MH closures in eyes with full-thickness MH treated with PVL is of interest. Surgery would result in nearly 100% hole closure and VMT release, making vitrectomy a poor control group choice. Spontaneous resolution of MH is highly unlikely, making an observation arm unnecessary. Therefore, these eyes will be enrolled into a non-randomized cohort treated with PVL to assess the outcomes of treatment.
Study Design	Single-arm study
Number of Sites	Approximately 50 sites
Endpoint	**Primary Outcome:** Proportion of eyes with MH closure of the inner retinal layers without rescue treatment at 8 weeks. **Key Secondary Outcomes:** proportion of eyes with central VMT release without rescue treatment, proportion of eyes with rescue treatment, mean change in visual acuity **Key Safety Outcomes:** retinal tear, retinal detachment, traumatic cataract, cataract extraction, vitreous hemorrhage, intraocular pressure (IOP) increase, and endophthalmitis.
Population	**Key Inclusion Criteria** • Age ≥ 18 years. • Able and willing to avoid high altitude until gas resolution (approximately 6 to 8 weeks) • For phakic patients, able and willing to avoid supine positioning until gas resolution (approximately 6 to 8 weeks) • Able and willing to position face down for at least 50% of the time for at least 4 days post-injection • At least 1 eye with:

PARTICIPANT AREA	DESCRIPTION
	○ Central vitreomacular adhesion on OCT that is no larger than 3000 microns, confirmed by a central reading center ○ Full-thickness MH that is ≤ 250 microns at the narrowest point as measured on OCT, confirmed by a central Reading Center ○ Best corrected E-ETDRS visual acuity equivalent of 20/25 to 20/400 **Key Exclusion Criteria** • Other condition that might affect visual acuity during the course of the study (e.g., retinal vein occlusion, advanced age-related macular degeneration, or macular edema induced by a condition other than VMT) • High level myopia (-8.00 diopters or more myopic if phakic or retinal abnormalities consistent with pathologic myopia if phakic or pseudophakic) • Prior gas injection, ocriplasmin injection, or intraocular injection for any reason • Prior vitrectomy • History of advanced glaucoma that contraindicates intraocular gas injection
Sample Size	50 eyes
Treatment Groups	C_3F_8 injection only
Participant Duration	24 weeks
Protocol Overview/Synopsis	1. Informed consent will be obtained. 2. Eligibility will be assessed, including reading center confirmation of VMT and MH on OCT. 3. Eligible eyes with VMT and MH will be treated with C_3F_8 injection. 4. Follow-up visits will occur at 1, 4, 8, and 24 weeks and consist of visual acuity testing, ocular exam, and OCT. 5. The primary outcome assessment will be the proportion of eyes at 8 weeks with full-thickness MH closure of inner retinal layers without rescue treatment.

SCHEMATIC OF STUDY DESIGN



SCHEDULE OF STUDY VISITS AND PROCEDURES

	Enrollment Visit*	1, 4, 8, and 24 weeks
E-ETDRS best corrected visual acuity [a]	X	X
OCT [b]	X	X
Eye exam [c]	X	X
Reading center eligibility confirmation [d]	X	
Gas injection	X	

* All baseline testing must occur within 8 days prior to enrollment.

a, Both eyes at all visits; includes protocol refraction in study eye only at each visit and in both eyes at enrollment and 8 weeks. Electronic ETDRS (E-ETDRS) testing using the Electronic Visual Acuity Tester that has been validated against 4-meter chart ETDRS testing.

b, Both eyes at baseline; study eye only at follow up visits.

c, Both eyes at baseline; study eye only at each follow-up visit. Includes slit lamp exam (including assessment of lens), measurement of intraocular pressure, and dilated ophthalmoscopy. Scleral depression is required at baseline to confirm eligibility. During follow up, the eye exam should be extensive enough to identify adverse events of interest. An extended ophthalmoscopy including a scleral depression is required at 1, 4, and 8 weeks.

d, Reading center review of the OCT for eligibility must occur prior to enrollment.

CHAPTER 1: BACKGROUND INFORMATION AND STUDY SYNOPSIS

1.1. Introduction

1.1.1 Vitreoretinal Interface Abnormalities

Disorders of the vitreoretinal interface represent a spectrum of abnormalities that develop as the posterior hyaloid separates from the internal limiting membrane. Vitreomacular adhesion (VMA) occurs when the posterior hyaloid remains attached to the internal limiting membrane centrally. Overall, about 1.5% of the population is estimated to have eye diseases caused by or associated with VMA.[1] The incidence of VMA diagnoses is expected to increase with widespread use of spectral-domain optical coherence tomography (SD-OCT). Vitreomacular traction (VMT) is diagnosed when VMA results in traction, distortion of retinal architecture, and patient symptomatology.[2]

Advanced VMT can lead to macular holes (MH), in which tractional forces create small full-thickness defects on the posterior fundus, often requiring surgical intervention.[2] Regarding the incidence of idiopathic full-thickness macular holes, a population-based study showed idiopathic macular holes occur at an age and sex-adjusted incidence in 8.69 eyes per 100,000 population per year.[3] The female to male ratio was determined to be 3.3 to 1, and bilateral idiopathic MHs occurred in 11.7% of patients and accounted for 20.9% of the affected eyes.[3] In another study of a large population of patients with age-related macular degeneration (15,196 with non neovascular age-related macular degeneration (AMD) and 12,716 with neovascular AMD), 0.7% were found to have MHs (1.1% with non-neovascular AMD and 0.3% with neovascular AMD).[4] Regarding MH prevalence globally, the Baltimore Eye Study reported a prevalence of 3.3 per 1,000 persons in Maryland,[5] the Beaver Dam Eye Study showed a prevalence of 2.9 per 1,000 persons in Wisconsin, the Blue Mountains Eye Study showed a prevalence of 0.2 per 1,000 persons in Australia,[6] the Beijing Eye Study showed a prevalence of 0.9 per 1,000 persons in China,[7] and Sen et al. reported a prevalence of 1.7 per 1,000 persons in Southern India.[8]

1.1.2 Treatments for VMT with Macular Hole

For eyes with VMT with MH, prompt treatment is indicated to restore central vision and prevent retinal detachment. In the MIVI-TRUST trial, ocriplasmin was successful in 60% in eyes with an MH of < 250 microns.[9] However, there have been multiple anecdotal reports of substantial ocular complications associated with intraocular administration of ocriplasmin,[10-14] including transient vision loss, persistent dyschromatopsia, electroretinographic abnormalities, subluxation of the crystalline lens likely related to zonulolysis, and disturbance or dehiscence of the ellipsoid layer documented by OCT. These adverse events have created major concerns among many retinal surgeons in the clinical use of this drug.[10-14] Therefore, vitrectomy is currently the first line therapy for VMT with MH. Although MH closure is usually successful after vitrectomy (approaching 100% success rate in several series), there are associated downsides including cost, patient discomfort, length of time a large bubble resides in the eye, and possible adverse events such as endopthalmitis, retinal detachment, and cataract progression.[15]

