Protocol Title: A Pilot Study for the Evaluation of Minocycline as a Microglia Inhibitor in the Treatment of Central Retinal Vein Occlusions

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Human Research Protections Program Investigator and Staff Training:

"Just in time" human subjects protection training courses are required for investigators and staff participating on this protocol: None

Total Requested Accrual: 20 Participants – A minimum of 10 and a maximum of 20 participants will be accrued.

0 Healthy Volunteers

Project Uses Ionizing Radiation: ☒ No ☐ Yes

IND/IDE: ☐ No ☒ Yes

Drug/Device #: 108,154
Sponsor: Catherine Cukras, MD, PhD

EudraCT: ☐ No ☒ Yes

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Chief Investigator: Clare Bailey, MD

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Multi-institutional Project: ☐ No ☒ Yes

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Coordinating Center: National Eye Institute (NEI)	FWA00005897
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Additional sites may be identified and added to this study in future amendments.

Data and Safety Monitoring Board: ☐ No ☒ Yes

Technology Transfer Agreement: ☒ No ☐ Yes

Samples Being Stored for Future Use: ☒ No ☐ Yes

Flesch-Kincaid Reading Level of Consent Form: 9.5

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PROTOCOL ABBREVIATIONS LIST

AE	Adverse Event
ALS	Amyotrophic Lateral Sclerosis
APD	Afferent Pupillary Defect
BCVA	Best Corrected Visual Acuity
BEH	Bristol Eye Hospital
BRB	Blood-Retinal Barrier
BRC	Biomedical Research Centre
BRVO	Branch Retinal Vein Occlusion
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CC	Clinical Center
CD	Clinical Director
CD-68	Clusters of Differentiation 68
CFP	Color Fundus Photography
CFR	Code of Federal Regulation
CNS	Central Nervous System
CNS IRB	Combined NeuroScience Institutional Review Board
COX-2	Cyclooxygenase-2
CRIS	Clinical Research Information System
CTCAE	Common Terminology Criteria for Adverse Events
CVA	Cerebral Vascular Event
DME	Diabetic Macular Edema
DSMC	Data and Safety Monitoring Committee
Emmes	The Emmes Corporation
EMR	Electronic Medical Record
ETDRS	Early Treatment Diabetic Retinopathy Study
EudraCT	European Clinical Trials Database
FA	Fluorescein Angiogram
FDA	Food and Drug Administration
FGF	Fibroblast Growth Factor
FSH	Follicle-Stimulating Hormone
HRPP	Human Research Protections Program
HRVO	Hemi-Retinal Vein Occlusion
ICE	IL-1b-Converting Enzyme
ICRRC	Intramural Clinical Research Review Committee
IL-1b	Interleukin-1b
IL-6	Interleukin-6
IL-8	Interleukin-8
IND	Investigational New Drug
IOP	Intraocular Pressure

PROTOCOL ABBREVIATIONS LIST (CONTINUED)

IP	Investigational Product
IRB	Institutional Review Board
MCP-1	Monocyte Chemoattractant Protein
MEH	Moorfields Eye Hospital
MHRA	Medicines & Healthcare products Regulatory Agency
MI	Myocardial Infarction
MMP	Matrix Metalloproteinase
MS	Multiple Sclerosis
NEI	National Eye Institute
NGF	Nerve Growth Factor
NIH	National Institutes of Health
NO	Nitric Oxide
OB/GYN	Obstetrics and Gynaecology
OCT	Optical Coherence Tomography
OHSRP	Office of Human Subject Research Protection
ONL	Outer Nuclear Layer
PI	Principal Investigator
PTMS	Protocol Tracking and Management System
QA	Quality Assurance
RVO	Retinal Vein Occlusion
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
SPF	Sun Protection Factor
STZ	Streptozotocin
SUSAR	Serious Unanticipated Serious Adverse Reaction
TNFa	Tumor Necrosis Factor-a
USP	United States Pharmacopeia
UV	Ultraviolet
VA	Visual Acuity
VEGF	Vascular Endothelial Growth Factor
YAG	Yttrium Aluminum Garnet

PRÉCIS

Objective: Retinal vein occlusions (RVOs) are significant sources of vision loss, affecting mostly healthy people over 55 years of age. The common source of vision loss is the macular edema accompanying the retinal injury. Very recently, studies employing monthly anti-vascular endothelial growth factor (VEGF) treatments have demonstrated a benefit to this line of treatment; however, the duration of effectiveness appears to be short lived and the length of time needed for these monthly injections remains unknown. A histologic study of human retinas with RVOs found the presence of activated microglia. Microglia are capable of migrating through the retina to sites of inflammation to associate closely with neurons and the vasculature, and are key cellular players in the mediation of processes of chronic inflammation. For these reasons, microglia represent a promising cellular target for forms of therapy that limit the deleterious inflammatory changes found in vein occlusions. Minocycline, a second-generation tetracycline, has been shown to exhibit anti-inflammatory properties, including microglial inhibition. The objective of this study is to investigate the safety and potential efficacy of minocycline as a microglia inhibitor in participants with central retinal vein occlusion (CRVO).

Study Population: A minimum of 10 and a maximum of 20 participants who meet the eligibility criteria may be enrolled. Eligibility criteria include: foveal center-involved macular edema secondary to a CRVO, retinal thickness in the central subfield > 350 microns as measured by optical coherence tomography (OCT); and visual acuity (VA) between 20/32 and 20/200 in the study eye.

Design: In this pilot, double-masked, randomized multi-center study, participants will receive monthly bevacizumab injections for the first three months, followed by PRN dosing. In addition, participants will take an oral dose of 100 mg of minocycline or placebo twice daily for 24 months. During each monthly visit, participants will have their visual acuity measured and will undergo OCT testing to measure retinal thickness. At the Month 3 visit and thereafter, participants will be evaluated for “improvement” and “worsening” and will be eligible for additional bevacizumab treatment and/or investigational product (IP) depending on which criteria they fulfill. Additionally, at Month 12, participants will also be evaluated for “no improvement.”

Outcome Measures: The primary outcome is the difference in mean change in best-corrected visual acuity (BCVA), as measured in ETDRS letters, between the minocycline and placebo groups in the study eye at 12 months compared to baseline. Secondary outcomes include the difference between the minocycline and placebo groups in the number of intravitreal bevacizumab injections between 12 and 24 months and baseline, changes in mean macular sensitivity as measured by microperimetry at 3, 6, 12, 18 and 24 months compared to baseline, the mean change in BCVA at 24 months compared to baseline, changes in retinal thickness as measured by OCT at 6, 12, 18 and 24 months compared to baseline, number of participants improving ≥ 1 logOCT scale step at 12 and 24 months compared to baseline, as well as and changes in fluid leakage in the macula as demonstrated by fluorescein angiography at 12 and 24 months compared to baseline. Safety outcomes include the number of participant withdrawals, number and severity of systemic and ocular toxicities and the number of adverse events (AEs).

1.0 INTRODUCTION/SCIENTIFIC RATIONALE

1.1 Central Retinal Vein Occlusions

Retinal vein occlusions are a significant source of vision loss, affecting mostly healthy people over 55 years of age. The common source of vision loss is the macular edema accompanying the retinal injury. The two main types of retinal vein occlusions are central retinal vein occlusions (CRVO) and branch retinal vein occlusions (BRVO). The prevalence of CRVO is approximately 0.1%.[1,2] The standard-of-care for CRVO is even more limited than for BRVO. While optic nerve sheathotomy and other invasive measures have been tried, these interventions have not been successfully proven in a large randomized clinical trial. Very recently the Central Retinal Vein OcclUsIon Study: Evaluation of Efficacy and Safety (CRUISE), a phase III multicenter trial, reported six-month outcome results demonstrating efficacy of intravitreal ranibizumab injections for the treatment of CRVO.[3] The CRUISE study, while encouraging, only has six-month results published and requires monthly intravitreal injections with no clear treatment endpoint. Ranibizumab has very recently gained FDA approval for the indication of vein occlusions and the labeling includes recommendations for monthly intravitreal injections.

The fundamental cause of macular edema associated with retinal vein occlusions is not understood completely, but it is increasingly clear that its pathophysiology extends beyond microvascular disease to involve immune mediators in the retina. While hydrostatic stress following the break-down of the blood-retinal barrier (BRB) can partly contribute to retinal edema, tissue responses to hypoxia are also likely to play a role. In the aftermath of a retinal vein occlusion, tissue hypoxia induces the expression of several cytokines, including monocyte chemoattractant protein (MCP-1), leading to the activation of inflammatory cells, such as macrophages and microglia, which are then attracted to the hypoxic retinal areas.[4] Activated microglia release TNF-α which triggers the production of interleukin-8 (IL-8), VEGF, basic fibroglast growth factor (FGF) and MCP-1 in retinal vascular cells and glial cells adjacent to the microvessels.[5] A study on patients with BRVO examined the concentrations of VEGF and interleukin-6 (IL-6) in the aqueous humor and found that these cytokine levels were increased in the setting of retinal vein occlusion.[6] Further, a histologic study of human retinas with retinal vein occlusions found numerous cluster of differentiation-68 (CD-68) positive cells surrounding the vasculature indicating the presence of activated microglia.[7]

Inflammation may represent an early adaptive response to metabolic derangements in the ischemic retina; over time; however, it leads to progressive vascular damage results, culminating in macular edema and neovascularization.[8] As such, there may be a feasible therapeutic rationale to broadly limit and down-regulate the level of microglia-mediated chronic inflammation in the treatment of RVOs.

1.2 Current Treatments of CRVO

Unlike branch retinal vein occlusions, CRVOs did not demonstrate an improvement in visual acuity with focal laser photocoagulation.[9] Several more invasive therapies have been pursued including optic neurotomy and sheathotomy. However, none have shown efficacy in a randomized controlled clinical trial. In fact, a review of CRVO intervention treatments in 2007 concluded that there was limited evidence for any intervention to improve visual acuity in patients with CRVO.[10]

Recently, phase III results of the CRUISE study, have demonstrated efficacy of monthly injections of ranibizumab for treatment of central retinal vein occlusions.[3] And in late June 2010, the FDA approved ranibizumab for use in the treatment of retinal vein occlusions.

However, while the treatment of monthly injections appears beneficial in the short term, the longer term result of this course of treatment is unknown. Also unknown, is the number of injections required, and the durability of these injections. While being a major breakthrough in the field of treatment of these retinal conditions, monthly anti-VEGF injections still have limitations for treating this disease.

We propose to study minocycline as a microglia inhibitor which can modulate the activation of microglia, alter the cytokine profile present, and thus may be useful in the treatment of CRVO.

