DRCR Retina Network

Randomized Clinical Trial Assessing the Effects of Pneumatic Vitreolysis on Vitreomacular Traction (Protocol AG)

IDE Sponsor: Jaeb Center for Health Research (JCHR)

Version 3.0

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Signature Page

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TABLE OF CONTENTS

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
SDH	Shape Discrimination Hyperacuity
SD	Standard Deviation
UADE	Unanticipated Adverse Device Effect
VMT	Vitreomacular Traction
VMA	Vitreomacular Adhesion

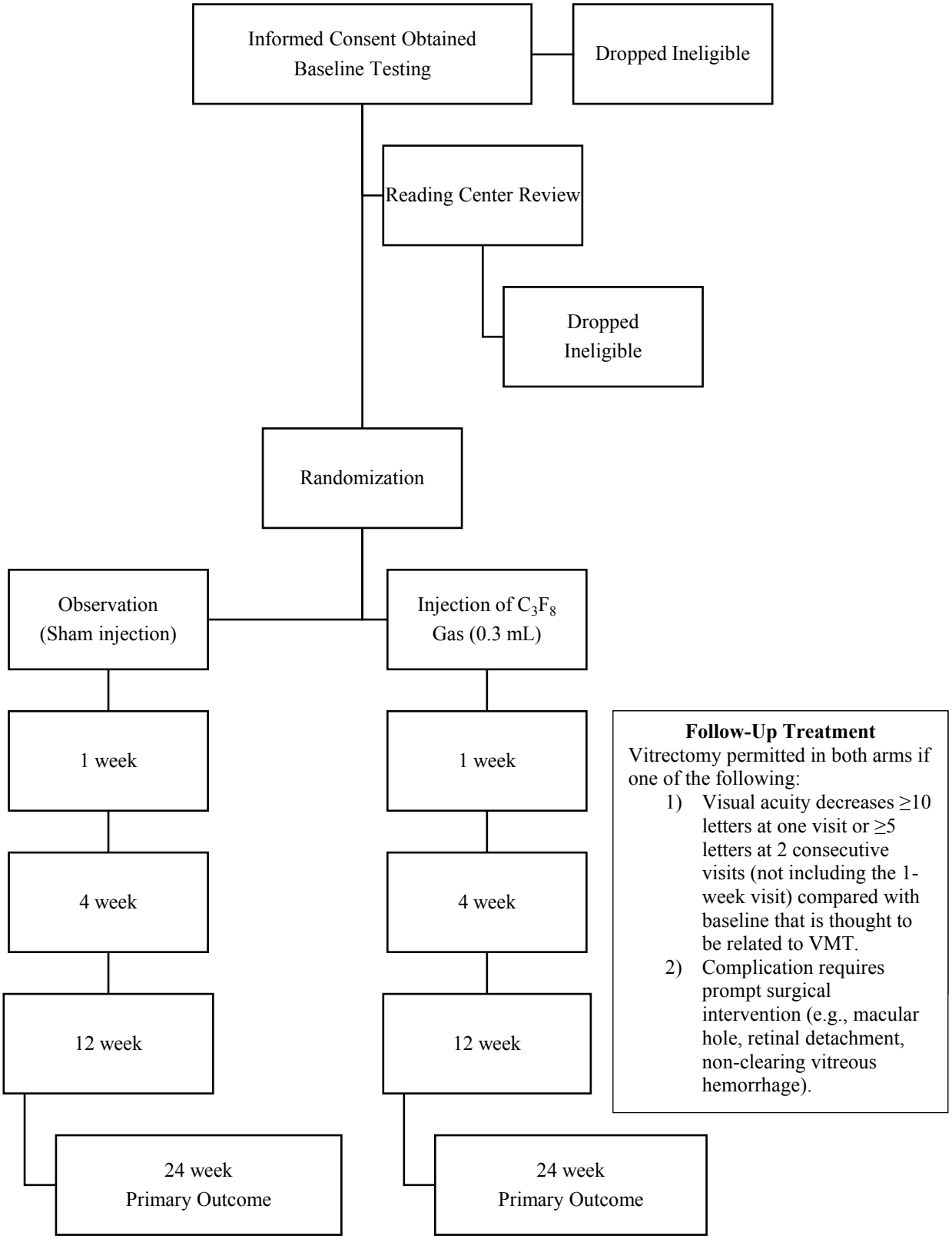
PROTOCOL SUMMARY

PARTICIPANT AREA	DESCRIPTION
Title	Randomized Clinical Trial Assessing the Effects of Pneumatic Vitreolysis on Vitreomacular Traction
Précis	Eyes with idiopathic symptomatic vitreomacular traction (VMT) without a macular hole will be randomly assigned to 0.3-mL intraocular gas (C_3F_8) injection or sham injection to determine if pneumatic vitreolysis (PVL) is effective in releasing VMT.
Investigational Device	0.3-mL intraocular gas (C_3F_8) injection
Objectives	Primary 1. To compare the proportion of eyes with central VMT release on OCT after pneumatic vitreolysis with gas injection versus observation (sham injection) in eyes with VMT without an associated macular hole. Secondary 2. To evaluate visual function outcomes at 24 weeks after gas injection is performed compared with sham injection.
Study Design	Multi-center, randomized clinical trial
Number of Sites	Approximately 50 sites
Endpoint	**Primary Outcome:** Proportion of eyes with central VMT release on optical coherence tomography (OCT) without rescue treatment at 24 weeks. **Key Secondary Outcomes:** Proportion of eyes with rescue treatment, mean change in visual acuity **Key Safety Outcomes:** Retinal tear, retinal detachment, macular hole development, 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 travel 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). • At least one eye with each of the following:

PARTICIPANT AREA	DESCRIPTION
	○ central vitreomacular adhesion on OCT that is no larger than 3000 microns, confirmed by central reading center, ○ decreased visual function (e.g. metamorphopsia or other visual symptom) that is attributed to VMT, ○ best corrected Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity equivalent of 20/32 to 20/400, and ○ prompt vitrectomy not required **Key Exclusion Criteria** • Macular or lamellar hole. • 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). ○ Note: Epiretinal membrane is not an exclusion nor a requirement. • High level myopia (-8.00 diopters or more negative if phakic; retinal abnormalities consistent with pathologic myopia if phakic or pseudophakic). • Prior gas injection, ocriplasmin injection, or intraocular injection for any reason. • Prior vitrectomy.