1.1.3 Pneumatic Vitreolysis (PVL)

Pneumatic vitreolysis (intraocular injection of expansile gas to induce a posterior vitreous detachment [PVD]) has been suggested as a potential treatment alternative to vitrectomy or ocriplasmin for VMT with MH. In 1995, Chan et al. first demonstrated and reported the utility of intraocular gas (C_3F_8) injection where 13 of 17 (76%) stage 1 or stage 2 macular holes closed after injection.[16] Subsequently, Jorge et al. showed success in the induction of PVD (6 of 6 eyes) and macular hole closure (5 of 6 eyes) with C_3F_8 in small case series.[17, 18] Mori et al.[19] reported 5

of 10 eyes had hole closure after gas.[19] Steinle et al. reported a success rate of 83% (25 of 30 eyes) with C_3F_8 gas in a retrospective case series for treatment of VMT syndrome.[20] In a 2016 retrospective review of 15 consecutive eyes receiving C_3F_8 gas for pneumatic vitreolysis performed in 2 centers, Chan and Mein reported a success rate of 100% for VMT release and 67% for hole closure in eyes with small stage 2 MH ≤ 250 microns.[21] In 2019, Chan and Mein provided an update on this study and reported a success rate of 95.7% for VMT release and 65.2% for hole closure among 23 eyes with small stage 2 MH ≤ 250 microns.[22]

1.2. Rationale

Understanding the rates of MH closure and VMT release in eyes with full-thickness macular holes treated with PVL is of interest given the low cost and convenience of gas injection in the office setting as well as a low rate of adverse events reported in prior retrospective studies. Although spontaneous macular hole closure is possible, this occurs infrequently.[23] It is likely that eyes with these characteristics would need prompt treatment, making randomization to a sham arm inappropriate. Prior studies have established the benefit of vitrectomy for treatment of macular holes, reporting 80 to 90% success rates in MH closure with associated visual benefit, making vitrectomy an unnecessary (and expensive) control group choice.[23-27] Therefore, these eyes will be enrolled into a non-randomized cohort treated with PVL to assess the outcomes of treatment.

If a large percentage of eyes can achieve MH closure with a simple in-office, low-cost procedure, while averting the invasiveness and expense of a vitrectomy for this condition, this would provide a viable first-line treatment option. Even if this proposed study finds that PVL is only moderately successful, physicians and patients may decide to attempt PVL in the office first, before proceeding with more costly, invasive surgery. Thus, even without a control group, the results from this study will provide data of value for physicians and patients to make informed decisions about treatment course.

1.3 Study Objectives

Primary

1. To obtain estimates for the proportion of eyes with MH closure of the inner retinal layers for eyes with VMT and full-thickness macular holes treated with PVL

1.4 Potential Risks and Benefits of C_3F_8 Gas Injection

1.4.1 Known Potential Risks

Potential risks of C_3F_8 gas injection include the following:

- Pain, discomfort, redness, or itching lasting for a few days is likely.
- Subconjunctival hemorrhage or floaters will commonly occur as a result of the injection. The floaters are typically reduced after 6 to 8 weeks, but some floaters may persist.
- Immediately following the injection, there may be elevation of IOP. Pressure usually returns to normal spontaneously, but may need to be treated with topical drugs or a paracentesis to lower the pressure. The likelihood of permanent loss of vision from elevated IOP is less than 1%.
- Although it has not been reported in prior case series, endophthalmitis could theoretically develop. The risk of endophthalmitis from other intraocular injections is less than 1%.

- A retinal tear or detachment could occur. The risk of retinal breaks or detachment after gas injection is approximately 5-13%. The risk of retinal detachment is increased if there are pre-existing peripheral retinal abnormalities such as lattice degeneration or cystic tufts.
- There is a possibility of traumatic cataract from the injection. The risk of developing a cataract from the injection is <1%
- If paracentesis is performed, there is a similar risk of traumatic cataract from paracentesis.
- If vitrectomy is required while gas is in the eye, there is high likelihood of cataract formation during surgery that may require cataract removal at the time of vitrectomy.
- Limited and transient uveitis may develop after gas injection. Persistent uveitis is uncommon.
- Limited transient conjunctival or episcleral hemorrhage is common shortly after gas injection. It is usually inconsequential and clears spontaneously from a few days to a week or two.
- Limited vitreous hemorrhage or opacities after gas injection is uncommon but may occur occasionally after gas injection, particularly given a history of active anticoagulation therapy or predisposing risk for hemorrhage. If present, it usually resolves from a few days to a few months. Marked intraocular hemorrhage requiring a surgical intervention after gas injection is exceedingly rare (< 1%).
- Pre-existing epiretinal fibrosis may sometimes progress or new epiretinal fibrosis may develop after gas injection.
- The development of excessive scarring on top of or under the retina after gas injection is exceedingly rare. When this occurs, it is usually associated with advanced retinal detachment, which is also uncommon after gas injection.

Additional risks if the participant does not follow post-injection instructions:

- Intraocular pressure may increase if the patient experiences changes in elevation (i.e. travel by air or over mountain ranges) while the gas bubble is still present in the eye.
- Loss of vision or blindness is possible if nitrous oxide anesthesia is administered with the gas bubble still present in the eye.
- Incorrect head positioning following the gas injection may lead to glaucoma or cataracts.

1.4.2 Known Potential Benefits

Potential benefits from participation in the study for eyes treated with PVL include, improved visual acuity, improved quality of vision, closure of MH, and avoidance of more invasive and expensive procedures, i.e., vitrectomy, ocriplasmin.

1.4.3 Risk Assessment

The risk level is considered to be research involving greater than minimal risk.

1.5 General Considerations

The study is being conducted in compliance with the policies described in the study policies document, with the ethical principles that have their origin in the Declaration of Helsinki, with the protocol described herein, and with the standards of Good Clinical Practice (GCP).

The DRCR Retina Network procedures manuals provide details of the procedures.

Data will be directly collected in electronic case report forms, which will be considered the source data.

There is no restriction on the number of subjects to be enrolled by each site towards the overall recruitment goal. However, recruitment will be monitored on an ongoing basis and the sponsor can decide to place recruitment at a particular site on hold as needed.