1.3 Role of Microglia

Retinal microglia, derived from the monocytic lineage, are resident cells in the healthy retina, and may be construed as representatives of the immune system in the immune-privileged environment of the retina. Microglia are related to macrophages but are a distinct entity[11] comprising approximately 5% to 20% of the total glial cell population in the central nervous system (CNS).[12] Microglia in the CNS are important sources of inflammatory cytokines and chemokines, and are the primary cellular mediators of immune function.[13] Most of the factors released by activated

microglia are proinflammatory and neurotoxic, including cytokines tumor necrosis factor-a (TNFa) and interleukin-1b (IL-1b), free radicals such as NO and superoxide, fatty acid metabolites such as eicosanoids, and quinolinic acid.[14] The activation of microglia has been implicated in the pathogenesis of many neurodegenerative diseases including Alzheimer's, Parkinson's disease, HIV-related dementia, Amyotrophic Lateral Sclerosis (ALS), Multiple Sclerosis (MS) and prion diseases.[15] The cytokines released by activated microglia are thought to speed neurodegeneration. Microglia have also been implicated in ischemic and mechanical injury to the brain.[16] In the retina, retinal glial cells consist of macroglia (Müller cells and astrocytes) and microglia. Microglia are able to migrate in various retinal disease states. In photic injury, microglia migrate from the inner retinal layers to the outer nuclear layer (ONL) and also into the subretinal space.[17,18] In sites of inflammation, these cells migrate through the retina to closely associate with neurons and the vasculature, and are key cellular players in the mediation of processes of chronic inflammation. As such, it is likely that retina microglia play an important role in the inflammatory etiology of diabetic retinopathy.

1.4 Role of Microglia in Retinal Vein Occlusions

There is accumulating evidence that retinal microglia are indeed associated with inflammation in the retina in conditions such as diabetic retinopathy and RVOs. While diabetic retinopathy is often thought of as a vasculopathy, there is a growing body of evidence to suggest that changes in the retinal neurons precede vascular changes.[19] Microglia function has been investigated in a diabetic animal model where diabetes was induced in rats with the injection of streptozotocin (STZ).

Animal models of diabetes have indicated a role of microglia activation in diabetic retinopathy. In a histologic study of microglia in eyes with diabetic retinopathy, Zeng et al. reported that microglia increase in number and exhibit hypertrophic features as well as an up-regulation of markers in diseased states and marked microglia surrounding the retinal vasculature, labeled "microglia perivasculitis" in further stages of disease.[20] This may lead to early fluorescein leakage present before frank vasculopathy is noticed clinically both in diabetic retinopathy and vein occlusion injury.

Animal models of ischemia/reperfusion injury mimics the mechanism of injury occurring in RVOs. Shin et al. used both an ischemia/reperfusion model as well as a venous cauterization method to demonstrate that microglia become activated under these circumstances and migrate to the inner plexiform layer near blood vessels.[21] Further research by Lin et al. on an ischemia/ reperfusion model in rats have shown that not only are microglia activated in this model, but also that they can be inhibited by minocycline.[22] In their model, they demonstrated a significant treatment effect of treatment in rats with minocycline inhibiting the vascular permeability response by inhibiting microglia.

Indeed, focal laser photocoagulation, the long-time standard-of-care treatment for Diabetic Macular Edema (DME) and for branch retinal vein occlusion (BRVO), recently has been found to induce prompt changes in the morphology and behavior of retinal microglia, and may thus also involve microglia in their therapeutic effect.[23] For these reasons, microglia represent a promising cellular target for forms of therapy that limit the deleterious inflammatory changes found in vein occlusions. While therapeutic agents that specifically inhibit individual pro-inflammatory molecules one at a time may have efficacy, an approach that targets the cellular mediator, the microglia cell, may have broader and more relevant effects.

1.5 Minocycline as a Microglia Inhibitor

1.5.1 Minocycline

Minocycline is a second-generation tetracycline, which has anti-inflammatory properties that are separate from its anti-microbial properties. Minocycline has high lipophilicity, which leads to both excellent bioavailability and ability to cross the blood-brain barrier[24] and appears to inhibit the activation of microglia, but not astrocytes.[25] The mechanism of neuroprotection by minocycline is likely several-fold and includes: anti-inflammatory effects, reduction of matrix metalloproteinase (MMP) activity,[26] and decrease in NO production, ultimately leading to an inhibition of microglia and of cell death.[27] Minocycline can exert its anti-inflammatory effects on microglia in culture, inhibiting their proliferation and release of IL-1B, IL-6, TNF-α, NO secretion, and NGF-production leading to neuroprotection with the inhibition of cell death.[28-32] Minocycline has been also found to inhibit induction of IL-1b-converting enzyme (ICE) mRNA[25] which means that minocycline may function by reducing cytotoxic properties of microglia which can be activated

by ischemia and neuroexcitation. In addition, minocycline can also help prevent cytokine-induced activation of microglia and prevent further production of proinflammatory stimuli.

1.5.2 Preclinical Studies with Minocycline

In animal models of global and focal cerebral ischemia, traumatic brain and spinal injury, minocycline treatment showed a reduction in lesion size in conjunction with the inhibition of microglial cells.[33-36] In rodent models of spinal cord injury, treatment with minocycline not only reduced lesion size and improved histological parameters but also improved neurological function such as hind limb function and strength.[37]

Minocycline has also been studied in the context of ocular diseases. In mouse models of glaucoma, minocycline successfully decreased microglial activation and retinal ganglion cell axonal transport and improved optic nerve integrity.[38] Minocycline was effective in decreasing retinal microglia activation in culture.[39] In models of inherited and light-induced retinal degeneration, minocycline was effective in delaying photoreceptor cell death.[39,40] As mentioned above, minocycline reduces apoptotic cell death in mouse models of diabetic retinopathy.[32] Animal studies have also indicated that inhibiting microglia activation with minocycline was useful in decreasing the level of pro-inflammatory mediators and preventing diabetes-induced cell death in the retina.[32] In particular, addition of minocycline to TNF-alpha-activated microglia was found to prevent the release of COX-2, an enzyme important in prostaglandin synthesis and is a robust marker of inflammation.[32] Ultimately, diabetic rats do demonstrate an increased rate of apoptosis, as evidenced by increased caspase-3 activity, and minocycline has been shown to decrease that activation.[32]

1.5.3 Clinical Use and Clinical Studies with Minocycline

Minocycline is a member of the tetracycline family of antibiotics that has good CNS bioavailability following oral administration. It is FDA-approved for treating many infections including skin infections caused by *Staphylococcus aureus* and is often used in ophthalmology to treat blepharitis and ocular rosacea. It has a favorable side-effect profile, although it should not be used in pregnancy as the use of tetracycline class of drugs during tooth development may cause permanent discoloration of the teeth.

Minocycline is used widely and has been shown to be well tolerated when used at the FDA-approved doses proposed in this study. Based on studies examining the adverse events (AEs) reported between 1998 and 2003, and the number of prescriptions for minocycline, the rate of AEs has been reported as 72 AEs per million prescriptions averaging to 13 per million per year.[41] The majority of these events were gastrointestinal upset, photosensitivity, hyperpigmentation and tooth discoloration.[41]

Its application in clinical trials as an anti-microglia agent include a large clinical trial of 152 patients with acute stroke in which patients treated with 200 mg minocycline for five days had a significantly better neurological outcome compared with placebo.[27] Other ongoing clinical studies include the use of minocycline to decrease symptom progression in Huntington's disease (NCT00277355), and to provide neuroprotective effects in Parkinson's disease (NCT00063193) and in ALS (NCT00047723).

A current clinical trial, conducted by the Penn State Diabetic Retinopathy Center, is commencing recruitment to examine the effects of a related tetracycline, doxycycline, in its ability to slow the deterioration or improve retinal function and/or induce regression, or slow progression, of diabetic retinopathy (NCT00511875).

2.0 STUDY OBJECTIVE

The study objective is to investigate the safety and potential efficacy of minocycline, a microglia inhibitor, in the treatment of CRVO.

3.0 PARTICIPANTS

The accrual ceiling is 20 participants who meet the eligibility criteria.

3.1 Participant Eligibility Criteria

3.1.1 Inclusion Criteria

To be eligible, the following participant-level inclusion criteria must be met, where applicable.

1. Participant is 18 years of age or older.
2. Participant must understand and sign the protocol's informed consent document.

3. Female participants of childbearing potential (see Appendix 1 for definition) must not be pregnant or breast-feeding and must be willing to undergo serum (BRC sites only) and urine pregnancy tests throughout the study.

4a. For the NEI Site: Female participants of childbearing potential (see Appendix 1 for definition) and male participants able to father children must have (or have a partner who has) had a hysterectomy or vasectomy, be completely abstinent from intercourse or must agree to practice two acceptable methods of contraception throughout the course of the study and for one week after study medication discontinuation (based on the half life of minocycline which is 11-22 hours). Acceptable methods of contraception include:

- hormonal contraception (i.e., birth control pills*, injected hormones, dermal patch or vaginal ring),
- intrauterine device,
- barrier methods (diaphragm, condom) with spermicide, or
- surgical sterilization (hysterectomy or tubal ligation).

*Oral birth control pills must be used with caution as minocycline decreases the effectiveness of some oral contraceptives. Participants already taking oral contraceptives may continue to use them, but must agree to use at least one other method of birth control while on study.

4b. For the BRC Sites: Female participants of childbearing potential (see Appendix 1 for definition) and male participants able to father children must have (or have a partner who has) had a hysterectomy or vasectomy, or be completely abstinent from intercourse. Male participants or male partners (of female participants) who have not had a vasectomy or are not abstinent are required to use a condom with spermicide throughout the course of the study and for one week after study medication discontinuation (based on the half life of minocycline which is 11-22 hours). Female participants of childbearing potential or female partners (of male participants) of childbearing potential must practice one of the below acceptable methods of contraception throughout the course of the study and for one week after study medication discontinuation:

- hormonal contraception (i.e., birth control pills*, injected hormones, dermal patch or vaginal ring),
- intrauterine device,
- barrier methods (e.g., diaphragm) with spermicide, or
- surgical sterilization (hysterectomy or tubal ligation).

Abstinence is only acceptable when it is the participant's preferred and usual lifestyle choice. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

*Oral birth control pills must be used with caution as minocycline decreases the effectiveness of some oral contraceptives. Participants already taking oral contraceptives may continue to use them, but must agree to use at least one other method of birth control while on study.

It should be noted that two forms of contraception (as specified above) will be used by sexually active participants for the duration of the study and for one week after study medication discontinuation.