Sample Size	Minimum of 124 eyes
Treatment Groups	Random assignment (1:1) to one of the following: Group A: PVL (0.3-mL C_3F_8 injection) Group B: Observation (Sham injection)
Participant Duration	24 weeks
Protocol Overview/Synopsis	1. Informed consent will be obtained for screening. 2. Eligibility will be assessed, including reading center confirmation of VMT on OCT. 3. Eligible eyes will be randomly assigned to C_3F_8 injection (0.3 mL) or sham injection, which will be performed on the day of randomization. 4. Follow-up will occur at 1, 4, 12 and 24 weeks and consist of vision testing (including visual acuity and visual function (myVisionTrack), ocular exam, and OCT. 5. Rescue vitrectomy may be performed if there is a 10 or more letter decrease at one visit or 5 or more letter decrease at two consecutive visits compared

PARTICIPANT AREA	DESCRIPTION
	with baseline that is thought to be associated with VMT, or a complication that requires prompt surgical intervention (e.g. macular hole, retinal detachment, non-clearing vitreous hemorrhage). Otherwise, alternative treatment may not be performed without discussion with and approval from the protocol chair or designee. 6. The primary outcome assessment will be the proportion of eyes with central VMT release on OCT at 24 weeks without rescue treatment.

SCHEMATIC OF STUDY DESIGN



SCHEDULE OF STUDY VISITS AND PROCEDURES

	Baseline Testing and Randomization*	1 and 4 weeks	12 and 24 weeks
E-ETDRS best corrected visual acuity [a]	X	X	X
Visual function testing (myVisionTrack) [b]	X		X
OCT [c]	X	X	X
Eye exam [d]	X	X	X
Reading center eligibility confirmation [e]	X		
Randomized treatment (gas or sham injection)	X		

* All baseline testing must occur within 8 days prior to randomization. Baseline testing and randomization can occur on the same day if eligibility is confirmed by reading center on day of screening.

a, Both eyes at all visits; includes protocol refraction in study eye only at 1, 4, and 12 weeks and in both eyes at baseline and 24 weeks; E-ETDRS refers to electronic ETDRS testing using the Electronic Visual Acuity Tester that has been validated against 4-meter chart ETDRS testing.

b, At baseline, 12, and 24 weeks.