All consented participants will be logged. The protocol is considered a significant risk device study because intraocular injection of C_3F_8 is experimental for this indication. Therefore, an investigational device exemption (IDE) from the U.S. Food and Drug Administration (FDA) is required to conduct the study.

CHAPTER 2: STUDY PARTICIPANT ELIGIBILITY AND ENROLLMENT

2.1 Participant Recruitment and Enrollment

Enrollment will proceed with the goal of at least 50 participants deemed eligible by Reading Center and treated with C_3F_8. Participants who have signed consent may be permitted to continue into the study, if eligible, even if the enrollment goal has been reached.

Study participants will be recruited from approximately 50 clinical centers in the United States. Approximately 5 participants are expected to be enrolled each month, resulting in 10 months of recruitment, for a total study duration of 16 months.

All eligible participants will be included without regard to gender, race, or ethnicity. There is no restriction on the number of participants to be enrolled by each site toward the overall recruitment goal.

Potential eligibility may be assessed as part of a routine-care examination. Before completing any procedures or collecting any data that are not part of usual care, written informed consent will be obtained.

The study protocol will be discussed with the potential study participant by study staff. The potential study participant will be given the Informed Consent Form to read. Potential study participants will be encouraged to discuss the study with family members and their personal physicians(s) before deciding whether to participate in the study.

As part of the informed consent process, each participant will be asked to sign an authorization for release of personal information. The investigator, or his or her designee, will review the study-specific information that will be collected and to whom that information will be disclosed. After speaking with the participant, questions will be answered about the details regarding authorization.

2.2 Participant Eligibility Criteria

2.2.1 Participant-Level Criteria

Inclusion

To be eligible, the following inclusion criteria must be met:

1. Age ≥ 18 years
    - *Participants < 18 years old are not being included because the condition is so rare in this age group that the diagnosis may be questionable.*
2. At least one eye meets the study eye criteria listed in section 2.2.2
3. Able and willing to provide informed consent
4. Able and willing to avoid high altitude travel, including airline travel, until gas resolution (approximately 6 to 8 weeks)
5. For phakic patients, able and willing to avoid supine position until gas resolution (approximately 6 to 8 weeks)
6. Able and willing to position face down for at least 50% of the time for at least 4 days post-injection (to facilitate macular hole closure)
7. Able and willing to wear wristband that informs any medical personnel that the patient has a gas bubble in the eye

Exclusion

A potential participant is not eligible if any of the following exclusion criteria are present:

8. A condition that, in the opinion of the investigator, would preclude participation in the study (e.g., unstable medical status that might preclude completion of follow up)
9. Participation in an investigational trial within 30 days of enrollment that involves treatment with any drug or device that has not received regulatory approval for the indication being studied at the time of study entry
    - *Note: study participants should not receive another investigational drug or device while participating in the study*
10. Known contraindication to any component of the treatment
11. Known allergy to any drug used in the procedure prep (including povidone iodine)
12. Potential participant is expecting to move out of the area of the clinical center to an area not covered by another clinical center during the 6 months following enrollment
13. Anticipated surgery requiring anesthesia within the 6 months following enrollment
    - *Participants cannot receive nitrous oxide until gas resolution*
14. For women of child-bearing potential: pregnant at the time of enrollment
    - *Women who are potential study participants should be questioned about the potential for pregnancy. Investigator judgement may be used to determine when a pregnancy test is needed*

2.2.2 Study Eye Criteria

The participant must have at least one eye meeting all of the inclusion criteria and none of the exclusion criteria listed below.

A participant can have only one study eye. If both eyes are eligible at the time of enrollment, the study eye will be selected by the investigator and participant before injection.

The eligibility criteria for a study eye are as follows:

Inclusion

a. Full-thickness macular hole that is ≤ 250 microns at the narrowest point, confirmed by central reading center
b. Central vitreomacular adhesion on OCT that is no larger than 3000 microns with visible separation of the vitreous on either side as seen on horizontal and vertical scans, confirmed by central reading center
    - *Presence of epiretinal membrane is neither a requirement nor exclusion*
c. Visual acuity letter score of at least 19 (approximate Snellen equivalent 20/400 or better) and at most 83 (20/25 or worse)

Exclusion

d. Other ocular condition that might affect visual acuity during the course of the study or require intraocular treatment (e.g., retinal vein occlusion, substantial age-related macular degeneration, or macular edema induced by a condition other than VMT)

- *If diabetic retinopathy is present, severity level must be microaneurysms only or better (≤ diabetic retinopathy severity level 20).*
- *Presence of drusen is acceptable; however, eyes with geographic atrophy or neovascular age-related macular degeneration involving the macula are excluded.*

e. High level of myopia (spherical equivalent of -8.00 diopters or more myopic if phakic, or retinal abnormalities consistent with pathologic myopia if phakic or pseudophakic)

f. History of prior gas injection, ocriplasmin injection, or intraocular injection for any reason

g. History of prior vitrectomy

h. History of uncontrolled glaucoma

- *Intraocular pressure must be < 30 mmHg, with no more than one topical glaucoma medication, and no documented glaucomatous field loss for the eye to be eligible*

i. History of major ocular surgery (including cataract extraction, scleral buckle, any intraocular surgery, etc.) within prior 4 months or major ocular surgery anticipated within the next 6 months following enrollment

j. History of YAG capsulotomy performed within 4 months prior to enrollment

k. Aphakia or anterior chamber intraocular lens

l. Exam evidence of severe external ocular infection, including conjunctivitis, chalazion, or substantial blepharitis

m. Uveitis

n. Retinal history or pathology that might predispose an eye to an increased risk of retinal detachment from the procedure

- *Untreated retinal tears, not retinal holes, are an exclusion. It is up to the investigator to determine whether extent of lattice degeneration or other pathology might increase the risk of retinal detachment*

o. Any contraindication to paracentesis (e.g., history of narrow angle glaucoma)

p. Lenticular or zonular instability

2.3 Screening Evaluation and Baseline Testing

After informed consent has been signed, the potential participant will be evaluated for study eligibility through the elicitation of a medical history and performance of an ocular examination by study personnel to screen for exclusionary conditions.

Individuals who do not initially meet study eligibility requirements may be rescreened at a later date per investigator discretion.

All testing does not need to be completed on the same day provided it is within the windows specified below. Reading Center confirmation of VMT with full-thickness macular hole on OCT must be completed prior to enrollment.