5. Participants must agree to notify the study investigator or coordinator if any of their doctors initiate a new medication during the course of this study.
6. Participant must have normal renal function and liver function, or have mild abnormalities not above grade 1 as defined by the Common Terminology Criteria for AEs v4.0 (CTCAE).
7. Participant must agree to minimize exposure to sunlight or artificial UV rays and to wear protective clothing, sunglasses, and sunscreen (minimum SPF 15) if s/he must be out in the sun.
8. Participant has at least one eye that meets the study eye criteria listed in Section 3.2 below.

3.1.2 Exclusion Criteria

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

1. Participant is in another investigational study and actively receiving IP for CRVOs.
2. Participant is unable to comply with study procedures or follow-up visits.
3. Participant has a known hypersensitivity to sodium fluorescein dye.

4. Participant has a condition that, in the opinion of the investigator, would preclude participation in the study (e.g., unstable medical status including blood pressure and glycemic control).
5. Participant has a history of chronic renal failure requiring dialysis or kidney transplant.
6. Participant has a history of chronic hepatitis or liver failure.
7. Participant has an allergy or hypersensitivity to minocycline or any drug in the tetracycline family.
8. Participant is currently taking a tetracycline medication.
9. Participant is taking any medication that could adversely interact with minocycline such as methoxyflurane.
10. Participant has a blood pressure of > 180/110 (systolic above 180 **OR** diastolic above 110).
    - *If blood pressure is brought below 180/110 by anti-hypertensive treatment, the participant can become eligible.*
11. Participant is currently being treated with systemic anti-VEGF agents or systemic steroids.
12. Participant had a cerebral vascular event (CVA) or myocardial infarction (MI) within three months prior study entry.
13. Participant has a history of thyroid cancer.

3.2 Study Eye Eligibility Criteria

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

3.2.1 Study Eye Inclusion Criteria

1. The study eye has a best-corrected ETDRS visual acuity score between 78 and 34 letters (i.e., between 20/32 and 20/200).
2. The study eye shows definite retinal thickening due to a CRVO based on clinical examination involving the center of the macula that is not refractory to further therapy as based on the investigator's clinical judgment. CRVO is defined as an eye that had retinal hemorrhage or other biomicroscopic evidence of RVO (e.g., telangiectatic capillary bed)

and a dilated (or previously dilated) venous system in at least three quadrants of the retina drained by the affected vein.

3. The study eye has retinal thickness in the central subfield on baseline OCT measurement > 350 microns, as measured by Zeiss Cirrus spectral domain OCT, or an equivalent retinal thickness on a similar OCT machine.
4. The study eye has media clarity and pupillary dilation sufficient for adequate fundus photographs. Furthermore, the participant must be able to cooperate during the procedure for accurate fundus photographs.

3.2.2 Study Eye Exclusion Criteria

1. Macular edema is considered to be due to a cause other than CRVO.
2. An eye should not be considered eligible if:
   - The macular edema is considered to be related to cataract extraction, or
   - Clinical examination and/or OCT suggest that vitreoretinal interface disease (e.g., a taut posterior hyaloid or epiretinal membrane) is the primary cause of the macular edema, or
   - Clinical examination, medical history and/or fluorescein angiography suggest that diabetic retinopathy is the primary cause of the edema.
3. The study eye has a history of a recurrent RVO.
4. The study eye has a history of RVO present for >18 months.
5. A brisk afferent pupillary defect (APD) is present in the study eye.
6. An ocular condition (other than RVO) is present such that, in the opinion of the investigator, visual acuity would not improve from resolution of macular edema (e.g., foveal atrophy, pigmentary changes, dense subfoveal hard exudates, laser scar at fovea, non-retinal condition).
7. An ocular condition (other than RVO) is present that, in the opinion of the investigator, might affect macular edema or alter visual acuity during the course of the study (e.g., vein occlusion, uveitis or other ocular inflammatory disease, neovascular glaucoma, Irvine-Gass Syndrome, etc.).

8. A substantial cataract that, in the opinion of the investigator, is likely to be decreasing visual acuity by three lines or more (i.e., cataract would be reducing acuity to 20/40 or worse if eye was otherwise normal) is present in the study eye.
9. The study eye has had panretinal or sectoral scatter photocoagulation (PRP) within four months prior to study entry.
10. The study eye has had pars plana vitrectomy within six months prior to study entry.
11. The study eye has undergone major ocular surgery (including cataract extraction, scleral buckle, any intraocular surgery, etc.) within three months prior to study entry.
12. A yttrium aluminum garnet (YAG) capsulotomy has been performed on the study eye within two months prior to study entry.
13. The study eye has had treatment < 3 months prior to study entry of intravitreal or periocular steroid injections.
14. The study eye has had treatment < 28 days prior to study entry of intravitreal anti-VEGF agents.

3.2.3 Study Eye Selection Criteria in Cases of Bilateral Disease

If both eyes of a participant meet the criteria described in Sections 3.2.1 and 3.2.2, the study eye will be determined at the investigator's discretion.

4.0 STUDY DESIGN AND METHODS

4.1 Study Overview

This is a pilot, double-masked, randomized, multi-center study to investigate the safety and potential efficacy of minocycline as a microglia inhibitor in participants with CRVO who are concurrently being treated with standard-of-care anti-VEGF (bevacizumab) intravitreal injections. This study is designed not to determine efficacy but rather to generate preliminary data to support a fully powered phase III study.

4.2 Recruitment

Up to 20 participants will be recruited from the NEI clinic and BRC sites, collectively. The recruitment totals are not fixed at either the NEI or BRC sites.

NIH recruitment will occur by self-referral and referral from outside physicians will be permitted.

A letter will be provided to local ophthalmologists to assist with recruitment. A recruitment flyer will be posted at community gathering places including but not limited to: hospitals, libraries, senior centers, senior housing facilities, area metro and bus stops, websites, newspapers and throughout the NIH campus. NIH investigators from this study working at the Veterans Affairs hospital will provide information to patients in the Veterans Affairs eye clinic, but will not perform a formal assessment of eligibility or conduct any part of the informed consent process at the Veterans Affairs hospital. Potentially interested patients will contact the investigators at a later time. Contact information from Veterans Affairs patients will be provided to the NIH investigators only if the patient specifically requests that someone call them to set up the initial visit. The interaction will be documented in the medical record at the Veterans Affairs clinic.

Participants at the BRC sites will be identified following attendance at routine appointments or sent a recruitment letter from existing databases of patients diagnosed with CRVO and who routinely attend the BRC sites for treatment.

4.3 Screening

Potential participants at the NEI will be screened under the NEI screening (08-EI-0102) or evaluation and treatment protocol (08-EI-0169) to establish eligibility. The participant must have an active signed consent for one of the above mentioned protocols on file for baseline examinations to occur. Baseline examinations will be performed as outlined in Appendix 2. Laboratory measures may be performed within 42 days of enrollment. Optical coherence tomography (OCT), color fundus photography and fluorescein angiography may be performed within seven days of the baseline visit. All other baseline examinations must occur on the day the participant reviews and signs this protocol's consent form, unless specified in Appendix 2. Treatment with the IP (minocycline or placebo) cannot occur until the participant has reviewed and signed this protocol's consent form.

Potential participants from the BRC sites will be screened under this study protocol. The participant must have signed this protocols consent form for any study examinations to occur. For the BRC sites, it is expected that the baseline examinations can be completed in one clinic visit over one day. If the baseline examinations cannot be performed in one clinic visit over one day, it

is acceptable that the baseline examinations be completed at subsequent clinic visits, if scheduled within fourteen days of the first visit.

4.4 Randomization, Masking and Unmasking

After the participant is determined eligible and has completed the informed consent process, the group will be assigned based on a predetermined randomization schedule developed by the Coordinating Center. Randomization will be stratified by site.

Randomization must occur on the same day as the initial administration of IP while the participant and the ophthalmologist are present and will not be permitted any time before. Participant eligibility must be confirmed before a participant is randomized. Participants will be randomized to one of two groups with equal probability of equal numbers of participants in each group: minocycline or placebo. Participants must continue to receive the same treatment regimen to which they were assigned for the duration of the study.

All clinic staff and participants will be masked to group assignments. Only designated pharmacy personnel, select individuals at the Emmes Corporation, and the NEI Data and Safety Monitoring Committee (DSMC) will have access to the group assignments.

Participants will be unmasked if deemed clinically necessary by the examining physician and if the study Principal Investigator (PI) and DSMC Chair are in agreement. In the case of a medical emergency, the examining or treating physician will have the final decision and unilateral right for unmasking. A request for unmasking, after approval by the PI and the DSMC Chair, will be made to the Coordinating Center personnel, who will inform the PI and the DSMC Chair of the group assignment. Attempts should be made to maintain the masking of the investigators prior to the study-wide unmasking. Unmasking will be recorded on an AE form. All instances of unmasking must be reported to the ICRRC, the CNS IRB and the DSMC.

4.5 Study Design and Procedures

For the NEI Site: The study duration will be 24 months. During this period, participants will be instructed to take the IP (either placebo or minocycline 100 mg capsule) twice daily. The primary outcome will be assessed at Month 12, and secondary outcomes will be assessed at 6, 12, 18 and 24 months. Visits will occur at baseline, and then monthly or as clinically indicated. The study will

require a minimum of 25 appointments (baseline and Months 1-24). All appointments must be conducted within a window of ± seven days from the target day. The tests scheduled at each visit will be completed in one day. At each visit, the participant will undergo a review of systems and an assessment of safety variables. A complete ophthalmologic examination will be performed at each visit to measure outcome variables.

During each clinic visit, participants will have their visual acuity measured and will undergo OCT testing to measure retinal thickness.

For the BRC Sites: The study duration will be approximately 24 months. During this period, participants will be instructed to take the IP (either placebo or minocycline 100 mg capsule) twice daily. The primary outcome will be assessed at Month 12, and secondary outcomes will be assessed at 6, 12, 18 and 24 months. Visits will occur at baseline, and then monthly or as clinically indicated. The study will require a minimum of 26 appointments (baseline and Months 1-24 and safety follow-up). All appointments must be conducted within a window of ± seven days from the target day, except for the safety follow-up visit which must occur at least five days after the cessation of IP. The tests scheduled at each visit are expected to be completed in one day, with the exception of the Baseline visit, Month 12 visit and Month 24 visit. If the baseline examinations cannot be performed in one clinic visit over one day, it is acceptable that the baseline examinations be completed at subsequent clinic visits, if scheduled within fourteen days of the first visit. If the Month 12 and Month 24 examinations cannot be performed in one clinic visit over one day, it is acceptable that the examinations be completed at subsequent clinic visits, if scheduled within seven days of the first visit. At each visit, the participant will undergo a review of systems and an assessment of safety variables. A complete ophthalmologic examination will be performed at each visit (except for the safety follow-up visit) to measure outcome variables. If the Baseline visit, Month 12 visit, or Month 24 visit are conducted over more than one day, BCVA, OCT and microperimetry testing must be conducted on the day of the injection.

