A Phase 2 Study Evaluating Short-Term Efficacy of Tonabersat (Xiflam) for Diabetic Macular Edema

(Protocol AN)

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DRCR Retina Network

A Phase 2 Evaluation of Tonabersat for Diabetic Macular Edema (Protocol AN)

Sponsor: Jaeb Center for Health Research (JCHR)

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A Phase 2 Evaluation of Tonabersat for Diabetic Macular Edema

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VERSION HISTORY

The following table lists versions of the protocol.

VERSION NUMBER	AUTHOR	APPROVER	EFFECTIVE DATE	REVISION DESCRIPTION
1.0	Crystal Franklin	Cynthia Stockdale	Not implemented	Initial (submitted to FDA)
2.0	Crystal Franklin	Cynthia Stockdale	Not implemented	Added 12-month visit and clarify throughout that study drug is stopped at 6 months, but participant remains in follow-up until 12-month visit; added lab collections to 1 & 3-month visits; added TSH to labs collected; statistics chapter updated to reflect all changes; study drug dose updated to 80mg (2 pills once per day); added following exclusion criteria: hepatic impairment, YAG within prior 2 months.
2.1	Crystal Franklin	Cynthia Stockdale	01FEB2023	Updated AE relatedness categories; serum sample for future use updated as optional for participant; clarified that participant must have capacity to consent on one's own behalf; clarified that drug is stopped following rescue treatment for DME (but not DR) prior to 6 months; clarified study drug dispensing procedure.
3.0	Crystal Franklin	Cynthia Stockdale	14SEP2023	Removed requirement to take 1st dose of study drug in-office at the randomization visit; Clarified drug should be taken at bed to reduce chance of side effects; Added option to take 40mg of study drug if 80mg dose is intolerable; Updated definition of Adverse Event; addition of clinical exam evidence of DR as inclusion criterion; miscellaneous minor clarifications
4.0	Crystal Franklin	Cynthia Stockdale	18APR2024	Modified eligibility criteria: expanded VA to 20/32 or better & modified prior injection criterion to no more than 4 injections in the past 12-36 months and changed allowable percent of cohort to have any prior injections from 15 to 25%; modified recruitment categories above the CI-DME thresholds. Updated gender to sex throughout. Minor wording/formatting edits. Effective date is the date all changes go live to the online case report forms.

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AE	Adverse Event
AMD	Age-Related Macular Degeneration
BCVA	Best-Corrected Visual Acuity
CI-DME	Center Involved-Diabetic Macular Edema
CST	Central Subfield Thickness
CBC	Complete Blood counts
CMP	Comprehensive Metabolic Panel
CI	Confidence Interval
CC	Coordinating Center
CRP	c-Reactive Protein
DSMC	Data & Safety Monitoring Committee
DME	Diabetic Macular Edema
DR	Diabetic Retinopathy
ETDRS	Early Treatment Diabetic Retinopathy Study
eCRF	Electronic Case Report Form
E-ETDRS	Electronic-Early Treatment Diabetic Retinopathy Study
ERG	Electroretinogram
eGFR	estimated Glomerular Filtration Rate
FDA	Food and Drug Administration
GFAP	Glial Fibrillary Acidic Protein
GCP	Good Clinical Practice
IRB	Institutional Review Board
ITT	Intention-to-Treat
IND	Investigational New Drug
IB	Investigator Brochure
JCHR	Jaeb Center for Health Research
LOCF	Last Observation Carried Forward
MCMC	Markov chain Monte Carlo
MedDRA	Medical Dictionary for Regulatory Activities
MCP	Monocyte Chemoattractant Protein
NIH	National Institutes of Health
OCT	Optical Coherence Tomography
PRP	Panretinal Photocoagulation
PI	Principal Investigator
QA	Quality Assurance

ABBREVIATION	DEFINITION
QC	Quality Control
RBM	Risk-Based Monitoring
SAE	Serious Adverse Event
SD	Sprague Dawley
SD	Standard Deviation
SUSAR	Unexpected Serious Adverse Reaction
TSH	Thyroid Stimulating Hormone
VEGF	Vascular Endothelial Growth Factor