2.3.1 Baseline Testing Procedures

The following procedures are needed to confirm eligibility and/or to serve as baseline measures for the study:

- If a procedure has been performed using the study technique and by study certified personnel as part of usual care, then it does not need to be repeated specifically for the study if it was performed within the defined time windows specified below.
- The testing procedures are detailed in the DRCR Retina Network Procedures Manuals. Visual acuity testing, ocular exam, and OCT will be performed by DRCR Retina Network certified personnel.

1. Self-reported demographics (date of birth, sex, race, and ethnicity)
2. Medical history (pre-existing medical conditions, concomitant medications, as well as ocular diseases, surgeries, and treatments)
    - Medical history will be obtained by medical charts if available at the enrolling site; otherwise, it will be self-reported
3. Electronic-ETDRS visual acuity testing at 3 meters using the Electronic Visual Acuity Tester (including protocol refraction) in each eye *(within prior 8 days)*
4. Spectral-Domain OCT using Zeiss Cirrus or Heidelberg Spectralis on each eye *(within prior 8 days)*
    - OCT scans of the study eye will be promptly sent to the central reading center for grading and a participant cannot be enrolled until reading center confirmation of eligibility has been received.
5. Ocular examination on each eye including slit lamp, measurement of IOP, lens assessment, and dilated ophthalmoscopy *(within prior 8 days)*
    - Scleral depression to rule out any retinal tears pre-operatively will be required for the baseline eye exam to confirm eligibility.

CHAPTER 3: BASELINE TREATMENT

3.1 Treatment

The C_3F_8 gas injection must be given on the day of enrollment.

3.2 Injection Procedure

3.2.1 Intravitreous Injection Technique

The injection is preceded by a povidone iodine prep of the conjunctiva. A subconjunctival injection of lidocaine may be administered at the discretion of the investigator. The injection will be performed using sterile technique. Pre-injection paracentesis should be considered due to the 4x expansion of C_3F_8 gas and the associated risk of shallowing of the anterior chamber. However, the choice of when or whether or not to do a paracentesis is ultimately at the investigator's discretion. The full injection procedure is described in the protocol-specific study procedures manual. Topical antibiotics in the pre-, peri-, or post-injection period should not be used without prior approval from the Protocol Chair or Coordinating Center designee.

3.2.2 Aqueous Sample Collection

Participation in the ancillary sample collection component is not a requirement for participation in this study. It is expected that sites with the capability to ship intraocular fluids will participate. At the time of consent into the main study, participants will have the option of signing the ancillary sample collection portion of the informed consent form to indicate their willingness to provide the sample for future use. If paracentesis is performed and participant consent is obtained, aqueous fluid already being drawn as part of paracentesis may be collected and shipped on dry ice to a central laboratory for storage for future analyses. Sites will be encouraged to collect samples when performing paracentesis, though sample collection will not be required. Details regarding collection, sample labeling, storage, and shipment can be found in the procedures manual.

3.3 Participant Instructions Post-Injection

Participants will be given a post-injection instruction sheet informing them of all post-injection requirements and risks if they do not follow these requirements. Participants will be instructed to position face down for at least 50% of the time for at least 4 days post-injection. Participants will be instructed to avoid high altitude travel until the surgeon confirms the gas bubble has cleared. Phakic participants will be asked to avoid the supine position and lie on one side or the stomach during sleeping hours until the surgeon confirms that the gas bubble has cleared. All participants will be instructed to wear a wristband to notify healthcare providers that they should not receive nitrous oxide anesthesia until the gas bubble has cleared.

CHAPTER 4: FOLLOW-UP VISITS AND TESTING

4.1 Study Visits

The schedule of protocol-specified follow-up visits is as follows:

- 1 week (- 4 days to + 3 days)
- 4 (±1) weeks
- 8 (±2) weeks
- 24 (±4) weeks

4.1.1 Procedures at Study Visits

The following procedures will be performed at each visit, unless otherwise specified:

1. E-ETDRS visual acuity testing (best corrected) in each eye
    - A protocol refraction in the study eye is required at each visit. Refraction in the non-study eye is only required at the 8 week visit. When a refraction is not performed, the most recently performed refraction is used for the testing.
2. Spectral-Domain OCT using Zeiss Cirrus or Heidelberg Spectralis on the study eye
    - The same machine type (Cirrus or Spectralis) used at baseline must be used during follow up.
3. Ocular exam in the study eye only, including slit lamp examination with lens assessment, measurement of IOP, and dilated ophthalmoscopy
    - The eye exam should be extensive enough to identify adverse events of interest
    - An extended ophthalmoscopy including a scleral depression is required at 1, 4, and 8 weeks to identify any retinal tears or detachments

All of the testing procedures do not need to be performed on the same day, provided that they are completed within the time window of a visit. If data from a testing procedure is unusable (e.g., if OCT is ungradable), the participant may be asked to repeat the procedure during an additional visit, whether part of usual care or solely to repeat the procedure.

4.1.2 Unscheduled Visits

Additional visits may occur as required for usual care of the study participant.

Testing procedures at unscheduled visits are at investigator discretion. However, it is recommended that procedures performed should follow the standard DRCR Retina Network protocol.

4.2 Treatment During Follow Up

4.2.1 Criteria for Vitrectomy

Vitrectomy should not be performed due to failure of macular hole closure prior to 4 weeks without chair approval. Between 4 and 8 weeks, vitrectomy may only be performed (but is not required) if the macular hole size is not improving from baseline. After 8 weeks, vitrectomy can be performed at investigator discretion.

4.2.2 Treatment for Other Conditions in the Study Eye

An eye should not be enrolled that is anticipated to require intraocular treatment for another condition during the study. If a condition requiring prompt treatment develops during follow up, the treatment is at investigator discretion.

4.2.3 Treatment in the Non-Study Eye

Treatment in the non-study eye is at investigator discretion, except that gas injection for VMT is not permitted in the non-study eye during the study.

CHAPTER 5: STUDY DEVICE

5.1 Description of the Investigational Device

Perfluoropropane C_3F_8 is an inert gas under pressure and is administered by injection into the vitreous cavity. It was approved by the FDA in February 1993 (P900066) for the use of placing pressure on detached retina.

5.2 Study Device Accountability Procedures

Each participating site will use their own commercially available perfluoropropane C_3F_8. It must be stored at room temperature. Prior to each injection, the investigator must confirm that the cylinder pressure is at least 50 psi and that the cylinder is not expired.

5.3 Safety Measures

Preparation of the perfluoropropane C_3F_8 injection will be performed in accordance with manufacturer labelling. The full injection procedure is described in the protocol-specific study procedures manual.

CHAPTER 6: ADVERSE EVENTS, DEVICE ISSUES, AND STOPPING RULES

6.1 Adverse Events

6.1.1 Definitions

Adverse Event (AE): Any untoward medical occurrence in a study participant, irrespective of the relationship between the adverse event and the device(s) under investigation (see section 6. 2 for reportable adverse events for this protocol).