During each study visit, with the exception of the Month 24 visit and safety follow-up visit at the BRC sites, IP will be dispensed to the participant unless the treatment is deemed to be “worsening” or to offer “no improvement,” and the participant and investigator decide to stop IP. An information sheet outlining instructions on taking the study medication and concomitant

medications will be distributed to participants and discussed in detail by study personnel. They will be asked to inform any physician who is prescribing new medications that they are currently taking minocycline. In addition, the participants will be asked to inform a study team member if a new medication is prescribed to them.

Participant compliance with IP will be prompted through ongoing encouragements and reminders during study visits and scheduled and unscheduled telephone contacts. Compliance will be assessed by pill counts conducted during study visits and the participant's pill diary. Once identified, occurrences in which the computed compliance rate between study visits falls below 50% will be reported as a protocol deviation to the CNS IRB within two weeks using PTMS.

4.5.1 Treatment Prior to Month 3

At baseline, the participant will receive an injection of 1.25mg bevacizumab, an anti-VEGF agent, and also start the randomized IP. At Months 1 and 2, the participant will again receive bevacizumab injections in the study eye and continue the randomized IP.

4.5.2 Treatment Beginning at Month 3

Visual acuity and macular edema will be evaluated at study visits to determine further treatment. Starting at Month 3 and at every visit thereafter, the following scenarios will be considered. (Also refer to Figure 1.) The participant will be reinjected with bevacizumab unless the participant meets the "improvement," "worsening," "new steady state" or "no improvement" criteria defined in Appendix 3.

Scenario 1: If the participant meets the "improvement" criteria defined in Appendix 3, the participant will continue to take the IP, but the bevacizumab injection will be withheld at that visit.

Scenario 2: If the participant meets the "worsening" criteria defined in Appendix 3, the participant will be offered any therapy available at the discretion of the treating investigator, including:

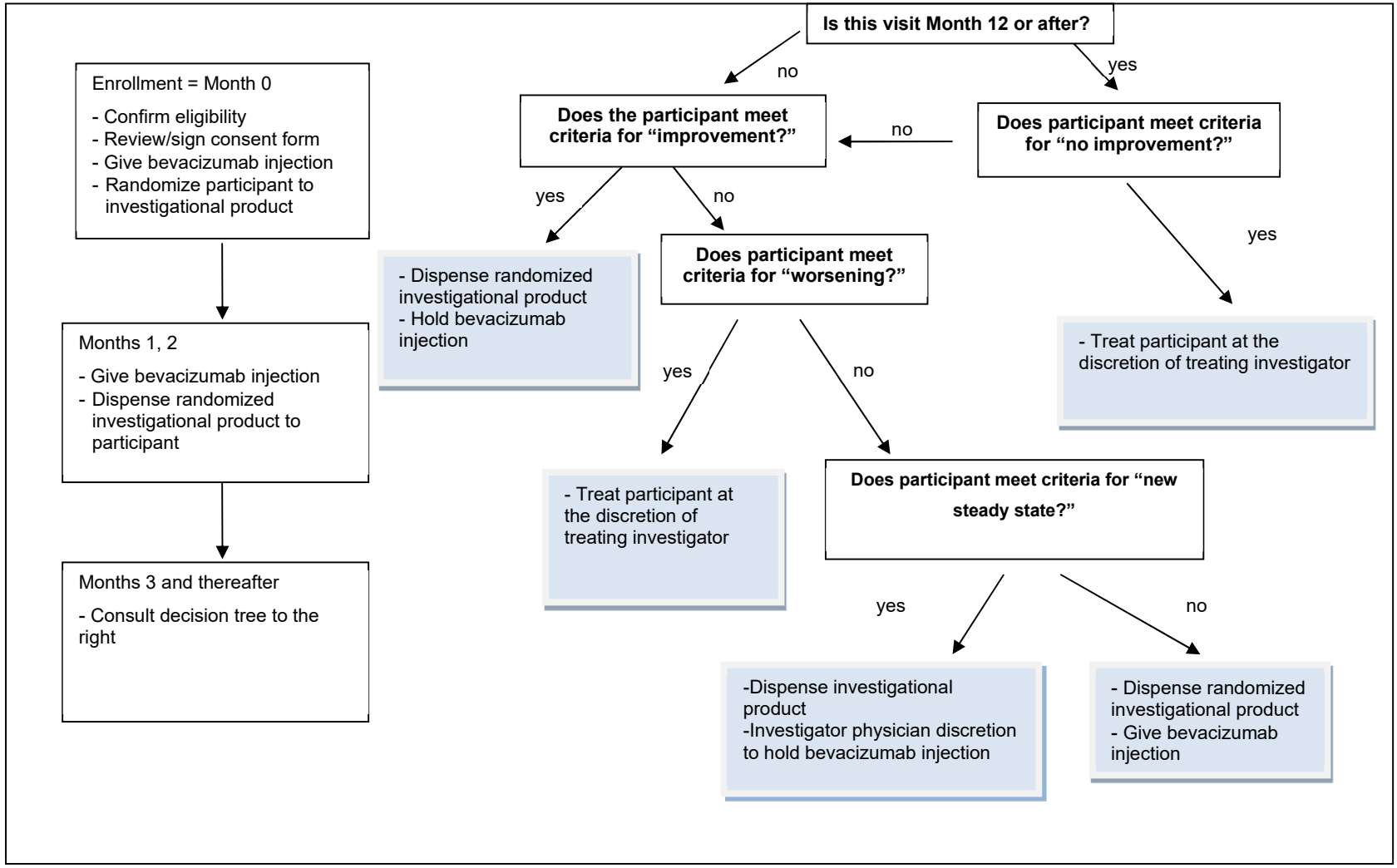
a. Anti-VEGF Treatment
b. Intravitreal or Periocular Steroid Injections

Scenario 3: If the participant does not meet the "improvement" or "worsening" criteria, but meets the "new steady state" criteria defined when the central subfield thickness remains ≥ 300 microns on OCT but has not changed > 25 microns over the last three consecutive injections (also defined in Appendix 3), the treating investigator physician will have the discretion to hold bevacizumab injections until the OCT changes (worsens) by > 25 microns from this "new steady state" level.

At the Month 12 visit and at every visit thereafter, Scenarios 1, 2 and 3 will be considered as well as the following scenario, which allows for a study physician to determine whether there has been "no improvement" in the participant's condition:

Scenario 4: If the participant meets the "no improvement" criteria defined in Appendix 3, or if the investigator's clinical impression is that there has been no improvement in the study eye, the participant will be offered therapy at the discretion of the treating investigator. The decision to re-inject the participant with bevacizumab (the anti-VEGF agent) will be made by the treating investigator after consulting with the participant. The decision to continue the IP will also be made by the treating investigator after consulting with the participant.

Figure 1. Treatment Scenarios



4.6 Ocular and Systemic Evaluations

Following is a detailed list of the study procedures. All of the study procedures, with the exception of the administration of the IP, are typical components of the clinical care required for a participant with macular edema secondary to CRVO. Examinations are performed at the study visits as indicated in the study flowsheet (Appendix 2).

1. Dispense IP and provide an information sheet outlining instructions on taking IP and concomitant medications and pill diary
2. Drug Accountability Review
3. Evaluation for "improvement," "worsening" and "no improvement" defined in Appendix 3
4. Medical/Ophthalmic History
5. Physical Examination
6. Vital Signs
7. Review of Systems
8. Allergies Assessment
9. Concomitant Medications Assessment
10. Adverse Event Assessment
11. Best-Corrected Visual Acuity (BCVA) and Manifest Refraction using Early Treatment Diabetic Retinopathy Study (ETDRS) Methods
12. Slit Lamp Examination
13. Dilated Fundus Examination
14. Color Fundus Photography (CFP)
15. Intraocular Pressure (IOP)
16. Spectral Domain Optical Coherence Tomography (OCT)
17. Microperimetry
18. Fluorescein Angiogram (FA)
19. Acute Care Panel[1]
20. Hepatic Panel[1]

21. Complete Blood Count (CBC)[1]
22. Thyroid Function Test[1,2]
23. Thyroid Palpation[3]
24. Pregnancy Test for Women of Childbearing Potential (serum and urine)[1, 4] (see Appendix 1 for definition)

[1] All abnormal values or positive tests will be reported to the participant's primary care physician, with the participant's permission.

[2] Participants with abnormal thyroid function test results will be referred to an endocrinologist.

[3] Abnormal findings during a thyroid palpation will be followed by a referral to an endocrinologist.

[4] Applicable to the BRC sites only: A serum pregnancy test will be performed with 24 hours prior to initiation of minocycline. After initiation of contraception, urine pregnancy tests will be conducted; however, a serum test will be performed anytime a urine test result creates doubt.

If any medications need to be initiated or altered to address any change in lab values, or symptoms, the participant's primary care doctor will be contacted, or the medical consultant team will be contacted to evaluate the participant and to prescribe the appropriate medications.

4.6.1 Reporting of Expected Events

It is anticipated that participants in this study will occasionally miss or fail to complete an assessment, procedure or study visit. These omissions will be considered expected events and not protocol deviations provided they are infrequent and do not include data needed to assess safety or the primary study outcome. If such an event is identified, the PI will submit a report to the IRB within two weeks using PTMS.

Cumulative proportions of these missed events in the study population will be presented to the IRB annually. In addition, these rates will be calculated and monitored biannually by the DSMC.

The following are considered deviations:

- An individual misses more than 15% of the required study assessments/procedures,
- An individual misses more than 15% of the required study visits,

- An individual completes more than 15% of the required study visits outside of the visit window,
- Collectively, participants missed more than 15% of a specific assessment/procedure,
- Collectively, participants missed more than 15% of the required study visits, or
- Collectively, participants complete more than 15% of the required visits outside the visit window.

If the total number of expected items (study visits or study assessments/procedures) is less than or equal to 16, and two or more items are missing, a deviation will be reported. If the total number of items expected is greater than 16, and 15% or more items are missing, a deviation will be reported.

The total number of items (study visits or study assessments/procedures) for an individual and collectively will be calculated cumulatively. If a protocol deviation (as defined above) is identified, the PI will submit a report to the IRB using PTMS.