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

d, Both eyes at baseline; study eye only at each follow-up visit. Includes slit lamp exam (including assessment of lens), measurement of IOP, 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 12 weeks.

e, Reading center review of the OCT for eligibility must occur prior to randomization.

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 (OCT). Vitreomacular traction (VMT) is diagnosed when VMA results in traction, distortion of retinal architecture, and patient symptomatology.[2] The prevalence of VMT has been reported to be 22.5 per 100,000 of the population, with an annual incidence of 0.6 per 100,000[2]. However, these rates, determined before widespread use of spectral-domain OCT, are likely an underestimation. 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]

1.1.2 Treatments for VMT

Treatment options for VMT include observation, vitrectomy, and intraocular injection of ocriplasmin. Observation may not be an optimal choice in symptomatic patients. There have been multiple reports in the literature of spontaneous resolution of VMT. [3-8] Based on a Cochrane Eyes and Vision systematic review of 4 trials with 932 eyes, the 28-day VMA release rate among eyes in the control groups (sham or saline injection) is estimated to be 97 of 1000 eyes.[9] In the Intravitreal Injection-Traction Release Without Surgical Treatment (MIVI-TRUST) trials,[10] 10% of the placebo-vehicle injected eyes developed a spontaneous posterior vitreous detachment (PVD) by 28 days, increasing to about 13% through 180 days. The Ocriplasmin for Treatment for Symptomatic Vitreomacular Adhesion Including Macular Hole (OASIS) Trial reported success of VMT release of 6% in the sham group at 28 days, increasing to 14% over 2 years.[11] In a 2014 retrospective case series, John et al[6] reported spontaneous PVD in 32% of eyes with focal VMA (documented by spectral-domain OCT) and good vision (mean visual acuity: 20/37) with a median follow-up time of 18 months. Thus, observation alone may yield a spontaneous resolution of visual impairment after a waiting period of sufficient time, although the precise timing of the spontaneous resolution is unpredictable and can be prolonged.

Vitrectomy is a viable alternative to observation. However, vitrectomy is costly and carries a risk of complications such as endophthalmitis, retinal detachment and cataract progression, even when performed by experienced surgeons. Therefore, vitrectomy is generally reserved for eyes with more advanced VMT associated with relatively poor visual acuity level.

In 2012, the MIVI-TRUST trial showed that ocriplasmin (Jetrea®), a proteolytic enzyme for treatment of symptomatic VMA, induced a PVD in 26.5% of eyes versus 10.1% of placebo-vehicle injected eyes.[10, 12] The success was shown to increase to 40% in eyes without epiretinal membrane.[10] In 2016, Lim et al reported in the Macula Society Collaborative Retrospective Study a rate of 45% in release of VMT after ocriplasmin.[13] Based on the Cochrane Eyes and Vision systematic review, the 28-day release rate among eyes treated with ocriplasmin is estimated to be 237 of 1000 eyes.[9] However, there have been multiple anecdotal reports of

substantial ocular complications associated with intraocular administration of ocriplasmin,[14-18] including transient visual 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.[14-18] In a recent American Society of Retina Specialists Preferences and Trends Survey, only 7% of retina specialists recommended ocriplasmin as first line therapy for VMT and visual acuity of 20/60 or worse.[19]