Serious Adverse Event (SAE): Any untoward medical occurrence that:

- Results in death.
- Is life-threatening (a non-life-threatening event which, had it been more severe, might have become life-threatening, is not necessarily considered a serious adverse event).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability, incapacity, or substantial disruption of the ability to conduct normal life functions (e.g, sight threatening).
- Is a congenital anomaly or birth defect.
- Is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the participant or may require medical or surgical intervention to prevent one of the outcomes listed above).

In general, an ocular adverse event should be reported as serious (considered sight threatening) if it meets one of the following criteria:

1. It causes a decrease of ≥ 30 letters in visual acuity compared with the last visual acuity measurement prior to onset (e.g. central retinal artery occlusion).
2. In the opinion of the investigator, it requires prompt surgical intervention (e.g. vitrectomy, vitreous tap, intravitreous antibiotics, laser or cryosurgical retinopexy) to prevent permanent loss of sight. Examples include endophthalmitis, retinal tear, or rhegmatogenous retinal detachment.

Ocular adverse events that do not have the potential to result in permanent loss of sight would not be considered serious.

Unanticipated Adverse Device Effect (UADE): Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of participants (21 CFR 812.3(s)).

Adverse Device Effect (ADE): Any untoward medical occurrence in a study participant which the device may have caused or to which the device may have contributed (Note that an Adverse Event Form is to be completed in addition to being reported on a Gas Injection Form).

Device Complaints: A device complication or complaint is something that happens to a device or related to device performance, whereas an adverse event happens to a participant. A device complaint may occur independently from an AE, or along with an AE. An AE may occur without a device complaint or there may be an AE related to a device complaint.

Device Malfunction: Any failure of a device to meet its performance specifications or otherwise perform as intended. Performance specifications include all claims made in the labeling for the device. The intended performance of a device refers to the intended use for which the device is labeled or marketed. (21 CFR 803.3)

6.1.2 Reportable Adverse Events

For this protocol, a reportable adverse event includes any untoward medical occurrence that meets one of the following criteria:

1) an ocular AE (study eye),
2) a serious AE,
3) an Adverse Device Effect (ADE) as defined in section 6.1.1, or
4) An AE occurring in association with a study procedure.

All reportable Adverse Events whether they are volunteered by the participant, discovered by study personnel during questioning, or detected through physical examination, testing procedure, or other means, will be reported on an Adverse Event Form online. Each Adverse Event Form is reviewed by the Medical Monitor to verify the coding and the reporting that is required.

6.1.3 Relationship of Adverse Event to Study Device

The study investigator will assess the relationship of any adverse event to be related or unrelated by determining if there is a reasonable possibility that the adverse event may have been caused by the study device.

To ensure consistency of adverse event causality assessments, investigators should apply the following general guideline when determining whether an adverse event is related:

Yes

There is a plausible temporal relationship between the onset of the adverse event and the study intervention, and the adverse event cannot be readily explained by the participant's clinical state, intercurrent illness, or concomitant therapies; or the adverse event follows a known pattern of response to the study intervention.

No

Evidence exists that the adverse event has an etiology other than the study intervention (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); or the adverse event has no plausible temporal relationship to study intervention.

6.1.4 Intensity of Adverse Event

The intensity of an adverse event will be rated on a 3 point scale: (1) mild, (2) moderate, or (3) severe. It is emphasized that the term severe is a measure of intensity: thus a severe adverse event is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

MILD: Usually transient, requires no special treatment, and does not interfere with the participant's daily activities.

MODERATE: Usually causes a low level of inconvenience or concern to the participant and may interfere with daily activities, but is usually ameliorated by simple therapeutic measures.

SEVERE: Interrupts a participant's usual daily activities and generally requires systemic drug therapy or other treatment.

6.1.5 Coding of Adverse Events

Adverse events will be coded using the MedDRA dictionary. The Medical Monitor will review the investigator's assessment of causality and may agree or disagree. Both the investigator's and Medical Monitor's assessments will be recorded. The Medical Monitor will have the final say in determining the causality.

Adverse events that continue after the participant's discontinuation or completion of the study will be followed until their medical outcome is determined or until no further change in the condition is expected.

6.1.6 Outcome of Adverse Event

The outcome of each reportable adverse event will be classified by the investigator as follows:

COMPLETE RECOVERY/RESOLVED – The participant recovered from the AE/SAE without sequelae. Record the AE/SAE stop date.

RECOVERED/RESOLVED WITH SEQUELAE – The event persisted and had stabilized without change in the event anticipated. Record the AE/SAE stop date.

FATAL – A fatal outcome is defined as the SAE that resulted in death. Only the event that was the cause of death should be reported as fatal. Adverse events and serious adverse events that were ongoing at the time of death; however, were not the cause of death, will be recorded as resolved at the time of death.

ONGOING – An ongoing AE/SAE is defined as the event was ongoing with an undetermined outcome.

- An ongoing outcome for which further improvement or worsening is possible will require follow up by the site in order to determine the final outcome of the AE/SAE.
- An ongoing outcome that is medically stable (further change not expected) will be documented as such and will not require additional follow up.
- The outcome of an ongoing event at the time of death that was not the cause of death, will be updated and recorded as resolved with the date of death recorded as the stop date.

All adverse events occurring during the study and continuing at study termination should be followed by the participant's physician and evaluated with additional tests (if necessary) until diagnosis of the underlying cause, or resolution. Follow-up information should be recorded on source documents.

If any reported serious, related, or unexpected adverse events or UADEs are present when a participant completes the study, or if a participant is withdrawn from the study due to a serious, related, or unexpected adverse event of UADE, the participant will be contacted for re-evaluation within 2 weeks. If the adverse event has not resolved, additional follow up will be performed as appropriate. Every effort should be made by the Investigator or delegate to contact the participant until the adverse event has resolved or stabilized.

6.2 Reportable Device Issues

All UADEs, ADEs, device complaints, and device malfunctions will be reported the Gas Injection Form irrespective of whether an adverse event occurred.

6.3 Pregnancy Reporting

If pregnancy occurs, the participant will remain in follow up for the duration of the study. The occurrence of pregnancy will be reported on an AE Form.

6.4 Timing of Event Reporting

Serious adverse events and unexpected device-related adverse events must be reported to the Coordinating Center within 24 hours via completion of the online case report form.

The Coordinating Center will notify all participating investigators of any adverse event that is serious, related, and unexpected. Notification will be made within 10 days after the Coordinating Center becomes aware of the event.