4.7 Study and Concomitant Therapy

4.7.1 Minocycline Formulation at Pine Pharmaceuticals

Pine Pharmaceuticals (100 Colvin Woods Parkway #300, Tonawanda, New York) will supply the minocycline hydrochloride capsules for oral administration containing 100 mg of minocycline (NDC number 00591-5695-50, manufactured by Actavis Pharma, Inc.). Pine Pharmaceuticals will provide the pink opaque capsules to the NIH Pharmacy.

4.7.2 Minocycline Formulation for BRC Sites

At the BRC sites, minocycline hydrochloride capsules for oral administration containing the equivalent of 100 mg of minocycline will be investigated under European Clinical Trials Database (pending EudraCT submission to M.H.R.A.) For the BRC sites, the commercial licensed tablets will be overencapsulated by the BEH pharmacy.

4.7.3 Placebo Formulation at Pine Pharmaceuticals

Pine Pharmaceuticals will formulate and supply a placebo pill with inactive ingredients in a pink opaque capsule. The prepared capsules will be provided to the NIH Pharmacy. The placebo capsules are indistinguishable from the minocycline capsules used in this trial.

4.7.4 Placebo Formulation for BRC Sites

The BEH pharmacy has formulated a placebo capsule with inactive ingredients. The placebo capsules are indistinguishable from the minocycline capsules used in this trial.

4.7.5 Dosage, Administration and Storage

Participants will be instructed to take the IP orally two times a day, once in the morning and once in the evening approximately 12 hours apart. The exception to this will occur on the day of the baseline visit. On the day of the baseline visit at which IP is dispensed, participants will take only the evening pill. Minocycline will be administered at an oral dose of 100 mg twice daily. Placebo pills contain no active ingredients.

At the baseline and Months 1-23 study visits, a 37-day supply will be dispensed. Participants will be given a pill diary upon which to record the morning and evening doses of the IP. The IP should be stored at 15 – 30° C (59 - 86° F). It should be protected from light, moisture and excessive heat. Participants will be required to return their bottles of IP to each visit for pill counts for compliance monitoring.

4.7.6 Concomitant Therapy

If the study eye develops neovascularization warranting panretinal photocoagulation, the participant will withdraw from this study because it is known that panretinal photocoagulation laser can exacerbate macular edema. This could interfere with the interpretation of the study data (see exclusion criteria Section 3.2.2).

The non-study eye will receive standard care treatment, as appropriate.

4.8 Investigational Product Accountability

The pharmacy and/or unmasked site staff designated to receive IP are responsible for the accountability of all used and unused study IP. Adequate drug accountability records include documentation of all IP and supplies shipped, received, formulated, dispensed and returned to the pharmacy. Participants will be asked to complete a pill diary documenting that they took the IP. At each visit, participants will be responsible for the return of a completed pill diary and any unused study medication and supplies. The investigator/study staff will review the pill diary with the participant for completeness and accuracy, document the number of returned pills and return

the unused study medication and supplies to the appropriate pharmacy. Each pharmacy will destroy unused IP and supplies following reconciliation by the Coordinating Center.

4.9 Follow-up/Termination Procedures

At the conclusion of the study, the participants will no longer be able to receive the IP under this protocol.

At the NEI, follow-up care will be arranged with an outside ophthalmologist or the participant will continue to be seen at the NIH under another protocol, if available, and if the participant is eligible. The participants and their physicians, with written consent, will be informed of their disease status during this study. Clinical data obtained during participation may be shared with the participants and, with written permission from the participants, their private physicians. Results from the overall study will be listed on http://www.clinicaltrials.gov once the data have been analyzed.

4.10 Storage of Samples and Data

No samples will be stored for this study. At the NEI, the clinical data will be stored in the NEI's electronic medical record system (EMR), The Emmes Corporation's database and the Clinical Research Information System (CRIS). At the BRC sites, the clinical data will be stored in The Emmes Corporation's database. Some of the baseline data for the NEI may be obtained under the NEI screening or evaluation and treatment protocols.

5.0 RISKS/DISCOMFORTS

There are risks associated with the procedures required for participants in this study. However, these are all standard procedures that are performed as part of a normal eye and medical examination. Some of the discomforts associated with the ocular examination include the following:

1. Dilating drops or anesthetic drops may sting. They can cause an allergic reaction, or if contaminated, can cause an infection, but neither of these problems is very likely to occur. Dilating drops can also cause a sudden increase of pressure (acute glaucoma) in eyes that are already predisposed to develop this condition. There is little risk of glaucoma being triggered in this way, but if it is, treatment is available. The participant's intraocular pressure will be obtained at each ophthalmic examination to determine whether there is an increased risk of developing glaucoma.

2. Color fundus photographs involve a bright flash to take pictures of the retina. This brief flash may cause temporary discomfort, but it does not damage the eye.
3. In rare instances, the cornea may be scratched during measurement of intraocular pressure or use of a funduscopic contact lens. A corneal abrasion of this sort may be painful, but it heals quickly with no lasting effects.
4. The fluorescein dye used in fluorescein angiography can make a participant's skin turn yellow for several hours. This yellow color is transient and usually disappears in one day. Because the dye undergoes renal excretion, the participant's urine will turn dark orange for up to 24 hours after the examination. The study team will educate the participant regarding this urine color change. Some participants may be slightly nauseated during the examination, but their nausea usually lasts only a few seconds. If the dye extravasates during the injection, the skin around the injection site may feel mildly uncomfortable or become yellow. The discomfort usually lasts only a few minutes, and the yellow color fades in a few days. There is a chance of ecchymosis at the site of injection and a remote possibility of cellulitis from the needle track.

   In rare cases, participants may have an allergic reaction to the dye. Treatment typically consists of an oral antihistamine medication, but may require intravenous antihistamine administration if the symptoms are severe. Very rarely (less than one in one million people), a participant experiences anaphylaxis. This would be treated immediately by trained personnel with medications or, if necessary, intubation.
5. OCT and microperimetry are non-invasive tests used to document and analyze retinal pathology. Neither test has any known medical risks.

Possible discomforts associated with non-ocular examinations include:

1. Blood draws can cause discomfort and bleeding/bruising at the site of venous puncture. There is a remote risk of fainting or local infection. If any of these conditions arise, they will be treated.
2. The medical/ophthalmic history, vital signs, review of systems and pregnancy testing entail no medical risk.

5.1 Intravitreal Bevacizumab Risks

The following data on the complications of intravitreal injections of bevacizumab were taken from a publication on the subject.[43] The following event rates were observed as described in Table 1. There is no statistically significant difference in event rates between bevacizumab and ranibizumab in any of these categories listed below. Risks related to the mechanical injection include endophthalmitis, retinal tears, vitreous hemorrhage, intraocular pressure elevation and lens damage. Risks related to the pharmacological agent include uveitis and endophthalmitis.

Table 1. Comparison of Ocular Adverse Events Occurred Among Patients Treated with Intravitreal Bevacizumab versus Ranibizumab

Ocular Adverse Event	Bevacizumab (n = 1,275) (%)	Ranibizumab (n = 725) (%)	*P*
Retinal detachment	1 (0.08)	0	1.0
Injection-site redness	821 (64.39)	474 (65.38)	>0.6
Corneal abrasion	6 (0.47)	3 (0.41)	1.0
Anterior uveitis	20 (1.57)	10 (1.38)	>0.6
Posterior or anterior-posterior uveitis	5 (0.39)	3 (0.41)	1.0
Subconjuctival hemorrhage	125 (9.80)	75 (10.35)	>0.6
Subconjuctival hemorrhage (> 1 quadrant)	6 (0.47)	5 (0.69)	>0.5

5.2 Investigational Product Risks

5.2.1 Minocycline-related

Due to oral minocycline's virtually complete absorption, side effects to the lower bowel, particularly diarrhea, have been infrequent. The following adverse reactions have been observed in patients receiving tetracyclines.

Gastrointestinal: Anorexia, nausea, vomiting, diarrhea, glossitis, dysphagia, enterocolitis, pancreatitis, inflammatory lesions (with monilial over growth) in anogenital region and increases in liver enzymes. Rarely, hepatitis and liver failure have been reported. Rare instances of esophagitis and esophageal ulcerations have been reported in patients taking the tetracycline-class antibiotics in capsule and tablet form. Most of these patients took the medication immediately before going to bed.

Skin: Maculopapular and erythematous rashes. Exfoliative dermatitis has been reported but is uncommon. Fixed drug eruptions, including balanitis, have been rarely reported. Erythema multiforme and rarely Stevens-Johnson syndrome have been reported. Photosensitivity manifested by an exaggerated sunburn reaction has been observed in some individuals taking tetracyclines. This has been reported with minocycline. Pigmentation of the skin and mucous membranes has been reported.

Renal toxicity: Elevations in BUN have been reported and are apparently dose related. While this is not a problem in those with normal renal function, in patients with significantly impaired function, higher serum levels of tetracycline may lead to azotemia, hyperphosphatemia and acidosis. If renal impairment exists, even usual oral or parenteral doses may lead to excessive systemic accumulations of the drug and possible liver toxicity.

Hypersensitivity reactions: Urticaria, angioneurotic edema, polyarthralgia, anaphylaxis, anaphylactoid purpura, pericarditis, exacerbation of systemic lupus erythematosus and rarely pulmonary infiltrates with eosinophilia have been reported. A transient lupus-like syndrome has also been reported.

Blood: Hemolytic anemia, thrombocytopenia, neutropenia and eosinophilia have been reported.

Central Nervous System: Central nervous side effects including light-headedness, dizziness, or vertigo have been reported with minocycline therapy. Serious neurologic events reported include bulging fontanels in infants and benign intracranial hypertension (Pseudotumor cerebri) in adults. Headache and blurred vision have also been reported.

Other: When given over prolonged periods, tetracyclines have been reported to produce brown-black microscopic discoloration of the thyroid glands. Very rare cases of abnormal thyroid function have been reported. Thyroid cancer has been reported in the post-marketing setting in association with minocycline products. Decreased hearing has been rarely reported in patients on minocycline hydrochloride. Tooth discoloration in children less than eight years of age and also, rarely, in adults has been reported. Use is not recommended for individuals of either gender attempting child conception. There is a risk of impaired spermatogenesis. Because tetracycline can reduce the amount of beneficial forms of bacteria in the body, participants will be counseled to include probiotics in their diet while participating in the study.

Pregnant and Nursing Women: Minocycline is in Pregnancy Category D and is excreted in human milk. Minocycline can cause fetal harm when administered to a pregnant woman. The use of drugs of the tetracycline class during tooth development (last half of pregnancy, infancy and childhood to the age of eight years) may cause permanent discoloration of the teeth (yellow-grey-brown).