PROTOCOL SUMMARY

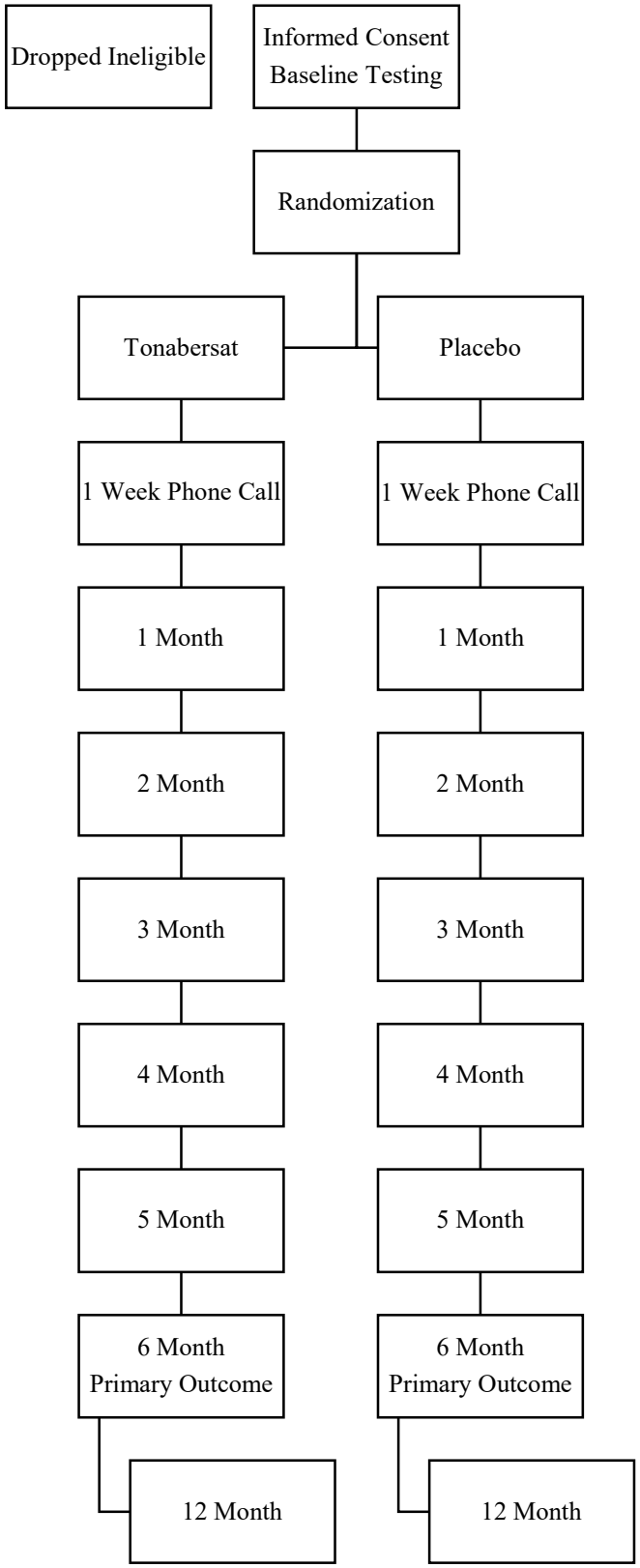
ITEM	DESCRIPTION
Title	A Phase 2 Evaluation of Tonabersat for Diabetic Macular Edema (DME)
Précis	This randomized clinical trial will evaluate the effect of tonabersat compared with placebo on central subfield thickness (CST) in eyes with center-involved DME and good visual acuity.
Investigational Drug	Tonabersat (SB-220453, Xiflam)
Objectives	The primary objective is to assess the effects of tonabersat, an orally administered Connexin43 hemichannel inhibitor, on CST (mean change) compared with placebo in eyes with center-involved DME and good visual acuity at 6 months. Exploratory objectives will evaluate biomarkers of kidney function for potential benefit. Furthermore, this phase 2 study is being conducted to determine whether the conduct of a phase 3 trial has merit and provide information on outcome measures needed to design a phase 3 trial.
Study Design	Randomized, double-masked, placebo-controlled clinical trial
Number of Sites	About 25
Outcome	**Primary Outcome:** • Mean change in central subfield thickness at 6 months* **Secondary Outcomes:** • Mean change in retinal volume from baseline at 6 months • Percentage of eyes with CST below OCT machine- and sex-specific threshold for DME and at least a 10% decrease from baseline at 6 months • Mean change in ETDRS BCVA from baseline at 6 months • Percentage of eyes receiving other treatment for DME prior to the 6 month visit • Percentage of eyes with ≥5 letter loss at the final two consecutive visits or ≥10 letter loss at the final visit in ETDRS BCVA **Exploratory Outcomes:** • Percent of eyes with worsening or improvement of diabetic retinopathy on fundus photographs • Mean change in eGFR from baseline to 6 months • Mean change in CRP from baseline to 6 months

ITEM	DESCRIPTION
	• Mean change in HbA1c from baseline to 6 months • Mean change in Cystatin C from baseline to 6 months • Mean change in TSH from baseline to 6 months • Mean change in Galectin 3 from baseline to 6 months • Mean change in IL-1 β from baseline to 6 months • Mean change in IL-6 from baseline to 6 months • Mean change in TNFα from baseline to 6 months • Mean change in urine creatinine from baseline to 6 months • Mean change in urine albumin from baseline to 6 months • Mean change in urine MCP1 from baseline to 6 months • Mean change in urine osteopontin from baseline to 6 months • Mean change in urine IL-18 from baseline to 6 months • Mean changes in OCTA vessel density and nonperfusion area • Change in CST from 6 to 12 months • Change in retinal volume from 6 to 12 months • CST below the OCT machine- and sex-specific threshold for DME at 12 months and a 10% or more decrease in CST from 6 to 12 months • Change in ETDRS BCVA from 6 to 12 months • Receiving other treatment for DME between the 6 and 12-month visit • Changes in blood and urine samples from 6 to 12 months **Safety Outcomes of Interest:** Headache, drowsiness, dizziness, effect on renal function (change in eGFR) and liver function, effect on blood counts and metabolic panel. * Change in CST will not be used to definitively establish efficacy. If an anatomical difference is observed in this phase 2 trial compared to placebo the results would suggest that this medication merits further investigation in a definitive, phase 3 trial.
Population	**Key Inclusion Criteria** • Adults with type 1 or 2 diabetes mellitus • At least one eye with: 1. Best corrected E-ETDRS visual acuity letter score ≥ 74 (i.e., 20/32 or better) 2. Ophthalmoscopic evidence of center-involved DME in study eye confirmed by central subfield thickness on spectral domain OCT

ITEM	DESCRIPTION
	• Zeiss Cirrus: ≥ 290 µm in females, ≥ 305 µm in males • Heidelberg Spectralis: ≥ 305 µm in females, ≥ 320 µm in males *Recruitment will be monitored with a goal to have approximately half of participants with measurements ≥75 µm above the CI-DME thresholds* 3. Media clarity, pupillary dilation, and study participant cooperation sufficient for adequate OCT **Key Exclusion Criteria** • Macular edema is considered to be due to a cause other than DME • Major ocular surgery within prior 4 months, or anticipated after randomization • History of focal/grid laser or other ocular surgical, intravitreal, or peribulbar treatment for DR or DME within prior 1 year, and no more than 4 anti-VEGF injections within prior 3 years • Anticipated need to treat DME or DR during the first 6 months, or anticipated need for cataract surgery during study period • Any history of vitrectomy • Systemic anti-VEGF or pro-VEGF treatment within 12 months prior to randomization • History of chronic renal failure requiring dialysis or kidney transplant • History of moderate to severe hepatic impairment, including known liver function test (LFT) values > 3x's the upper limit of normal
Sample Size	128
Phase	Phase 2
Treatment Groups	Random assignment (1:1) to tonabersat or placebo
Participant Duration	12 months (6 months on study drug, followed by 6 months not on study drug for each participant)
Study Duration (planned)	Anticipated 24 months total duration, from first enrollment until last participant visit.
Protocol Overview/Synopsis	1. Informed consent will be obtained. 2. Study eligibility will be assessed.