1.1.3 Pneumatic Vitreolysis (PVL)

Pneumatic vitreolysis (intraocular injection of expansile gas to induce a PVD) has been suggested as a potential treatment alternative to ocriplasmin for resolving VMT without surgery. Chan et al first demonstrated and reported the utility of intraocular gas (C_3F_8) injection in eliciting a PVD (95%) in 1995.[20] Subsequently, Costa et al, and Jorge et al showed a high rate of success in the induction of PVD (100%) with C_3F_8 in small case series.[21, 22] Recently, Rodrigues et al, in a series of fifteen eyes, showed resolution of VMT in 40% at one month and 60% by 6 months after injection of C_3F_8 gas.[23] Steinle et al reported a success rate of 83% with C_3F_8 gas in a retrospective case series for treatment of VMT syndrome.[24] In a 2016 retrospective review of 50 consecutive eyes receiving C_3F_8 gas for PVL performed in 2 centers, Chan and Mein reported a success rate of 86% in VMT release (80% success for VMT-only eyes and 100% for small stage 2 MH [$\leq$ 250 microns]).[25] In 2019, Chan and Mein provided an update on this study and reported a success rate of 85% among 80 eyes (80.7% in eyes with VMT-only, 95.7% in eyes with macular hole).[26] However, all of these cited case series involving PVL involved relatively small numbers of cases and did not incorporate control arms for comparison.

1.2. Rationale

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, PVL may serve as an alternative to either potentially less effective observation or much more costly treatments with well-established associated adverse events such as ocriplasmin or vitrectomy for managing VMT. Although many eyes with VMT have good vision and are not symptomatic, there are also many patients who experience central visual loss or symptoms such as metamorphopsia due to persistent VMT. Therefore, a randomized clinical trial is indicated to evaluate the safety and efficacy of intraocular gas injection for treatment of VMT without a MH in patients who are symptomatic and desire intervention.

The purpose of this study is not to compare release rates directly with vitrectomy. Instead, the goal is to evaluate PVL compared with observation to determine if it is a viable first-line treatment option to consider before resorting to vitrectomy. If PVL is successful for managing VMT, its use may reduce the need for current therapies by both resolving VMT and preventing MH formation in early cases and by closing small stage-2 MH in the advanced cases.

1.3 Study Objectives

Primary

1. To compare the proportion of eyes with central VMT release on OCT from PVL with gas injection versus observation (sham injection) in eyes with VMT without an associated MH.

Secondary

2. To evaluate visual function outcomes at 24 weeks after gas injection is performed compared with observation.

1.4 Potential Risks and Benefits of C_3F_8 Gas Injection

1.4.1 Known Potential Risks

- 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. It 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 and/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 the 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.
- Progression to MH after gas injection is a potential complication. Previous retrospective studies have shown this complication to be <5%
- 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 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 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:

- IOP 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 randomly assigned to PVL include release of VMT, improved visual acuity, improved quality of vision, prevention of full-thickness macular hole, 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.

VA and OCT technicians will be masked to treatment group at all visits. The goal is for study participants to remain masked to their treatment group assignment, although it is likely that the gas bubble will be visible to participants in the PVL group. Investigators and study coordinators are not masked to treatment group.

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 Randomized Trial Participant Recruitment and Enrollment

Enrollment will proceed with the goal of at least 124 participants being randomized. Participants who have signed consent may be permitted to continue into the trial, if eligible, even if the randomization goal has been reached.

Study participants will be recruited from approximately 50 clinical centers in the United States. Approximately 10 participants are expected to be randomized each month, resulting in 13 months of recruitment, for a total study duration of 19 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.

Once a study participant is randomized, that participant will be counted regardless of whether the assigned treatment is received. Thus, the investigator must not proceed to randomize an individual until he/she is convinced that the individual is eligible and will accept assignment to either of the two treatment groups.

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 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:

7. 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)
8. Participation in an investigational trial within 30 days of randomization 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/device while participating in the study*
9. Known contraindication to any component of the treatment
10. Known allergy to any drug used in the procedure prep (including povidone iodine)
11. 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 next 6 months following randomization
12. Anticipated surgery requiring anesthesia within the next 6 months following randomization
    - *Participants cannot receive nitrous oxide until gas resolution*
13. 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 randomization, the study eye will be selected by the investigator and participant before randomization.

The eligibility criteria for a study eye are as follows:

Inclusion

a. 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

    Note: presence of epiretinal membrane is neither a requirement nor exclusion.

b. Decreased visual function (e.g. metamorphopsia or other visual symptom) that is attributed to VMT.