5.2.2 Drug Interaction-related

Because tetracyclines have been shown to depress plasma prothrombin activity, participants who are on anticoagulant therapy may require downward adjustment of their anticoagulant dosage. Since bacteriostatic drugs may interfere with the bactericidal action of penicillin, it is advisable to avoid giving tetracycline-class drugs in conjunction with penicillin. Absorption of tetracyclines is impaired by antacids containing aluminum, calcium, or magnesium, and preparations that contain iron. The concurrent use of tetracycline and methoxyflurane has been reported to result in fatal renal toxicity. Concurrent use of tetracyclines with oral contraceptives may render oral contraceptives less effective.

5.2.3 Placebo-related

There are no medical risks associated with the placebo capsules.

6.0 Participant Monitoring

Participants will be monitored during all study visits for AEs by the study investigators. At each visit, the participant will be asked about any new ocular or systemic symptoms including rashes, hospitalizations or new/changed medications. General and ophthalmic assessments will be performed according to Appendix 2. Stopping guidelines for individual participants and the entire study are outlined below.

6.1 Individual Withdrawal Criteria

Participants may choose to withdraw from this study for any reason at any time without penalty, without loss of benefits, and without prohibition from enrolling in other clinical protocols. Participants may stop the study at any time. The investigators will ask all study participants to continue to return for study visits even if they stop using the IP. However, participants are free to withdraw from the study at any time. Also, investigators may withdraw a participant from IP if the investigator determines that the study is not in the participant's best interest.

Reasons for participant discontinuation may include, but are not limited to, the following:

- Investigator determination that it is not in the best medical interest of the participant to continue participation;
- Findings during the course of the trial that may affect willingness to participate;
- Participant requires additional medicines or intravitreal injections that may interfere with the IP;
- Serious suspected adverse reaction;
- VA loss of ≥ 15 letters from baseline;
- Any other safety concerns;
- Inability to keep study visits or to comply with study requirements; or
- Participant requires ocular surgery which cannot safely be postponed until the end of the study.

Discontinuation of IP does not require participant withdrawal from the study. If the participant agrees to continue in the study after discontinuing IP, s/he will continue to return for his/her subsequent study visits and undergo the required examinations. Otherwise, the participant will exit the study and continue his/her ophthalmic care either with an outside ophthalmologist or at the NEI. Participants will be monitored by study investigators and clinical staff at each visit to the clinic.

6.2 Pregnancy Monitoring

If the staff becomes aware that a female study participant has become pregnant during the study, the investigator will advise the participant to stop taking the IP immediately. The investigator and participant will determine whether to continue any remaining study visits or to exit the study.

If the staff becomes aware that a male study participant has impregnated his partner during the study, the investigator will remind the participant of the potential risks to the unborn fetus.

For the NEI site, in either case of reported pregnancy, the NEI participant (and/or partner) will be referred to the NIH OB/GYN consultation service for evaluation and counseling. The investigator must follow the participant (or partner) until the pregnancy outcome. The data must be recorded on the *Confirmed Pregnancy & Outcome* case report form, which is also reported to the Coordinating Center.

7.0 OUTCOME MEASURES

7.1 Primary Outcome

The primary outcome is the difference in mean change of BCVA, as measured in ETDRS letters, between the minocycline and placebo groups in the study eye at 12 months compared to baseline.

7.2 Secondary Outcomes

Secondary outcomes include the difference between the minocycline and placebo groups in the:

- Number of bevacizumab injections from baseline to 12 months and from baseline to 24 months,
- Changes in mean macular sensitivity as measured by microperimetry at 3, 6, 12, 18 and 24 months compared to baseline,
- Mean change in the ETDRS BCVA in the study eye at 24 months compared to baseline,
- Changes in retinal thickness as measured by OCT at 6, 12, 18 and 24 months compared to baseline,
- Number of participants improving ≥ 1 logOCT scale step at 12 and 24 months compared to baseline,
- Changes in fluid leakage in the macula as demonstrated by fluorescein angiography at 12 and 24 months compared to baseline.

Also, a decrease of ≥ 1-step on the logOCT scale, where Change in logOCT = log (follow-up thickness/300) – log(baseline thickness/300) is considered clinically significant.[42] A one-step decrease is equivalent to at least a 20% improvement of central macular thickness and represents greater than twice the variability of retinal thickness measurements (approximately 25-30 microns). Table 2 presents examples of OCT measurements with their corresponding LogScore, where LogScore = 10xlogOCT.

Table 2. OCT and Corresponding LogScores[42]

LogScore	OCT (µm)
0	300
0.5	337
1	378
1.5	424
2	475
2.5	533
3	599
3.5	672
4	754
4.5	846
5	949
5.5	1064
6	1194
6.5	1340

7.3 Safety Outcomes

Safety outcomes will be the number and severity of systemic and ocular toxicities and AEs. The number of participants withdrawn from the study therapy due to vision loss or AEs and the number of participants deemed to have worsening disease will also contribute to the assessment of safety.

8.0 STATISTICAL ANALYSIS

As this is a pilot study, analyses will be primarily descriptive and by eye. Additionally, all exploratory analyses carried out may be stratified by site. No formal sample size calculation has been performed. The accrual ceiling for this study is 20 participants (10 per group) with a minimum of 10 participants (five per group). This number was selected to obtain at least five participants in each group reaching the final study visit in order to obtain preliminary data to potentially support a larger trial if promising results are revealed.

BCVA will be evaluated at each scheduled visit, and the primary outcome is defined as the difference between groups (those randomized to placebo and those randomized to minocycline) in mean change in BCVA in the study eye at 12 months compared to baseline. This study will be unable to determine whether there is a statistically significant difference in mean change in BCVA between placebo and minoycline groups. Difference in mean change (± standard deviation) in BCVA across the two groups will be presented, and interval estimates of mean change in BCVA will also be constructed. Exploratory nonparametric statistical analyses will be conducted where appropriate. Mean (per group) and individual BCVA will be plotted against time.

The number of bevacizumab injections required by participants will be assessed as a secondary outcome. The number of bevacizumab injections will be summarized for each participant from baseline to 12 months and from baseline to 24 months in each group (those randomized to minocycline versus placebo) and the means will be compared.

Changes in mean macular sensitivity as measured by microperimetry will also be assessed as a secondary outcome at months 3, 6, 12, 18 and 24 as compared to baseline. A number of recent publications have demonstrated the usefulness of microperimetry in assessing macular function in vein occlusions.[44-55] In addition, some of these studies have found that retinal sensitivity as measured by microperimetry correlates more precisely with macular edema thickness measurements than with visual acuity measurements.[51-52]

Additional secondary outcome measures include changes in retinal thickness in the study eye as measured by OCT. Individual and mean changes in retinal thickness will be summarized and plotted against time. Other secondary outcomes include change in BCVA in the study eye at 12 and 24 months compared with baseline, proportion of participants improving ≥ 1 logOCT scale step, and change in fluid leakage in the macula of the study eye as demonstrated by fluorescein angiography at 12 and 24 months compared to baseline. In general, changes in BCVA, changes in retinal thickness, and changes in fluid leakage in the macula will be monitored throughout the study period in both the study and fellow eyes. Analyses pertaining to these outcomes will be descriptive and will include tabulations with point and interval estimates and plots of outcome values over the study period for all participants. Exploratory nonparametric statistical analyses will be conducted where appropriate.

Participants who were exposed to recent (< three months) systemic steroid or systemic anti-VEGF prior to enrolling will also be analyzed separately to assess for any possible confounding effects for treatment benefit. Safety outcomes, including the number and severity of systemic and ocular toxicities and AEs will be summarized by severity, type and assessed relatedness to the IP throughout the study period. The number of participants withdrawn from the IP due to vision loss or AEs and the number of participants deemed to have worsening disease will also be summarized. Worsening disease is defined as loss of 15 or more ETDRS letters of vision compared to baseline OR a ≥ 1-step increase in the logOCT scale. These safety analyses will use data of all participants who were exposed to IP regardless of adherence to the protocol.

Participants' compliance with IP administration will be assessed through capsule counts during study visits. If study IP compliance falls below 80% of the level prescribed for any participant, the participant will be queried by the site about the reasons for decreased compliance and counseled on the importance of compliance for study integrity. The level of compliance will be reported to the IRB on annual review and taken into account in the analyses of study outcomes. When IP compliance cannot be determined for a participant during any visit due to missing capsule counts, IP compliance will be imputed by taking the median of all available capsule counts recorded at previous study visits.

9.0 HUMAN PARTICIPANTS PROTECTION

9.1 Equitability

Accrual for this study will be equitable among participants with RVOs meeting the enrollment criteria. All participants will have received the standard care therapy prior to enrollment.

9.1.1 Justification for Exclusion of Children

Children are not eligible for this study as minocycline has not been fully studied in children and is not indicated for use in children.

9.1.2 Justification for Exclusion of Pregnant or Lactating Women

Pregnant or lactating women are not eligible for this study because of the known and unknown risks of minocycline. One of the known risks of minocycline is that it can cause permanent discoloration of the teeth (yellow-grey-brown) of a fetus when given to a pregnant woman during the last half of pregnancy. Minocycline also passes into breast milk and can cause permanent discoloration of the teeth of a nursing infant. Given the known and unknown effects on pregnancy outcomes, two forms (one form for BRC sites) of contraception will be required for female participants of childbearing potential (see Appendix 1 for definition). In addition, female participants of childbearing potential will undergo urine pregnancy tests throughout the study.

9.2 Qualifications of Investigators

The following physicians who will be treating the participants are experienced in caring for patients with RVOs. In addition, they are experienced in conducting studies similar to this protocol. Credentialed NEI staff physicians may also conduct procedures under the direction of these investigators.

The NEI PI has verified that all individuals working on this protocol at the NEI site required to take HRPP training under OHSRP SOP 25 (Training requirements for the NIH Human Research Protections Program) have completed all required training.

The BRC site PIs have verified that all individuals working on this protocol at their site are sufficiently trained to complete the tasks delegated to them on the study Authorized Staff Signature log.

Catherine Cukras, MD, PhD is the PI with the ability to obtain informed consent. She is responsible for obtaining medical history, performing scheduled ophthalmic study visits, dispensing/administering medication, maintaining regulatory documentation, submitting IRB materials, generating clinical source documents, resolving data queries and overseeing the protocol. Dr. Cukras is currently a staff clinician at NEI. She completed the medical retina clinical fellowship at NEI and has mentored medical students in the NEI retina clinic. Dr. Cukras currently serves as the PI on a protocol investigating minocycline for the treatment of macular edema. She has also served as an Associate Investigator on other clinical research studies on related ocular diseases.