Each principal investigator is responsible for reporting serious study-related adverse events and abiding by any other reporting requirements specific to his or her Institutional Review Board or Ethics Committee.

Upon receipt of a UADE report, the JCHR will investigate the UADE and if indicated, report the results of the investigation to the sites' IRBs, and the FDA within 10 working days of the JCHR becoming aware of the UADE per 21CFR 812.46(b) (2). The Medical Monitor must determine if the UADE presents an unreasonable risk to participants. If so, the Medical Monitor must ensure that all investigations, or parts of investigations presenting that risk, are terminated as soon as possible but no later than 5 working days after the Medical Monitor makes this determination and no later than 15 working days after first receipt notice of the UADE.

Device malfunctions will be handled by the site and manufacturer.

6.5 Stopping Criteria

6.5.1 Criteria for Suspending or Stopping Overall Study

The Data and Safety Monitoring Committee (DSMC) will be informed of all unanticipated adverse device events that occur during the study and will review compiled safety data at periodic intervals. The DSMC may request suspension of study activities or stoppage of the study if deemed necessary based on the totality of safety data available.

The study may be discontinued by the Executive Committee (with approval of the DSMC) prior to the preplanned completion of follow up for all study participants.

6.6 Independent Safety Oversight

A Data and Safety Monitoring Committee will approve the protocol, template Informed Consent Form, and substantive amendments and provide independent monitoring of adverse events. Cumulative adverse event data are tabulated semi-annually for review by the DSMC. Following each DSMC data review, a summary will be provided to IRBs. A list of specific adverse events to be reported expeditiously to the DSMC will be compiled and included as part of the DSMC Monitoring Plan document.

6.7 Risks

The potential risks associated with use of the study device are described in section 1.4.1.

Additional risks are minor or infrequent and include the following:

Risks Related to Testing Procedures

Many of the testing procedures in this study are part of daily ophthalmologic practice in the United States and pose few if any known risks.

- Dilating eye drops will be used as part of the exam. There is a small risk of inducing a narrow-angle glaucoma attack from the pupil dilation. However, all participants will have had prior pupil dilation usually on multiple occasions and therefore the risk is extremely small.

Risks Related Specifically to the Pre-Injection Preparation

- There are potential side effects to subconjunctival anesthetic, which are rare. They include, but are not limited to, the following: damage to the eyeball by the needle, damage to the optic nerve, double vision lasting 24 hours or more.
- Complications associated with paracentesis are uncommon, but may include uveitis, flat anterior chamber, corneal wound leak, hyphema, anterior vitreous prolapse, or pupillary block glaucoma and cataract. Under certain circumstances, such complications may lead to vision loss.

Risks if Pregnant

According to the C_3F_8 package insert, there are no known teratogenic effects when injected into the eye; however, caution should be used in pregnant women. Therefore, patients will not be allowed to participate in this study if pregnant. Women who become pregnant during the study will be asked to stay in the study since there is no follow-up treatment with the investigational product.

CHAPTER 7: MISCELLANEOUS CONSIDERATIONS

7.1 Prohibited Medications, Treatments, and Procedures

The participant will be instructed that nitrous oxide anesthesia must not be administered unless the investigator has confirmed that the gas bubble is no longer present. Wristbands notifying healthcare providers of this will be given to participants following the intravitreous injection, and must be worn until the investigator confirms that the gas bubble has cleared.

7.2 Participant Withdrawal

Participation in the study is voluntary and a participant may withdraw at any time. If a study participant is considering withdrawal from the study, the principal investigator should personally speak to the individual about the reasons and every effort should be made to accommodate him or her.

The goal for the study is to have as few losses to follow up as possible. The Coordinating Center will assist in the tracking of study participants who cannot be contacted by the site. The Coordinating Center will be responsible for classifying a study participant as lost to follow up. For participants who withdraw, their data will be used up until the time of withdrawal.

7.3 Discontinuation of Study

The study may be discontinued by the Executive Committee (with approval of the DSMC) prior to the preplanned completion of follow up for all study participants.

7.4 Confidentiality

For security and confidentiality purposes, participants will be assigned an identifier that will be used instead of their name. Protected health information gathered for this study will be shared with the coordinating center, the Jaeb Center for Health Research in Tampa, FL. The Coordinating Center will be provided with contact information for each study participant. Permission to obtain such information will be included in the Informed Consent Form. The contact information may be maintained in a secure database and will be maintained separately from the study data. Phone contact from the Coordinating Center will be made with each study participant in the first month after enrollment. Additional phone contacts from the Coordinating Center will be made if necessary to facilitate the scheduling of the study participant for follow-up visits. A participant-oriented newsletter and a study logo item may be sent once. Study participants will be provided with a summary of the study results in a newsletter format after completion of the study by all participants.

7.5 Participant Compensation

Participant compensation will be specified in the informed consent form.

CHAPTER 8: STATISTICAL CONSIDERATIONS

8.1 Statistical and Analytical Plans

The approach to sample size and statistical analyses are summarized below. A detailed statistical analysis plan will be written and finalized prior to first review of data. The analysis plan synopsis in this chapter contains the framework of the anticipated final analysis plan.

8.2 Statistical Hypotheses

As this is a single-arm study, there are no formal statistical hypotheses that will be evaluated.

8.3 Sample Size

8.3.1 Outcome Projections

Several case series provide estimates of stage 2 MH closure with PVL using C_3F_8 gas. Chan et al. (1995) reported MH closure in 3 of 6 eyes (50%) within 9 weeks of injection.[16] Jorge et al. (2006) reported MH closure in 5 of 6 eyes (83%) one month after injection.[17, 18] Finally, Chan et al. (2017) reported MH closure in 8 of 15 eyes (53%) within 9 weeks of injection.[21]

8.3.2 Sample Size Estimates

Table 1 shows anticipated confidence interval half widths for various sample sizes and true proportions of MH closure. These calculations assume a Type I error rate of 5%.

Table 1: Anticipated Confidence Interval Half Widths

MH Closure	Sample Size		
	25	**50**	**75**
85%	13.8%	9.9%	8.1%
70%	16.9%	12.3%	10.2%
50%	18.2%	13.4%	11.0%

The sample size for this study was chosen for convenience and set at 50 eyes.

8.4 Outcome Measures

For the outcomes below, rescue treatment includes vitrectomy, ocriplasmin, or additional pneumatic vitreolysis during the course of the study.