ITEM	DESCRIPTION
	3. Prior to randomization, the participant's willingness to proceed into the randomized trial will be confirmed. 4. Eligible participants will be randomly assigned 1:1 to tonabersat or placebo for a duration of 6 months. 5. A follow-up phone call is performed at 1 week. 6. Participants will return for monthly follow-up visits for the first 6 months, then one final follow-up visit at 12 months. 7. Participation in the study will be considered completed upon completion of the 12-month visit.

SCHEMATIC OF STUDY DESIGN



SCHEDULE OF STUDY VISITS AND PROCEDURES

Visit Procedures	Baseline	Month						
		1*	2*	3*	4*	5*	6†	12††
Randomization	X							
Best-Corrected Visual Acuity [a]	X	X	X	X	X	X	X	X
OCT [b]	X	X	X	X	X	X	X	X
Fundus Photography [c]	X						X	
OCTA [d]	X						X	
Eye Exam [e]	X	X	X	X	X	X	X	X
Blood Pressure [f]	X	X	X	X	X	X	X	X
Routine Lab Samples [g] • Whole Blood for HbA1c and CBC • Serum for CRP, CMP, Cystatin C, eGFR and TSH • Urine for creatinine, albumin, and pregnancy test	X	X	Pregnancy test only	X	Pregnancy test only	Pregnancy test only	X	X
Additional Biomarker Samples [h] • Serum for biomarkers such as Galectin 3, IL-1 β, IL-6, and TNFα • Urine for biomarkers such as MCP1, Osteopontin, IL-18 • Optional serum for biobanking	X						X	
Safety Assessment		X	X	X	X	X	X	X
Study Drug Compliance Assessment [i, j]		X	X	X	X	X	X	

Notes

Ocular testing is only required for the study eye unless otherwise specified below.

* Visit window = +/- 2 weeks. In addition, if rescue treatment for DME is initiated in the study eye prior to the 6-month visit, 6-month study eye testing will be completed. For testing that is to be performed pre-dilation, post-dilation testing will be accepted if the decision to treat is made at a usual care visit, after dilation. Tonabersat is discontinued following rescue treatment for DME.

† Visit window = +/- 4 weeks

†† Visit window = +/- 6 weeks

a Visual acuity testing includes protocol refraction on both eyes at baseline and the 6 & 12-month visits, and on the study eye(s) only at all other visits followed by electronic-ETDRS using the EVA Tester in both eyes, which has been validated against 4-meter chart ETDRS testing.

b OCT will be done on both eyes during the baseline and the 6 & 12-month visits, and on the study eye(s) only at all other visits

c Fundus photographs are required in both eyes at the baseline and 6-month visits.

d OCTA performed in the study eye(s) only

e Includes slit lamp exam (including assessment of lens), measurement of intraocular pressure, and dilated ophthalmoscopy on study eye(s) at every visit, and on both eyes at baseline and the 6 & 12-month visits.

f Weight also taken at the baseline, 6 and 12-month visits and height at baseline.

g CBC = Complete Blood Count, CRP = C-Reactive Protein; CMP = Comprehensive Metabolic Panel; eGFR = estimated glomerular filtration rate; TSH = thyroid stimulating hormone. Pregnancy test completed on site at all visits for women of child-bearing potential.

h IL-1 β = Interleukin-1 beta; IL-6 = Interleukin-6; TNFα = Tumor Necrosis Factor alpha, MCP1 = Monocyte chemoattractant protein-1; IL-18 = Interleukin-18. Collected at baseline, 6 months, and when treatment is initiated in the study eye prior to the 6-month visit, when feasible. Biobanking for future use optional for participant.

i Participants may consent to daily text message reminders to increase Study Drug compliance. Study drug will be discontinued at the 6-month visit.

j Approximately one week after randomization, site will contact participant via telephone to assess study drug compliance.

Chapter 1: Background Information

1.1 Introduction

1.1.1 Diabetic Macular Edema

The number of adults living with diabetes mellitus today is higher than ever, having tripled over the past 20 years. Estimates suggest that by the year 2045, 700 million people worldwide will be affected by this chronic disease.[1] The steadily increasing rates of what is effectively a global epidemic likewise implies an increase in rates of associated vascular complications. In the United States at least 7.7 million adults are estimated to have diabetic retinopathy (DR) and an additional 800,000 have DME .[2,3] Despite advances in diagnosis and management of ocular disease, eye complications from diabetes mellitus continue to be a leading cause of blindness in American adults. [3]

1.1.2 Current Treatment for DME in Eyes with Good Visual Acuity

DME is a manifestation of DR and the leading cause of moderate vision loss in patients with diabetes. Without intervention, 33% of the 221 eyes included in the Early Treatment Diabetic Retinopathy Study (ETDRS) with center-involved DME (CI-DME) experienced "moderate visual loss" (defined as a 15 or more letter score decrease in visual acuity) over a three-year period.[4] Results of the DRCR Retina Network Protocol I (Intravitreal Ranibizumab or Triamcinolone Acetonide in Combination with Laser Photocoagulation for Diabetic Macular Edema) indicated that treatment with anti-vascular endothelial growth factor (anti-VEGF) agents was superior to that of focal/grid laser (both alone and combined with intravitreous corticosteroids) in the management of DME.[5] While subsequent studies have confirmed this superiority further with different anti-VEGF agents, this treatment is not without its disadvantages including the need for recurrent injections as often as once a month, and the injections themselves having potentially serious side effects such as the development of endophthalmitis.[6] Intravitreal injections are also associated with high incremental cost effectiveness ratios and additionally,[7] not all eyes treated with anti-VEGF have resolution, needing other interventions such as laser, a treatment with well-documented adverse events.[8] Thus, there is an ongoing need to identify novel therapies that are both effective for DME treatment and that also avoid potential adverse events or costs associated with current ocular interventions.