Clare Bailey, MD is the PI for the Bristol Eye Hospital with the ability to obtain informed consent at the UK sites. She is responsible for obtaining medical history, performing scheduled ophthalmic study visits, administering and dispensing medication, completing regulatory documentation and regulatory submissions, generating clinical source documents, resolving data queries and the overall oversight of the protocol for the BEH site. Dr. Bailey is a consultant ophthalmologist in medical and surgical retina at the BEH and a member of the Royal College of Ophthalmologists.

Bishwanath Pal, MBBS, MS, Ophth, FRCOphth, FRCSEdin, is the PI for Moorfields Eye Hospital with the ability to obtain informed consent from patients at the MEH site. He is responsible for obtaining medical history, performing scheduled ophthalmic study visits, administering and dispensing medication, completing, generating clinical source documents, resolving data queries and the overall oversight of the protocol for the Moorfields site. Dr. Pal is a consultant ophthalmologist in medical retina at Moorfields Eye Hospital and a member of the Royal College of Ophthalmologists.

Wai Wong, MD, PhD is the Lead Associate Investigator with the ability to obtain informed consent. He is responsible for obtaining medical history, performing scheduled ophthalmic study visits, dispensing/administering medication and generating clinical source documents. Dr. Wong is a staff clinician and the head of the unit of neuron-glial interactions in retinal diseases at NEI. Dr. Wong currently serves as an Associate Investigator on another protocol for the treatment of macular edema targeting microglia. Dr. Wong has clinical experience and conducted research studies on related ocular diseases as a Principal and Associate Investigator.

Emily Chew, MD is an Associate Investigator and Accountable Investigator with the ability to obtain informed consent. She is responsible for obtaining medical history, performing scheduled ophthalmic study visits, dispensing/administering medication and generating clinical source documents. Dr. Chew is the deputy clinical director (CD) and director of the medical retina fellowship program at NEI. Dr. Chew is on the editorial board of a diabetic journal and serves on several committees focused on the care of patients with diabetes. Dr. Chew has conducted numerous research studies as a Principal and Associate Investigator on related ocular diseases.

Henry Wiley, MD is an Associate Investigator with the ability to obtain informed consent. He is responsible for obtaining medical history, performing scheduled ophthalmic study visits, dispensing/administering medication and generating clinical source documents. Dr. Wiley has clinical experience and has conducted research studies as a Principal and Associate Investigator on related ocular diseases.

Alexander Kaplan, MD, Christopher Hwang, MD and Munir Iqbal, MD, FRCSC are Associate Investigators with the ability to obtain informed consent. They are responsible for obtaining medical history, performing scheduled ophthalmic study visits, dispensing/administering medication and generating clinical source documents. They are currently clinical fellows at the NEI.

Angela Kibiy, MPH, BSN, RN is an Associate Investigator and the Research Contact without the ability to obtain informed consent. She is responsible for providing assistance to the PI with study procedures, generating clinical source documents, entering data and resolving data queries. Ms. Kibiy has been involved in clinical research studies on ocular diseases as an Associate Investigator and primary study coordinator/research contact.

Awilda V. Holland, DNP, MSHSc, RN, CCRP is an Associate Investigator and back-up coordinator without the ability to obtain informed consent. She is responsible for providing assistance to the PI with study procedures, generating clinical source documents, entering data and resolving data queries. Dr. Holland is the NEI Clinical Trials Coordinating Section Chief and has been involved in clinical research studies on ocular diseases.

Prior to screening or enrolling potential participants, the PI will verify all individuals working on this protocol have met the following training and certification requirements as applicable to their indicated role:

9.2.1 Professional Licensure

Physicians must provide evidence of current medical licensure applicable to the study location(s) if they are practicing medicine and undertake to diagnose and/or treat participants (including administration of the IP) in this study. A physician who is a site PI must also provide satisfactory evidence of ophthalmology training before study initiation.

10.0 ANTICIPATED BENEFITS

During this study, all participants will be eligible to receive intravitreal bevacizumab (Avastin®), an anti-VEGF agent. Additionally, participants who are randomized to minocycline may benefit directly from this study, as we hypothesize that minocycline may result in resolution or reduction of macular edema or improved visual acuity or need for fewer intravitreal bevacizumab injections. The study will also lead to generalizable knowledge regarding macular edema associated with CRVO.

11.0 CLASSIFICATION OF RISK

Risk is classified as more than minimal risk. The risks will be minimized with careful monitoring and are reasonable given that the participants are experiencing visual loss due to CRVO.

12.0 CONSENT DOCUMENTS AND PROCESS

Applicable study investigators with consenting privileges will obtain informed consent. All NIH study investigators obtaining informed consent have completed the National Institute of Mental Health (NIMH) Human Subject's Protection Unit (HSPU) "Elements of Successful Informed Consent" training. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study. The participants must have the ability to understand and sign an informed consent form, which must be signed prior to enrollment. The participants will have an opportunity to carefully review the consent and ask questions regarding this study prior to signing and they will be informed that they may withdraw from the study at any time without penalty to themselves or benefits lost.

If the participant requires the consent to be in larger font in order to read it well, this will be provided. If participants are visually impaired to the point of being unable to read the consent, they can take the consent back with them to read it over with a family member or with the use of magnifying devices. If the participant chooses, the investigator can also read the consent verbatim to the participant and answer any questions that may arise.

An investigator present during the consent process will document the consent process in the participant's medical record. A signed copy of the informed consent form will be provided to the participant to take home.

12.1 Unanticipated Enrollment of Non-English Speaking Participants at the NEI

If a non-English speaking participant is eligible for enrollment at the NIH Clinical Center (CC), the participant will be provided with the *CC Short Written Consent Form for Non-English Speaking Research Subjects* in the participant's native language and a verbal explanation of the purpose, procedures and risks of the study. The IRB-approved English consent form will serve as basis for the verbal explanation of the study. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is not related to the participant (i.e., not a family member). Interpreters provided by the CC will be used whenever possible. The interpreters will interpret the IRB-approved English consent form and facilitate discussion between the participant and investigator.

The IRB-approved English consent form will be signed by the investigator obtaining consent and a witness to the oral presentation. The CC Short Written Consent Form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent document as the witness and, in this case, will note "Interpreter" with his/her signature. If not also serving as the witness, the interpreter will be separately identified on the short form in the space provided. A copy of both signed forms (the long form serving as the script and the short form) will be provided to the participant. The short form process, including the use of an interpreter, will be documented in the medical record in accordance with NIH policy.

All instances of use of the CC Short Written Consent Form will be reported to the IRB at the time of annual review. If the CC Short Written Consent Form is used three times or more for the same language within an IRB approval period, this will be reported to the IRB immediately.

Interpreters will also be present for other protocol procedures as necessary.

13.0 DATA AND SAFETY MONITORING

The NEI Data and Safety Monitoring Committee (DSMC) is responsible for monitoring data and safety, and will exercise oversight of the clinical investigation independently from the study investigators.

13.1 Coordinating Center

The Emmes Corporation has been assigned as the coordinating center for this trial to conduct data collection, protocol monitoring, data analysis and reporting. The coordinating center provides routine monitoring of study participants' data. Monitoring visits will occur on a schedule depending on the status of the study. More frequent monitoring visits will be performed at the beginning of the study when enrollment is open. Monitoring will decrease as enrollment closes and as participant follow-up continues. The protocol monitor will monitor documentation for the informed consent process. In addition, the protocol monitor will review source documentation for the data collected throughout the trial to ensure that the data points are collected and reported.

Communications between the investigational site and the Coordinating Center will be maintained through regular telephone and e-mail contacts between the Coordinating Center Protocol Monitor and the site PI and/or Coordinator. Substantive communications must be maintained both at the site and at the Coordinating Center containing the date and a summary of communications where instructions are given or received, an interpretation of protocol requirements is made, recommendations for corrections to study documentation are made, or where the reporting of possible AEs is discussed.

Although the Coordinating Center advises the NEI CD and PI on data and statistical activities, the Coordinating Center staff does not have direct access to or interaction with participants.

13.2 Data and Safety Monitoring Committee

The NEI DSMC is responsible for reviewing and approving the study design and, as appropriate, recommending design changes. In addition, the DSMC assesses study data with particular consideration of participant safety. The DSMC will convene prior to the initiation of the trial to review the protocol. Emmes will provide accumulated data from all study sites to the NEI DSMC for review. The Committee will review accumulated data on a biannual basis, but will convene ad hoc meetings to address any significant problems related to participant safety brought to its attention by any study participant or investigator. The Committee will review the accumulated data and consider whether a protocol modification is necessary and approve protocol modifications. If changes in protocol are indicated, recommendations will be made to the NEI Director and Clinical Center Director who will consider and act on such recommendations in a timely manner.

All protocol amendments must be reviewed and approved by the NEI DSMC prior to submission to the IRB.

13.3 Criteria for Stopping the Study

The DSMC may recommend temporarily suspending or closing enrollment, or stopping the study at any time due to safety concerns, demonstration of efficacy or lack of efficacy, or slow recruitment. Criteria for stopping the study include the following:

- A sufficiently large number of dropouts occurs as to make the trial likely to be uninformative;
- A participant experiences a drop in BCVA in the study eye of ≥ 30 letters from baseline attributed to the IP;
- A participant experiences a serious adverse event (SAE), drug reaction or complication, whether attributed to the IP or not, which has an impact on visual function or any other body system or precludes continuation of the IP. This would include the development of hypersensitization, allergic responses or other potentially serious drug reactions to medications required by the protocol.

Following premature IP discontinuation, not due to an AE, participants will continue to be followed as per the protocol.

14.0 QUALITY ASSURANCE

The NEI and Emmes maintain quality control by adhering to standard operating procedures (NEI QA program and NEIS standard operating procedures). These procedures cover the full protocol cycle beginning with staff credentialing and training, and protocol development and approval, through database development, data collection, monitoring and analysis, and finally manuscript preparation at the conclusion of the study. Data quality assurance is of the utmost importance to the NEI and Emmes. The two groups use a quality assurance system that relies on real-time data checks and reports throughout the course of a study to ensure the accuracy of information. This system is a secure and confidential data management system that stores data and provides quality assurance and reporting. Emmes has developed a number of routine reports specifically designed for monitors (e.g., listings of SAEs, etc.).

Additionally, Emmes has developed summary reports of discrepancies, as well as reports of the exceptions databases, which include requests and reasons for exceptions. The results of the reports are communicated back to site staff, and, along with protocol compliance issues, to the DSMC (if applicable).

Following the monitoring plan for this study, Emmes will perform monitoring activities, including on-site audits, review of database entries and the resolution of study issues. In addition to monitoring, Emmes performs various detailed automated and manual data quality checks. The results from these checks and any protocol compliance issues are communicated back to site staff and to the NEI Project Officer, NEI CD and applicable regulatory bodies.