    Examples of visual symptoms include:

a) Distortion and/or reduction in visual acuity
b) Recognized difficulty with reading, driving, or using a computer
c) Patient recognized interference with quality of life because of a and/or b.

c. Visual acuity letter score of at least 19 (approximate Snellen equivalent 20/400 or better) and at most 78 (20/32 or worse)

d. Investigator and participant willing to wait 6 months before surgical intervention, provided visual acuity remains stable

- *An eye that requires prompt treatment for VMT should not be enrolled*

Exclusion

e. 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*

f. 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)

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

h. History of prior vitrectomy

i. History of uncontrolled glaucoma

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

j. 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 randomization

k. History of YAG capsulotomy performed within 4 months prior to randomization

l. Aphakia or anterior chamber intraocular lens

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

n. Uveitis

o. Presence of any macular hole or lamellar hole (according to reading center grading)

p. 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.*

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

r. Lenticular or zonular instability

2.3 Screening Evaluation and Baseline Testing

After informed consent has been signed, a 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 on OCT must be completed prior to randomization.

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, visual function testing, 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. Visual function testing (myVisionTrack) *(within prior 8 days)*
    - Visual function testing will be performed using the myVisionTrack application on an iPad or tablet. myVisionTrack® is an FDA cleared application to track disease progression in patients with certain retinal diseases. During the test, the patient will select the circle that is shaped differently among a series of side-by-side circles on the screen. The series continues with increasing difficulty until the patient's visual function is determined. Visual function is measured using the shape discrimination hyperacuity (SDH) (logMAR) score.
5. 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 randomized until reading center confirmation of eligibility has been received

6. 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

2.4 Randomization of Eligible Participants

Randomization may occur on the same day as baseline testing if eligibility is confirmed by the reading center on the day of screening.

1. Prior to randomization, the participant's understanding of the trial, willingness to accept the assigned treatment group, and commitment to the follow-up schedule should be reconfirmed.
2. The baseline injection (or sham) must be given on the day of randomization; therefore, a participant should not be randomized until this is possible.
3. Randomization is completed on the DRCR Retina Network website.
    - Study eyes will be randomly assigned with equal probability to one of two treatment groups:
        - Group A: PVL (Intravitreous injection of 0.3 mL C_3F_8 gas)
        - Group B: Observation (Sham injection)

Randomization will be stratified by clinical site and presence of epiretinal membrane (ERM) within 1 mm of the center of the macula, determined by Reading Center grading. Previous reports have suggested that the proportions of eyes with VMT release differ depending on the presence of ERM.

Once a study participant is randomized, that participant will be counted regardless of whether the assigned treatment is received. Thus, the investigator must not proceed to randomize an individual until he/she is convinced that the individual is eligible and will accept whichever treatment group is assigned through randomization.

CHAPTER 3: BASELINE TREATMENT

3.1 Treatment

For both groups, the baseline injection (sham or intravitreous) must be given on the day of randomization.

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 aqueous 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.

3.2.3 Sham Injection Technique

The prep will be performed as for an intravitreous injection. For the sham injection procedure, a syringe without a needle will be used, with the hub pressed against the injection site to simulate the force of an actual injection.

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. All participants will be instructed to avoid high altitude travel until the surgeon confirms the gas bubble has cleared. Phakic participants in both groups 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. For observation group participants, this will be the 4-week visit.

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
- 12 (±2) weeks
- 24 (±4) weeks

4.1.1 Procedures at Study Visits

The following procedures will be performed in both groups at all visits, 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 24-week visit. When a refraction is not performed, the most recently performed refraction is used for the testing.
    - VA technicians will be masked to treatment group at all visits.
2. Visual function testing (myVisionTrack) at 12 and 24 weeks only
3. 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
    - OCT technicians will be masked to treatment group at all visits.
4. 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 12 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 that are performed should follow the standard DRCR Retina Network protocol for each procedure.