1.1.3 Tonabersat

Tonabersat is an oral small-molecule agent that targets connexin43 (Cx43) hemichannels that are often overexpressed in autoinflammatory disease, including retinal disease such as diabetic retinopathy.[9] It acts by blocking the pathologically overexpressed Cx43 hemichannels in the cell membranes, preventing release of ATP into the extracellular space which is a signal for the assembly of the NLRP3 inflammasome, a key component of the innate immune system.[10] Preclinical *in vitro* studies suggest that blocking of the Cx43 hemichannel shuts down the inflammasome and reduces the activation and recycling of multiple proinflammatory cytokines including IL-1β, IL-18, TNF-α, IL-6, IL-8, MCP-1, sICAM-1 and VEGF, which are all

associated with DR/DME pathology.[11-13] This is further supported in both animal disease models and human tissue cultures where inflammasome assembly is prevented through the inhibition of Cx43 overexpression resulting in inflammasome assembly and proinflammatory cytokine release (Figures 1,2).

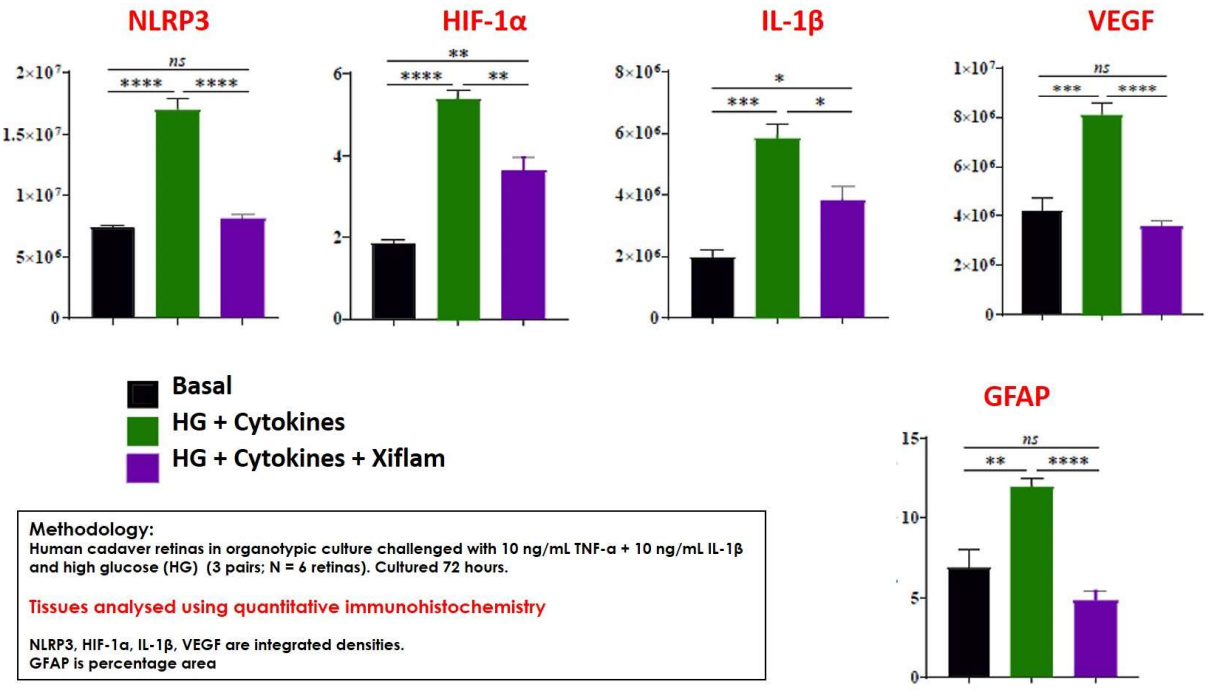


Figure 1: *Ex vivo* organotypic culture model of cadaveric human retina challenged with high glucose and inflammatory cytokines IL-1β and TNF-α as a model of DR. Immunohistochemistry shows that tonabersat blocks inflammasome complex assembly (a multimeric oligomer labeled NLRP3), reducing the production of inflammatory cytokines, and preventing signs of inflammation. (GFAP = glial fibrillary acidic protein; astrocytosis)

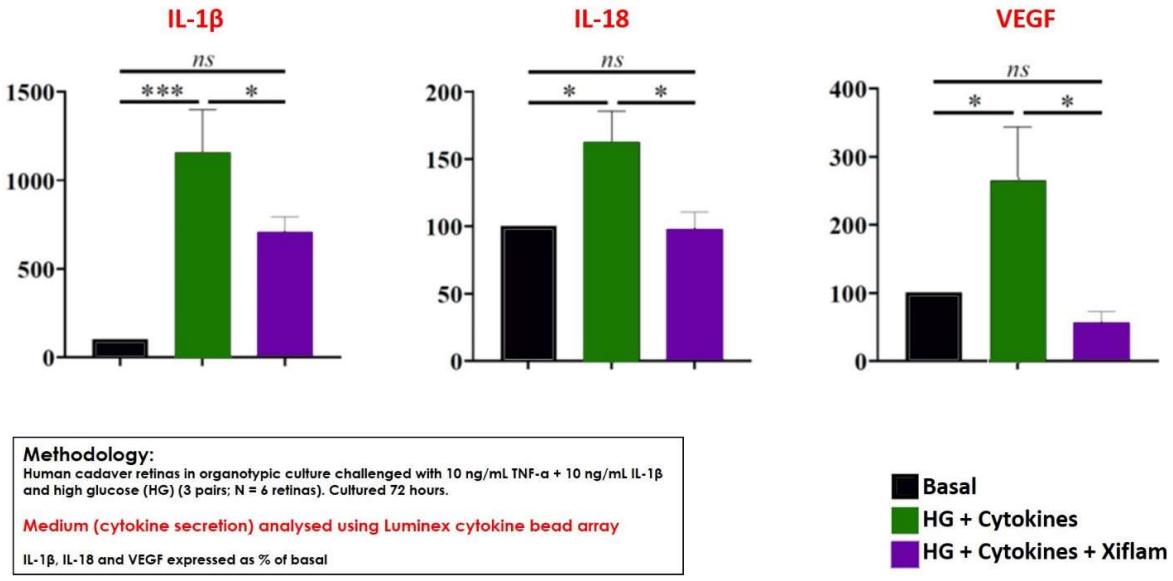


Figure 2: *Ex vivo* organotypic culture model of cadaveric human retina challenged with high glucose and the inflammatory cytokines IL-1β and TNFα as a model of DR. A cytokine bead array has been used to analyze the secretion of inflammatory cytokines into the media. Tonabersat blocked the release of key inflammatory cytokines, including VEGF.