15.0 REPORTING OF UNANTICIPATED PROBLEMS, ADVERSE EVENTS AND PROTOCOL DEVIATIONS

The PI is responsible for detecting, documenting, and reporting unanticipated problems, AEs, including SAEs, and deviations in accordance with NIH policy, IRB requirements, and federal regulations. For the BRC sites, site PI is responsible for detecting, documenting, and reporting unanticipated problems, AEs, including SAEs, and deviations, occurring at their site. Relatedness to the research of all SAEs will be determined by the PI in consultation with the CD. Additionally, both the Sponsor and Investigator will report AEs (SAEs/SUSARs) from the sites in accordance with the European Directive 2001/20/EC. SAEs must be reported to the Sponsor by the Investigators within 24 hours of awareness. Non-SAEs that are expected as part of the natural history of the underlying disease will be summarized at the time of annual review to the IRB.

Serious unanticipated problems, SAEs (including deaths) that are not unanticipated problems, and serious protocol deviations at the NIH will be reported to the CNS IRB and CD as soon as possible and in writing not more than seven days after the PI first learns of the event, unless immediate reporting is waived for specific SAEs as noted below. Not serious unanticipated problems and not serious deviations at the NIH will be reported to the CNS IRB and CD as soon as possible and in writing on the NIH Problem Report Form not more than 14 days after the PI first learns of the event. Written reports will be submitted in PTMS. For the BRC sites, the site PI is responsible for reporting events, including SAEs and protocol deviations to Emmes and the Sponsor, and then further reporting to regulatory authorities if reporting requirements are met.

All unanticipated problems, SAEs, and protocol deviations at the BRC sites will be monitored and reported per local regulations. These events will be reported to the NEI DSMC and unanticipated problems will be reported to the CNS IRB in the timeframes specified above.

All AEs, deviations, and unanticipated problems will be summarized and reported to the CNS IRB at the time of Continuing Review.

16.0 ALTERNATIVE THERAPIES

Alternatives to participation include further repeat treatment with intraocular injections of anti-VEGF agents such as bevacizumab (Avastin®) or ranibizumab (Lucentis®) without randomization to minocycline or placebo. Ranibizumab (Lucentis®) recently has been demonstrated to improve vision and edema, but the durability of the injections remains unknown. Other alternatives include the use of periocular and intraocular corticosteroids. Another approach would be observation. Other therapies have been administered with varying rates of success. All of these options are associated with significant side effects and complications if the treatment is extended and increased. Participants can alternatively receive minocycline outside of the study if prescribed off-label by their own physician.

17.0 PRIVACY

All research activities will be conducted in as private a setting as possible.

18.0 CONFIDENTIALITY

No blood, tissue or other samples will be stored in this study. All medical records will be kept confidential and will only be reviewed by the participating investigators. Data will be kept in password-protected computers held at the NEI and the Coordinating Center. Only study investigators and authorized Coordinating Center staff will have access to the study data. The participants' names will not appear on any of the data forms reported to the Coordinating Center. A unique identifier will identify the participant if their information is shared with the Coordinating Center for research purposes. The participants' names will not appear in any publication of the study results. Participants' personal information will be kept as private as possible. However, records can be inspected by organizations for quality assurance and data analysis. Potential inspectors include the members of the FDA, IRB, DSMC and the Coordinating Center.

19.0 CONFLICT OF INTEREST

The NIH guidelines were distributed to all the investigators, and none of the investigators had any conflicts of interest.

20.0 TECHNOLOGY TRANSFER

There are no technology transfer agreements for this study.

21.0 RESEARCH AND TRAVEL COMPENSATION

For this study, there is no compensation for participation. This protocol does not include reimbursement for travel and subsistence. Participants needing financial assistance will be able to receive supplemental reimbursement based upon need. Requests for supplemental reimbursement will be evaluated on a case-by-case basis for valid financial and/or medical need through a standardized process.

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APPENDIX 1: DETERMINING CHILDBEARING POTENTIAL

A female participant who is considered non-childbearing due to a medical condition (i.e., participant has previously undergone a hysterectomy) does not need a pregnancy test, Follicle-stimulating Hormone (FSH) test or contraception.

If a female participant is considered non-childbearing due to menopause, it must be in accordance with the CNS IRB/NIH Ob-Gyn guidance on the definition of menopause. This guidance defines menopause as:

- Women over age 55 who have not had a period for one year will be considered menopausal and do not need a pregnancy test, FSH test or contraception.
- Women between 50 and 55, who have not had a period for one year, should have an FSH test. If their FSH level is ≥ 20 mIU/mL, they will be considered menopausal and do not need pregnancy testing or contraception. If their FSH level is < 20 mIU/mL, they will need pregnancy testing and contraception as required by the protocol.
- Women between 45 and 50 who have not had a period for one year will need both an FSH test and a pregnancy test. If they are not pregnant and their FSH level is ≥ 20 mIU/mL, they will be considered menopausal and will not require contraception or additional pregnancy testing. If their FSH test is < 20 mIU/mL, they will need pregnancy testing and contraception as required by the protocol.

APPENDIX 2: STUDY FLOWSHEET

Study Visit Number* (Month)	0	1	2	3-11	12	13-23	24	SFTY[13]
Visit Number	00	01	02	03-11	12	13-23	24	SFTY
Target Day from Baseline Visit	000	030	060	090-334	365	395-699	730	735
Treatment[14]								
Dispense Investigational Product[1] (taken twice daily[2])	X	X	X	X[3]	X[3]	X[3]		
Drug Accountability Review		X	X	X	X	X	X	
Bevacizumab Injection	X	X	X	X[3]	X[3]	X[3]		
General Assessments[14]								
Medical/Ophthalmic History	X							
Vital Signs	X							
Physical Examination	X[6]							
Thyroid Palpation[4]	X	X	X	X	X	X	X	
Review of Systems	X	X	X	X	X	X	X	
Allergies Assessment	X							
Concomitant Medications Assessment	X	X	X	X	X	X	X	X
Adverse Event Assessment		X	X	X	X	X	X	X
Ophthalmic Assessments[14]								
BCVA (ETDRS)	X	X	X	X	X	X	X	X
Manifest Refraction[5]	X				X		X	
Slit Lamp Examination	X	X	X	X	X	X	X	X
Dilated Fundus Examination	X	X	X	X	X	X	X	
Intraocular Pressure (IOP)	X	X	X	X	X	X	X	X
Color Fundus Photography (CFP)	X[6]				X		X	
Spectral Domain Optical Coherence Tomography (OCT)	X[6]	X	X	X	X	X	X	X
Microperimetry	X[6]			X[7]	X	X[7]	X	
Fluorescein Angiogram (FA)	X[6]				X		X	
Laboratory Assessments[8, 14]								
Acute Care Panel	X[9]		X	X[10]		X[10]	X	X
Hepatic Panel	X[9]		X	X[10]		X[10]	X	X
Complete Blood Count (CBC)	X[9]							X
Thyroid Function Test[11]	X[9]		X	X[10]		X[10]	X	
Pregnancy Test (urine)[12]	X	X	X	X	X	X	X	

* These visit numbers correspond with the protocol visit numbers. All follow-up visits will be conducted within a window of ±7 days of the target day, with the exception of the safety follow-up visit at the BRC sites, which can only occur at least five days following IP cessation.

[1] At the time the study medication is dispensed, participants will be provided a pill diary and an information sheet outlining instructions on taking study medication and concomitant medications.

[2] Participants will take an oral pill two times a day, once in the morning and once in the evening, approximately 12 hours apart. The exception to this will occur on the day of the baseline visit, in which participants will take only the evening pill.

[3] The decision to continue dispensing IP or injecting bevacizumab is determined by the scenarios described in Section 4.5.2.

[4] Abnormal findings during a thyroid palpation will be followed by a referral to an endocrinologist.

[5] BCVA with manifest refraction must also be performed when scheduled and when there is a change in VA of ≥10 ETDRS letters (≥ 0.20 logMAR) as compared with relevant baseline.

[6] These procedures may be completed under another NEI protocol if performed within 1-7 days prior to the baseline visit.

[7] Microperimetry will be performed at baseline and at Months 3, 6, 12, 18 and 24.

[8] All abnormal values or positive tests will be reported to the participant's primary care physician, with the participant's permission.

[9] These procedures may be completed under another NEI protocol if performed within 42 days prior to the baseline visit.

[10] The acute care and hepatic panels and thyroid function testing are performed at baseline, Month 2 and every four months thereafter (Months 6, 10, 14, 18 and 22), as well as the final visit at Month 24.

[11] Participants with abnormal thyroid function test results will be referred to an endocrinologist.

[12] For women of childbearing potential only and they must have a negative test within 24 hours prior to initiation of IP. For the BRC sites, for women of childbearing potential only and they must have a negative serum pregnancy test within 24 hours prior to initiation of IP. After initiation of contraception, urine pregnancy tests will be conducted; however, a serum test will be performed anytime a urine test result creates doubt. See Appendix 1 for guidance on determining whether a female is considered to be of childbearing potential. Some women may require a follicle-stimulating hormone (FSH) test to determine childbearing potential.

[13] Applicable to the BRC sites only: This visit must be conducted at least five days after the last study drug administration. Additional assessments may be performed if deemed necessary by the investigator.

[14] Applicable to the BRC sites only: In the event that the baseline testing was not completed within one day, subsequent clinic visits may be scheduled within 14 days of the first visit to complete the scheduled evaluations and treatments. If the Month 12 or Month 24 visit was not completed within one day, subsequent visits may be scheduled within 7 days of the first visit. If the baseline visit, Month 12 visit or Month 24 visit are conducted over more than one day, BCVA, OCT and microperimetry testing must be completed on the day of the injection.

APPENDIX 3: LIST OF DEFINITIONS

- ***Improvement*:** Visual acuity in the study eye of ≥ 84-88 letters (20/20) OR OCT central subfield thickness < 300 microns.

- ***Worsening*:** A decrease in visual acuity of ≥15 or more letters in the study eye compared to baseline, AND an increase in OCT central subfield thickness ≥ 1-step log unit compared to baseline for at least two consecutive visits.

- ***New Steady State:*** Improvement and worsening criteria not met and central subfield thickness remains ≥ 300 microns on OCT but thickness is stable and has not changed > 25 microns over the last three consecutive injections.

- ***No improvement*:** Improvement and worsening criteria are not met and visual acuity failed to increase by ≥ 10 letters in the study eye compared to baseline AND OCT central subfield thickness failed to decrease by ≥ 1-step log unit compared to baseline.