4.2 Treatment During Follow-Up

4.2.1 Criteria for Vitrectomy

Vitrectomy may be performed if one of the following occurs:

1. Visual acuity decreases at least 10 letters at a single visit (not including the 1-week visit) or at least 5 letters at two consecutive visits (not including the 1-week visit) compared with baseline that is thought to be related to VMT
2. Complication requires prompt surgical intervention (e.g., macular hole, retinal detachment, non-clearing vitreous hemorrhage)

Otherwise, protocol chair approval must be obtained for alternative treatment.

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 develops during follow-up requiring prompt treatment, it 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 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/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 $\geq$ 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 in the 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; and/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., pre-existing medical condition, underlying disease, intercurrent illness, or concomitant medication), and/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, that 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 or 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 on 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 Adverse Event 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/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 DSMC 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 the IRB. 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 and/or infrequent and include:

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, and/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 or sham injection, and must be worn until the investigator confirms that the gas bubble has cleared (at 4-week visit for observation group participants).

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 the first review of data by treatment group. The analysis plan synopsis in this chapter contains the framework of the anticipated final analysis plan.

8.2 Statistical Hypotheses

A test of superiority will be used in evaluating the following hypotheses for the primary outcome:

Null Hypothesis (H_0): There is no difference in the proportion of eyes with central VMT release without rescue treatment between the PVL and observation groups at 24 weeks.

Alternative Hypothesis (H_a): There is a difference in the proportion of eyes with central VMT release without rescue treatment between the PVL and observation groups at 24 weeks.

Similar hypothesis tests will be conducted for all secondary, exploratory, and safety outcomes.

8.3 Sample Size

8.3.1 Outcome Projections:

Observation Group (sham injection)

In a consecutive case series of 106 eyes with a median follow-up time of 18 months,[6] the proportion of eyes with spontaneous VMT release was 32%. The proportion of eyes with VMT release at 6 months in the Sham Groups of the randomized controlled trials MIVI-TRUST[10] (N = 188) and OASIS (N = 74),[11] which compared ocriplasmin versus sham for VMT, were approximately 13% and 10%, respectively. Dugel et al.[11] also found that the proportion of eyes with VMT release was greater among eyes that had MH at baseline. Macular hole was present at baseline in approximately 36% of the OASIS Sham Group and 25% of the MIVI-TRUST Sham Group.

PVL group

Three case series provide estimates of VMT release with C_3F_8 gas. A study of 15 eyes (1 with MH) from Rodrigues et al.[23] showed release of VMT in 60% of eyes through 6 months. Steinle et al.[24] showed 83% success among 30 eyes (3 with MH) through 62 days. Finally, Chan et al.[25] (2017) showed 86% success among 50 eyes (15 with MH) over 9 weeks. In addition, Chan et al. reported higher rates of VMT release among eyes with MH. The release rate among eyes with VMT only in Chan et al. was 80%.[25]

8.3.2 Sample Size Estimates

Table 1 shows sample size estimates for the primary analysis under varying assumptions for the proportion of eyes with VMT release within the 2 groups. These calculations assume a Type I error rate of 5%, 90% power, and a two-sided test of superiority (See Section 8.6) with a null hypothesis of no difference between the groups (Section 8.2).

Table 1: Comparison of Proportion with VMT Release: Total Sample Size

Release in PVL Group	Release in Sham Group		
	10%	**20%**	**30%**
50%	52	104	248
60%	34	60	**112**
70%	24	38	62

For true outcome proportion of 60% (PVL) vs 30% (sham injection), a sample size of 112 (56 per group) gives 90% power to reject the null hypothesis of no difference. Adjusting for possible loss to follow-up of 10% gives a final sample size of 124 (62 per group).

8.4 Outcome Measures

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

Primary Efficacy Outcome:

- Proportion of eyes with central VMT release* without rescue treatment at 24 weeks.
    - For purposes of description only, the distribution of eyes within treatment group by the following categories at 24 weeks will be tabulated without statistical comparison:
        - Central VMT release without rescue treatment
        - Central VMT release with rescue treatment
        - No central VMT release and no rescue treatment
        - No central VMT release despite rescue treatment

Secondary Efficacy Outcomes:

- Proportion of eyes with central VMT release* without rescue treatment through 24 weeks (time-to-event analysis).
- Proportion of eyes with central VMT release and vitreopapillary traction release* without rescue treatment at 24 weeks.
- Mean change in visual acuity letter score from baseline at 24 weeks.
- Proportion of eyes with at least 10-letter gain (increase) in visual acuity from baseline at 24 weeks.
- Proportion of eyes with at least 10-letter loss (decrease) in visual acuity from baseline at 24 weeks.
- Proportion of eyes receiving rescue treatment before the 24-week visit.
    - For purposes of description only, the following will be tabulated within treatment group 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 Efficacy Outcomes:

- Mean change in shape discrimination hyperacuity from baseline at 24 weeks.
- Proportion of eyes with ellipsoid zone* integrity at 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 from both treatment groups combined at 24 weeks.

8.5 Analysis Cohorts

- Intention-To-Treat (ITT) Analysis Cohort: all randomized participants irrespective of treatment received, and analyzed according to treatment assignment.
- Safety Analysis Cohort: all randomized participants irrespective of treatment received, and analyzed according to treatment assignment.
- Per-Protocol Analysis Cohort: only participants that complete the initial treatment (PVL or sham injection) 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. It will include all randomized participants. The data from the ITT cohort will be analyzed according to the group to which the participants were assigned through randomization, regardless of treatment actually received.

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 more than 10% of randomized participants would be excluded by these criteria (e.g., 13 or more participants if exactly 124 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 differences.

8.6 Analysis of the Primary Efficacy Outcome

The primary outcome of central VMT release without rescue treatment at 24 weeks is a binary variable that is graded by the central reading center. Logistic regression with robust variance estimation will be used to test the hypothesis of superiority (Section 8.2). Presence of ERM within 1 mm of the center of the macula at baseline will be included as a covariate, as previous reports have indicated the rate of VMT release differs by presence of ERM and the randomization schedules were therefore stratified by presence of ERM. The relative risk (estimated using the method of Localio et al. 2007[27]) for the treatment group effect, 95% confidence interval, and *P* value will used to compare treatment groups. To aid in interpretation of the relative risk, observed outcome proportions will be reported for each treatment group.

Since the chance of re-attachment after release is highly unlikely before 24 weeks, an eye with central VMT release without rescue treatment 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 will have been given.

Multiple imputation will be used to impute missing data for eyes lost to follow-up that did not have prior release or rescue treatment documented. The imputation model will include presence of ERM within 1 mm of the center of the macula at baseline, treatment group, and VMT status at 1, 4, 12, 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 Efficacy Outcomes

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

8.7.1. Secondary Efficacy Outcomes

Development of central VMT release without rescue treatment through 24 weeks is a time-to-event outcome that will be modeled with Cox proportional hazards regression and robust variance estimation. The hazard ratio along with the 95% confidence interval and *P* value will be used to compare treatment groups. To aid in interpretation, a Kaplan-Meier plot will be constructed. Data from eyes not observed to have release or that receive rescue treatment will be censored on the date of their final visit.

The proportion of eyes with central VMT release and vitreopapillary traction release (without rescue treatment) at 24 weeks is a binary variable graded by the central reading center that will be analyzed with logistic regression. Analysis and imputation of missing data will be handled in a manner similar to the primary outcome. The analysis will be adjusted for vitreopapillary traction status at baseline.

Change in visual acuity letter score from baseline to 24 weeks is a continuous variable that will be analyzed using a general linear model with robust variance estimation. Presence of ERM within 1 mm of the center of the macula at baseline and baseline visual acuity will be included as covariates. The treatment group difference, 95% confidence interval, and *P* value will be presented. To aid in interpretation, least squares means and associated 95% confidence intervals for each group will used to compare treatment groups. Serious violations of statistical assumptions may be addressed by transformation of variables, non-parametric methods, or categorizing continuous covariates. 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, treatment group, baseline visual acuity, and change in visual acuity from baseline at 1, 4, 12, and 24 weeks, and VMT status at 1, 4, 12, and 24 weeks.

The proportion of eyes with at least 10-letter gain (increase) or loss (decrease) in visual acuity from baseline are binary variables that will be analyzed with logistic regression and will use the imputed data sets from the analysis of mean change in visual acuity from baseline.