InflammX Therapeutics, Inc. is a development stage biopharmaceutical company that develops novel therapeutics for clinical use in ophthalmology. The company specializes in development of drug candidates involved in a mechanism of action called Hemichannel Modulation. It currently has four candidates that target the dysregulated Cx43 protein, a key component of the gap junction hemichannel. All four connexin hemichannel modulators are proprietary, with distinct modes of action for topical, intravitreal and systemic oral administration opportunities. Each candidate has robust and repeatable clinical and pre-clinical data, with efficacy shown in internationally accepted ocular models including models of DR and dry age-related macular degeneration (AMD).

Tonabersat is one of a series of novel benzoylamino benzopyran compounds initially developed by GSK for the treatment of migraine and completed full GLP toxicology studies including 2-year carcinogenicity studies. Additionally extensive CMC development was carried out to develop an orally administered drug product. This data enabled an IND to be filed with the FDA to support human clinical trials. A series of Phase 1 and Phase 2B clinical studies were carried out under the IND in which more than 1000 patients were dosed. As a result a significant safety database in human patients was generated demonstrating tonabersat to be well tolerated with no significant safety issues.[13] It is orally available, and dosed once daily, with a T_{max} 0.5 to 3 hour, and terminal half-life of 24 to 40 hours, but no accumulation.

Its efficacy in the treatment of DR was evaluated at the University of Auckland in animal models using a spontaneously hyperglycemic strain of Sprague Dawley (SD) rats at birth that exhibited clinical signs of DR at 4 weeks.

Ten rats per group (10 normal SD and 10 hyperglycemic SD) were grown to 5 weeks of age and assessed with optical coherence tomography (OCT) and electroretinogram (ERG) at baseline. The hyperglycemic rats were then separated into two groups which were treated once-daily with either 0.28 mg/kg of orally administered tonabersat or placebo over a period of 14 days. All animals were assessed again at 8 weeks of age before being euthanized, with tissue collected for immunohistochemical analysis.

The results demonstrated significant regression of micro- and macro-aneurysms on fundus imaging and OCT, restored retinal function on ERG, and reduced inflammatory markers (Iba-1, microglial marker, astrocyte marker glial fibrillary acidic protein).[14]

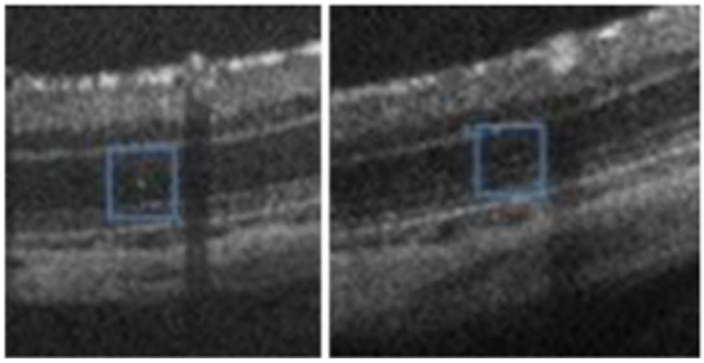


Figure 3: OCT Imaging of tonabersat-treated hyperglycemic rats at 8 weeks[15]. 1) microaneurysms and macroaneurysms were specifically in the INL and ONL; 2) microaneurysms defined as 20 to 30 µm diameter, macroaneurysms defined as 140 to 160 µm in diameter.

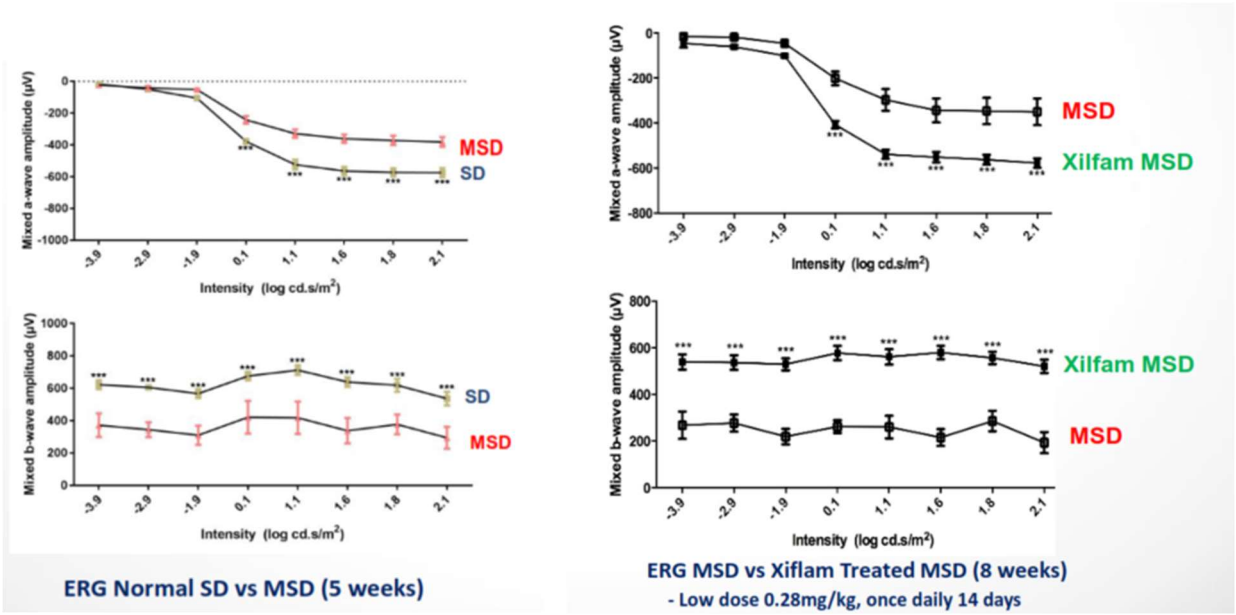


Figure 4: ERG analysis of hyperglycemic rats (MSD) as compared to hyperglycemic rats that received 0.28 mg/kg of tonabersat, once daily for 14 days. Tonabersat-treated rats had significantly improved retinal function by 8 weeks, with both a-wave and b-wave level closely matching those of untreated control. ERG remains impaired in placebo-treated MSD.[16].