Primary Outcome:

- Proportion of eyes with MH closure of the inner retinal layers* without rescue treatment at 8 weeks.
    - For purposes of description only, the distribution of eyes by the following categories at 8 weeks will be tabulated without statistical comparison:
        - MH closure without rescue treatment
        - MH closure with rescue treatment
        - No MH closure and no rescue treatment
        - No MH closure despite rescue treatment

Secondary Outcomes:

- Proportion of eyes with MH closure of the inner retinal layers* without rescue treatment through 24 weeks (time-to-event analysis).

- Proportion of eyes with central VMT release* without rescue treatment through 24 weeks (time-to-event analysis).
    - For purposes of description only, the following will be tabulated without statistical comparison at 8 weeks and 24 weeks:
        - MH closure with central VMT release without rescue treatment
        - MH closure without central VMT release without rescue treatment
        - MH closure with central VMT release with rescue treatment
        - MH closure without central VMT release with rescue treatment
- Proportion of eyes with central VMT release and vitreopapillary traction release* without rescue treatment through 24 weeks (time-to-event analysis).
- Mean change in visual acuity letter score from baseline at 8 and 24 weeks.
- Proportion of eyes with at least 10-letter gain (increase) in visual acuity from baseline at 8 and 24 weeks.
- Proportion of eyes with at least 10-letter loss (decrease) in visual acuity from baseline at 8 and 24 weeks.
- Proportion of eyes receiving rescue treatment before the 8-week visit.
- Proportion of eyes receiving rescue treatment before the 24-week visit.
    - For purposes of description only, the following will be tabulated without statistical comparison:
        - Proportion of eyes receiving rescue treatment before the 24-week visit or for which rescue treatment is planned at the 24-week visit and medical records confirm rescue treatment occurred within the subsequent 12 weeks.
        - Type of rescue treatment.

Exploratory Outcomes:

- Proportion of eyes with MH closure of the inner retinal layers with outer retinal lucency* without rescue treatment at 8 and 24 weeks.[28]
- Proportion of eyes with ellipsoid zone* integrity at 8 and 24 weeks.

*Determined by masked grader at the central reading center.

To ensure that statistical outliers do not have undue impact on analyses of continuous outcomes, change in continuous outcomes from baseline will be truncated to ± 3 standard deviations based on the overall mean and standard deviation at 8 weeks.

8.5 Analysis Cohorts

- Intention-To-Treat (ITT) Analysis Cohort: all enrolled participants irrespective of treatment received.
- Safety Analysis Cohort: all enrolled participants irrespective of treatment received.
- Per Protocol Analysis Cohort: only participants that complete the initial treatment (PVL) and do not receive any non-protocol treatments during follow up. Vitrectomy performed according to the criteria in section 4.2.1 is considered per-protocol and will be included in this analysis.

The primary analysis will follow the ITT principle and include all enrolled participants.

A per-protocol analysis will be performed to provide additional information regarding the magnitude of the treatment effect. The per-protocol analysis will only be performed if at least 10% of enrolled participants would be excluded by these criteria (e.g., 5 or more participants if exactly 50 are enrolled).

The ITT analysis is considered the primary analysis. If the results of the per-protocol and ITT analyses give inconsistent results, the per-protocol analysis will be interpreted with caution. In this scenario, exploratory analyses will be performed to evaluate possible factors contributing to the difference.

8.6 Analysis of the Primary Outcome

The primary outcome of MH closure of the inner retinal layers without rescue treatment through 8 weeks is a binary variable that is graded by the central reading center. The proportion of eyes meeting the primary outcome will be determined and the 95% Wilson (Score) confidence interval will be calculated.

Since the chance of re-opening after closure before 8 or 24 weeks is highly unlikely, an eye with MH closure of the inner retinal layers without rescue treatment at any time prior to 24 weeks will be considered to have met the outcome through 24 weeks if the patient is lost to follow-up. Similarly, any eye receiving rescue treatment prior to 24 weeks will be considered not to have met the outcome through 24 weeks because rescue treatment has been given.

Multiple imputation will be used to impute missing data for eyes lost to follow-up that did not have prior MH closure or rescue treatment documented. The imputation model will include presence of ERM within 1 mm of the center of the macula at baseline and MH status at 1, 4, 8, and 24 weeks.

A sensitivity analysis will be conducted using the same approach as above but without multiple imputation (i.e., complete-case analysis).

8.7 Analysis of the Secondary and Exploratory Outcomes

The ITT analysis cohort will be used for all secondary and exploratory outcomes.

8.7.1 Secondary Efficacy Outcomes

Macular hole closure of the inner retinal layers without rescue treatment through 24 weeks is a time-to-event outcome. A Kaplan-Meier curve will be constructed and the cumulative probability with 95% confidence interval will be estimated for the final time point. Data from eyes not observed to have closure or that receive rescue treatment will be censored on the date of their final visit.

Central VMT release without rescue treatment and central VMT release with vitreopapillary traction release without rescue treatment through 24 weeks are time-to-event outcomes graded by the central reading center and will be analyzed as above. The latter analysis will be adjusted for vitreopapillary traction status at baseline.

Change in visual acuity letter score from baseline at 8 and 24 weeks is a continuous outcome. The mean and 95% confidence interval will be calculated for each time point. Missing data will be imputed with multiple imputation. The imputation model will include presence of ERM within 1 mm of the center of the macula at baseline, baseline visual acuity, change in visual acuity from baseline at 1, 4, 8 and 24 weeks, and MH status at 1, 4, 8, and 24 weeks.

The proportion of eyes with at least 10-letter gain (increase) or loss (decrease) in visual acuity from baseline at 8 and 24 weeks are binary variables. Wilson (Score) 95% confidence intervals will be calculated for each time point. The imputed data sets described above for the mean change in visual acuity from baseline will be utilized.

The proportion of eyes receiving rescue treatment before 8 and 24 weeks is a binary variable. Wilson (Score) 95% confidence intervals will be calculated for each time point. Complete-case analysis (no imputation of missing data) will be used for this outcome.

8.7.2 Exploratory Efficacy Outcomes

The proportion of eyes with MH closure of the inner retinal layers with outer retinal lucency at 8 and 24 weeks is a binary variable graded by the central reading center. Wilson (Score) 95% confidence intervals will be calculated for each time point. Complete-case analysis (no imputation of missing data) will be used for this outcome.

The proportion of eyes with ellipsoid zone integrity at 8 and 24 weeks is a binary variable graded by the central reading center (loss of integrity and no loss of integrity). Wilson (Score) 95% confidence intervals will be calculated for each time point. Complete-case analysis (no imputation of missing data) will be used for this outcome.

8.8 Safety Analyses

All reportable adverse events will be categorized as study eye or systemic. All events will be tabulated in a listing of each reported Medical Dictionary for Regulatory Activities (MedDRA) term and summarized over each MedDRA System Organ Class. All enrolled participants will be included in safety analyses.