The proportion of eyes receiving rescue treatment before the 24-week visit is a binary variable that will be analyzed with logistic regression. The presence of ERM within 1 mm of the center of the macula at baseline will be included as a covariate. Complete-case analysis (no imputation of missing data) will be used for this outcome.

8.7.2 Exploratory Efficacy Outcomes

Change in shape discrimination hyperacuity (measured in logMAR) is a continuous variable that will be analyzed as above but substituting baseline and follow-up shape discrimination hyperacuity for visual acuity. Complete-case analysis (no imputation of missing data) will be used for this outcome.

The proportion of eyes with ellipsoid integrity at 24 weeks is a binary variable graded by the central reading center (loss of integrity and no loss of integrity). Logistic regression adjusted for ellipsoid zone status at baseline and the presence of ERM within 1 mm of the center of the macula at baseline will be used to compare treatment groups. The relative risk for the treatment group effect, 95% confidence interval, and *P* value will used to compare treatment groups. To aid in interpretation of the relative risk, observed outcome proportions will be reported for each treatment group. 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 by treatment group in a listing of each reported Medical Dictionary for Regulatory Activities (MedDRA) term and summarized over each MedDRA System Organ Class. All randomized participants will be included in safety analyses. Any events occurring between randomization and study treatment will be noted.

8.8.1 Ocular Adverse Events

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 compared between treatment groups with Barnard's unconditional exact test. The following ocular adverse events are of primary interest:

- Retinal detachment
- Retinal tear
- Macular hole development
- 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 number of eyes with endophthalmitis and traumatic cataract will be tabulated without statistical comparison.

8.8.2 Systemic Adverse Events

The frequency of each systemic adverse event occurring at least once per participant will be calculated. The proportion of participants experiencing each outcome will be compared with Barnard's unconditional exact test. 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 and will be tabulated without statistical comparison:

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

For each treatment group, the number of adverse events (ocular or systemic) considered related to treatment will be tabulated.

8.9 Intervention Adherence

Adherence will be defined as completion of the treatment assigned at randomization, either PVL or sham injection.

8.10 Protocol Adherence and Retention

Protocol deviations and visit completion rates (excluding deaths) will be tabulated for each treatment group.

8.11 Baseline Descriptive Statistics

Baseline characteristics will be tabulated by treatment group 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 (interaction), will be conducted for the primary outcome. These analyses will be considered exploratory. Additionally, interpretation of the analyses will depend on whether the primary analysis demonstrates a significant treatment group difference; in the absence of such a difference, subgroup analyses will be interpreted with caution.

The general approach for these exploratory analyses will be to add an interaction term for the subgroup factor by treatment into the primary analysis model. In addition, within-subgroup treatment effects and 95% confidence intervals will be estimated from the interaction model if the interaction *P* value is less than .05. Subgroup analyses will use data from eyes that complete the 24-week visit or have VMT release or rescue treatment prior to 24 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. In previous studies, eyes with ERM treated with C_3F_8 for VMT had lower release rates compared with eyes not having ERM.[10, 11, 25] It is unknown what effect ERM may have on the release rate in the observation group.

Secondary subgroup analyses will include ERM presence at the site of vitreous adhesion, lens status (phakic or pseudophakic), components of VMT severity grade,[6] 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 20 eyes in each subgroup for each treatment group.

8.14 Multiple Testing

There will be no formal adjustment for multiple testing. Only $P \leq .05$ will be considered of interest.

8.15 Assessment of Confounding

Imbalances between groups in important covariates are not expected to be of sufficient magnitude to produce confounding. However, a sensitivity analysis using observed data (no multiple imputation) will be conducted if there is an imbalance between treatment groups in any of the following: presence of ERM, lens status, extent of VMT, diabetes status, age, or sex. Imbalance by treatment group will not be judged using statistical testing. Instead, imbalance will be judged by whether the imbalance is large enough to have a clinically important effect on the primary outcome. The sensitivity analysis will mimic the primary analysis but add any potentially imbalanced factors as covariates.

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 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:

- 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, 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 principal 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 Ethical Standard

The principal 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/or 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; 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, which is for 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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