Additional models of ischemic retinal injury demonstrated that treatment with a Cx43 hemichannel blocker, including tonabersat, reduced vascular leakage (up to 86%), and edema, by shutting down the NLRP3 inflammasome.

There is evidence from preclinical studies, that suggests a possible correlation between the role of the NLRP3 inflammasome in the microvascular pathology of DR and chronic kidney disease in diabetes.[17] However, additional data in human trials is needed to further understand whether tonabersat treatment leads to improvement of renal outcomes in diabetes.

1.1.4 Public Health Impact

At present, at least 5 million people over the age of 40 in the United States are estimated to have diabetic retinopathy (DR) in the absence of diabetic macular edema (DME), and an additional 800,000 have DME, according to data from the Centers for Disease Control and Prevention. Current treatment regimens of intravitreal anti-VEGF injections result in many clinic visits and injections during the course of treatment and approximately 50% of patients with DME may not respond fully to anti-VEGF treatment. If this oral therapy is effective in improving DME, injections and other invasive treatments may be avoided or delayed and treatment burden, such as frequent visits and costs, may be reduced. This would be a major public health contribution if this non-invasive oral treatment is effective in treating DME and can be implemented into clinical care for patients with diabetic eye disease.

1.2 Rationale

Given the visit burden, costs, and potential adverse events associated with current treatments for DME, a well-tolerated, safe, and effective oral medication for this indication would have a substantial positive public health impact. The application of an oral therapy targeting the dysregulated NLRP3 inflammasome seeks to modify the course of DME through blocking its inflammatory component. The proposed study will evaluate tonabersat compared with placebo for eyes with CI-DME and good vision to determine whether there is a short-term effect on central subfield thickness on OCT. If there is a short-term anatomic effect in these eyes, a larger study would be needed to definitively establish the efficacy of this novel treatment targeting the activated NLRP3 inflammasome.

1.3 Potential Risks and Benefits of Tonabersat

1.3.1 Known Potential Risks Related to Common Procedures

Many of the procedures in this study are part of daily ophthalmologic practice in the United States and pose few if any known risks. Dilating eye drops will be used as part of the exam. There is a small risk of inducing a narrow-angle glaucoma attack from the pupil dilation. However, all participants will have had prior pupil dilation usually on multiple occasions and therefore the risk is extremely small. Possible risks from blood draws include the following: bruising, arm discomfort, clotting, excess bleeding, infection, or fainting.

1.3.2 Known Potential Risks Related to Tonabersat

As described in the Investigator Brochure (IB),[18] tonabersat was initially developed for the treatment of migraine and, as required by FDA, underwent IND-enabling high dose toxicity testing including a 2-year carcinogenicity study. Human clinical studies were completed with more than 1,500 patients with QD oral administration dosing up to 80 mg/day for up to 5 months. The safety database generated by the trials indicated that the drug is well tolerated with no significant safety issues.[13] It is orally available, and dosed once daily, with a T_{max} 0.5 to 3 hour, and terminal half-life of 24 to 40 hours, but no accumulation. The most common adverse events in previous human trials included headache, drowsiness and dizziness. Less common side effects included dry mouth, chills, tremors (shakes), nausea, vomiting, and heart palpitations

(occurring in 2 in 100 people taking tonabersat), as well as abdominal pain, constipation, diarrhea, abnormal vision, and a runny nose (occurring in 1 in 100 people taking tonabersat).

Systemic effects of tonabersat will be evaluated via routine lab testing (at the 1, 3, 6, and 12-month visits) and collection of adverse events at every follow-up visit. Participants will stop study drug at the 6-month visit, and will have a final follow-up visit at 12 months. The investigator may choose to discontinue tonabersat in advance of the 6-month visit if necessary for safety concerns, but the participant will remain in the study and followed for safety.

Participants will be asked to provide contact information for their primary health care provider (PCP) who will, if patient agrees, be notified of their enrollment in the study and study lab results. Laboratory results collected at the baseline, 1, 3, 6, and 12-month visits will be evaluated centrally using predetermined thresholds for abnormal values. The results will be provided to the site investigator, the participant, and the participant's PCP (if participant agreed). The site investigator will review the results and record any clinically significant values as an adverse event.

1.3.3 Risks Related to Confidentiality

The risk of disclosure of protected health information is very small. Efforts are taken to assure that this does not occur, in compliance with HIPAA.

1.3.4 Risks if Pregnant

The effects of tonabersat on a human fetus (unborn baby) or nursing (breast feeding) infant are unknown. It is possible that use of these drugs may be associated with unknown risks to a pregnancy or fetus. Therefore, patients will not be allowed to participate in this study if pregnant, planning to become pregnant within the next 12 months, or if nursing an infant. During the study, females who are of child-bearing potential (i.e., have started menstruation, are not surgically sterile, and are not $\geq$ 1 year post-menopausal) must agree to use an effective method of birth control to prevent pregnancy and will complete a pregnancy test prior to randomization and at every follow-up visit. Child-bearing potential and/or pregnancy prevention plans must be documented. In the event of pregnancy during the first 6 months, tonabersat/placebo will be discontinued. The site staff and participant will be unmasked, but the participant will remain in the study through 12 months, and further followed for safety through the end of pregnancy.