The frequency of each ocular adverse event occurring at least once per eye will be calculated. The proportion of eyes experiencing each outcome will be calculated along with 95% Wilson (Score) confidence intervals. The following ocular adverse events are of primary interest:

- Endophthalmitis
- Retinal detachment
- Retinal tear
- Traumatic cataract
- Cataract extraction in eyes phakic at baseline
- Vitreous hemorrhage
- Adverse IOP events (composite outcome)
    - Increase in IOP $\geq$ 10 mmHg from baseline (at a follow-up visit)
    - IOP $\geq$ 30 mmHg (at a follow-up visit)
    - Initiation of medication to lower IOP that was not in use at baseline
    - Glaucoma procedure

The frequency of each systemic adverse event occurring at least once per participant will be calculated. The following systemic adverse events are of primary interest:

- Death
- Serious adverse event (at least one)

The following systemic adverse events are of secondary interest:

- For each MedDRA System Organ Class, proportion of participants with at least one serious adverse event

In addition, the following will be tabulated:

- Number of adverse events thought by investigator to be related to treatment

8.9 Intervention Adherence

Adherence will be defined as completion of PVL.

8.10 Protocol Adherence and Retention

Protocol deviations and visit completion rates (excluding deaths) will be tabulated.

8.11 Baseline Descriptive Statistics

Baseline characteristics will be tabulated and summary statistics appropriate to the distribution will be reported.

8.12 Planned Interim Analyses

There is no formal interim analysis planned for this study. The Data and Safety Monitory Committee (DSMC) will review safety and outcome data approximately every 6 months while the study is ongoing.

8.13 Subgroup Analyses

Subgroup analyses, i.e., assessments of effect modification, will be conducted to detect factors associated with the primary outcome. These analyses will be considered exploratory.

Subgroup analyses will be conducted using logistic regression. The relative risk for the subgroup factor (estimated using the method of Localio et al. 2007[29]), 95% confidence interval, and *P* value will be presented. To aid in interpretation of the relative risk, observed outcome proportions will be reported for each subgroup. Subgroup analyses will use data from eyes that complete the 8-week visit or have MH closure or rescue treatment prior to 8 weeks (i.e., complete case analysis as described in section 8.6).

The primary subgroup analysis will evaluate the effect of ERM presence within 1 mm of the center of the macula at baseline. Previous reports have suggested rates of VMT release and MH closure differ depending on the presence of ERM.

Secondary subgroup analyses will include ERM presence at the site of vitreous adhesion, lens status (phakic or pseudophakic), components of VMT severity grade,[30] length of adhesion on OCT (less than or equal to 1500 microns or greater than 1500 microns), and diabetes status (has diabetes or does not have diabetes).

There are no data to suggest that the treatment effect will vary by sex or race/ethnicity. However, both of these factors will be evaluated in exploratory subgroup analyses as mandated by National Institutes of Health (NIH) guidelines.

Subgroup factors will be analyzed as categorical and continuous or ordinal variables where possible. Secondary and exploratory subgroup analyses will only be conducted if there are at least 10 eyes in each subgroup.

8.14 Multiple Testing

Because this is a single-arm study without treatment group comparisons, there will be no adjustments made for multiple testing.

CHAPTER 9: DATA COLLECTION AND MONITORING

9.1 Case Report Forms and Device Data

The main study data are collected through electronic case report forms (CRFs). These electronic CRFs from the study website are considered the primary source documentation.

Each participating site will maintain appropriate medical and research records for this trial in compliance with ICH E6 and regulatory and institutional requirements for the protection of the confidentiality of participants.

9.2 Study Records Retention

Study documents should be retained for a minimum of 3 years following the NIH grant cycle for which the last visit was completed (expected to be December 31, 2026). These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of JCHR.

9.3 Quality Assurance and Monitoring

Designated personnel from the Coordinating Center will be responsible for maintaining quality assurance (QA) and quality control (QC) systems to ensure that the clinical portion of the trial is conducted and data are generated, documented, and reported in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements. Adverse events will be prioritized for monitoring.

A risk-based monitoring (RBM) plan will be developed and revised as needed during the course of the study, consistent with the FDA "Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring" (August 2013). Study conduct and monitoring will conform with 21 Code of Federal Regulations (CFR) 812.

The most important data for monitoring at the site are participant eligibility and adverse events. Therefore, the RBM plan will focus on these areas. As much as possible, remote monitoring will be performed in real time with on-site monitoring performed to evaluate the verity and completeness of the key site data. Elements of the RBM may include the following:

- Qualification assessment, training, and certification for sites and site personnel
- Oversight of Institutional Review Board (IRB) coverage and informed consent procedures
- Central (remote) data monitoring: validation of data entry, data edits, audit trail, protocol review of entered data and edits, statistical monitoring, study closeout
- On-site monitoring (site visits): source data verification, site visit report
- Communications with site staff
- Patient retention and visit completion
- Quality control reports
- Management of noncompliance
- Documenting monitoring activities

- Adverse event reporting and monitoring

Coordinating Center representatives or their designees may visit the study facilities at any time in order to maintain current and personal knowledge of the study through review of the records, comparison with source documents, or observation and discussion of the conduct and progress of the study.

9.4 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or procedure requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

The site primary investigator and study staff are responsible for knowing and adhering to their IRB requirements. Further details about the handling of protocol deviations will be included in the monitoring plan.

CHAPTER 10: ETHICS/PROTECTION OF HUMAN PARTICIPANTS

10.1 Ethics/Protection of Human Participants Ethical Standard

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Participants of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and the ICH E6.

10.2 Institutional Review Boards

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved. In the event of an amendment, a determination will be made regarding whether previously consented participants need to be re-consented.

10.3 Informed Consent Process

10.3.1 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing.

The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.3.2 Participant and Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the JCHR and their agents. This confidentiality is extended to cover biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the JCHR.

The study monitor, other authorized representatives of the JCHR, or representatives of the IRB may inspect all documents and records required to be maintained by the investigator, including but not limited to medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and institutional regulations.

Study participant research data that is for the purposes of statistical analysis and scientific reporting will be transmitted to and stored at the coordinating center, the Jaeb Center for Health Research (JCHR) in Tampa, FL. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by JCHR research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at JCHR.

10.3.3 Future Use of Stored Specimens

With the participant's approval, de-identified biological samples will be stored at a central repository for future research into the causes, complications, and treatments of retinal diseases. The repository will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the masking of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage will not be possible after the study is completed.

CHAPTER 11: REFERENCES

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