1.3.5 Known Potential Benefits

There is potential that the treatment could result in improvements in DME. However, there is no guarantee that the treatment with tonabersat will be successful. It is recognized that a 6-month change in central subfield thickness is not a definitive efficacy outcome. This phase 2 study is being conducted to determine whether the conduct of a phase 3 trial has merit based on an anatomic outcome and to provide information on outcome measures needed to design a phase 3 trial. If the results of this study support proceeding with a phase 3 trial after evaluation of all the data and discussion within DRCR, information from this study will contribute to designing the trial.

If definitive efficacy were established based on a phase 3 trial, the benefits to society may be substantial. Patients with diabetes are at high risk of developing advanced diabetic eye

complications. DME is a common complication, affecting approximately 800,000 persons in the United States aged 40 years and older. Widespread use of an oral agent effective at reducing worsening of DR would decrease the numbers of patients who undergo more invasive and much more expensive treatment for DR and DME and who are consequently at risk for side effects that adversely affect visual function.

Future patients may benefit from this medical research if tonabersat is subsequently demonstrated to be effective in this cohort with an acceptable safety profile, and its use becomes more widespread. By preventing progression of the disease, patients may avoid the potential adverse events and costs associated with the frequent injections necessary to treat complications of advanced diabetic eye disease.

1.3.6 Risk Assessment

The protocol risk assessment for this study has been categorized as greater than minimal risk.

1.4 General Considerations

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

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

Study personnel and participants will be masked to treatment group.

Chapter 2: Study Enrollment and Randomization

2.1 Participant Recruitment and Enrollment

A minimum of 128 participants are expected to be randomized. It is anticipated that up to 300 participants may be enrolled in the study (defined as signing the consent form) in order to achieve this goal. Participants who have signed consent and started the screening process may be permitted to continue into the trial, if eligible, even if the randomization goal has been reached.

Study participants will be recruited from approximately 25 clinical centers in the United States. All eligible participants will be included without regard to sex, race, or ethnicity. There is no restriction on the number of participants to be enrolled by each site toward the overall recruitment goal.

2.1.1 Informed Consent and Authorization Procedures

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

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

As part of the informed consent process, each participant will be asked to provide authorization for release of personal information. The investigator, or his or her designee, will review the study-specific information that will be collected and to whom that information will be disclosed. After speaking with the participant, questions will be answered about the details regarding authorization. A separate signature section will be used to collect consent for optional text message reminders.

A participant is considered enrolled when the informed consent form has been signed. Once a study participant is randomized, that participant will be counted regardless of whether the assigned treatment is received. Thus, the investigator must not proceed to randomize an individual until he/she is convinced that the individual is eligible, will accept assignment to either of the two treatment groups, and will be compliant with the drug use schedule.

2.2 Participant Inclusion Criteria

2.2.1 Patient-level Criteria

Inclusion

To be eligible for the randomized trial, the following inclusion criteria must be met:

1. Age ≥ 18 years
   - *Subjects <18 years old are not being included because DME is so rare in this age group that the diagnosis may be questionable.*
2. Diagnosis of diabetes mellitus (type 1 or type 2)

- Any one of the following will be considered to be sufficient evidence that diabetes is present:
  - *Current regular use of insulin for the treatment of diabetes.*
  - *Current regular use of oral anti-hyperglycemia agents for the treatment of diabetes.*
  - *Documented diabetes by American Diabetes Association and/or the World Health Organization criteria.*

3. At least one eye meets the study eye criteria listed in section 2.2.2.
4. Able, willing, and has the capacity to provide informed consent on one's own behalf.
5. Willing to take an oral medication, two pills once per day.

Exclusion

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

6. A condition, that in the opinion of the investigator, would preclude participation in the study (e.g., unstable medical status that might preclude completion of follow-up)
7. History of chronic renal failure requiring dialysis or kidney transplant
8. History of moderate to severe hepatic impairment, including known liver function test (LFT) values > 3x's the upper limit of normal
9. Initiation of intensive insulin treatment (a pump or multiple daily injections) within 4 months prior to randomization or plans to do so in the next 6 months
10. Participation in an investigational trial that involved treatment within 30 days of randomization with any drug/device that has not received regulatory approval for the indication being studied
    - *Note: study participants cannot participate in another investigational trial that involves treatment with an investigational drug or device while participating in the study.*
11. Blood pressure > 160/100 (systolic above 160 or diastolic above 100)
    - *If blood pressure is brought below 160/100 by anti-hypertensive treatment, individual can become eligible.*
12. Systemic anti-VEGF or pro-VEGF treatment within 12 months prior to randomization
13. For females of child-bearing potential: pregnant, intending to become pregnant within the next 12 months, or plans to nurse an infant within the next 12 months
    - *Females who are potential study participants should be questioned about the potential for pregnancy and be willing to complete a pregnancy test at each in-person visit.*
14. Individual is expecting to move out of the area during the first 6 months of the study
15. Known allergy to any component of the study drug
    - Active ingredient: SB-220453
    - Inactive ingredients: lactose, microcrystalline cellulose, sodium starch glycollate, colloidal silicon dioxide, and magnesium stearate

2.2.2 Study Eye Criteria

To be eligible, the study participant must have at least one eye meeting all of the inclusion criteria and none of the exclusion criteria listed below. A study participant can have two study eyes only if both are eligible at the time of randomization.

The eligibility criteria for a study eye are as follows:

Inclusion

a. Best corrected E-ETDRS visual acuity letter score ≥ 74 (i.e., 20/32 or better)

b. Clinical exam evidence of diabetic retinopathy

c. Ophthalmoscopic evidence of center-involved DME, confirmed by CST on spectral domain OCT:
   - Zeiss Cirrus: ≥290µm in females, and ≥305µm in males
   - Heidelberg Spectralis: ≥305µm in females, and ≥320µm in males

   Recruitment will be monitored with a goal to have approximately half of participants with measurements ≥75 µm above the CI-DME thresholds

d. Media clarity, pupillary dilation, and study participant cooperation sufficient for adequate OCT

Exclusion

e. Macular edema is considered to be due to a cause other than DME
   - *An eye should not be considered eligible if: (1) the macular edema is considered to be related to ocular surgery such as cataract extraction or (2) clinical exam and/or investigator assessment of OCT suggests that vitreoretinal interface abnormalities (e.g., a taut posterior hyaloid or epiretinal membrane) are contributing to the macular edema.*

f. An ocular condition is present such that, in the opinion of the investigator, any visual acuity loss would not improve from resolution of macular edema (e.g., foveal atrophy, pigment abnormalities, dense subfoveal hard exudates, nonretinal condition)

g. An ocular condition is present (other than DME) that, in the opinion of the investigator, might affect visual acuity during the course of the study or require intraocular treatment (e.g., vein occlusion, uveitis or other ocular inflammatory disease, neovascular glaucoma, etc.)

h. Cataract is present that, in the opinion of the investigator, may alter visual acuity during the first 6 months of the study

i. History of major ocular surgery (including cataract, scleral buckle, any intraocular surgery, etc.) within prior 4 months, or anticipated after randomization

j. History of focal/grid laser or other ocular surgical, intravitreal, or peribulbar treatment for DME or DR within prior 12 months
   - *If treatment was given more than 12 months prior, no more than 4 intraocular injections within the prior 3 years*
   - *Enrollment will be limited to a maximum of 25% of the planned sample size with any history of anti-VEGF treatment.*

k. Anticipated need to treat DME or DR during the first 6 months of the study

l. History of topical steroid or topical NSAID treatment within 2 months prior to randomization

m. History of YAG capsulotomy performed within 2 months prior to randomization

n. Any history of vitrectomy
o. Any history of PRP
p. Aphakia
q. Evidence of uncontrolled glaucoma

2.2.3 Non-Study Eye

There are no eligibility or exclusion criteria with respect to the non-study eye.

2.3 Screening Procedures

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

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

All testing does not need to be completed on the same day provided it is within the windows specified below.

2.3.1 Data Collection and Testing

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

- If a procedure has been performed using the study technique and by study certified personnel as part of usual care, then it does not need to be repeated specifically for the study if it was performed within the defined time windows specified below.
- The testing procedures are detailed in the DRCR Retina Network procedures manuals. See Chapter 5 for which testing requires certified personnel.
- OCTs meeting DRCR Retina Network criteria for manual grading will be sent to a reading center, but study participant eligibility is determined by the site (i.e., individuals deemed eligible by the investigator will be randomized without pre-randomization reading center confirmation).

1. Self-reported demographics (date of birth, sex, race and ethnicity)
2. Medical history (pre-existing medical conditions, concomitant medications, as well as ocular diseases, surgeries, and treatment)
    - Medical history will be obtained by medical charts if available at the enrolling site; otherwise, it will be self-reported
3. Electronic-ETDRS visual acuity testing at 3 meters using the Electronic Visual Acuity Tester (including protocol refraction) in both eyes *(within prior 14 days of randomization)*
4. Spectral-Domain OCT using Zeiss Cirrus or Heidelberg Spectralis in both eyes *(within prior 14 days of randomization)*

5. Digital fundus photographs on both eyes using the widest approach (e.g. ultra-widefield imaging device) available *(within prior 14 days of randomization)*
6. Ocular examination on each eye including slit lamp, measurement of intraocular pressure, lens assessment, and dilated ophthalmoscopy *(within prior 14 days of randomization)*
7. OCT Angiography on study eye(s) only *(within prior 14 days of randomization)*
8. Physical examination to include *(within prior 14 days of randomization)*:
    - Weight and Height
    - Measurement of blood pressure
9. Blood draw *within 8 days of randomization* for central-lab measurement of routine and biomarker labs:

    HbA1c, C-reactive protein (CRP), complete blood counts (CBC), comprehensive metabolic panel (CMP), cystatin C, thyroid stimulating hormone (TSH), serum creatinine (used for estimated glomerular filtration rate [eGFR]), and biomarkers such as Galectin 3, IL-1 β, IL-6, TNFα

    - *Includes one whole blood sample for HbA1c and CBC tests, one sample for extraction of serum for eGFR, CRP, CMP, TSH, and cystatin C, and one sample for biomarker serum.*
    - *If a participant consents to serum biobanking for future use, the sample will be collected from the same tube as the biomarker serum tube which is collected for all participants (no additional tubes are needed). For participants who do not consent, any remaining serum following completion of study tests will be destroyed by the central laboratory at the end of the study.*
    - ***Dry ice is required** for shipment of biomarker serum samples; see study lab procedure manual for collection, processing, and shipment procedures. Plan in advance for the availability of dry ice as samples must be shipped the same day.*
    - *If HbA1c value is not obtained from central laboratory because sample cannot be analyzed, it does not need to be repeated if available in the prior 3 months.*
    - *The blood draw does <u>not</u> need to be fasting.*
10. Urine sample *within 8 days of randomization* for central lab measurement of routine and biomarker labs:

    - Creatinine, albumin, and biomarkers such as MCP1, osteopontin, and IL-18 (by central lab)
        - ***Dry ice is required** for shipment of biomarker samples; see study lab procedure manual for collection, processing, and shipment procedures.*
    - Pregnancy test for all females who are premenopausal and are not surgically sterile
        - *Pregnancy test is conducted at the site and must be negative before proceeding to randomization.*

Screening procedures will last approximately two to three hours.

2.4 Randomization of Eligible Participants

Randomization does not have to be completed the same day as screening, provided all screening procedures are completed within the windows specified in section 2.3.1.

1. Prior to randomization, the study participant's understanding of the trial, willingness to accept the assigned treatment group, and commitment to the 12-month follow-up schedule should be reconfirmed.
2. The initial supply of Study Drug (tonabersat or placebo) will be provided on the day of randomization. The tonabersat and placebo pills will be provided in masked bottles by the Coordinating Center, or designee, to the clinical sites for distribution to the study participants.
3. Randomization is completed on the DRCR.net website.
    1. Study participants will be randomly assigned [stratified by CST category of the study eye on OCT (<75 μm above threshold, 75 to <175 μm above threshold, ≥175 μm above threshold), using the thicker eye if participant has two study eyes] with equal probability to one of the treatment groups:
        - 80 mg tonabersat
        - Placebo

Each group will be asked to start daily supplementation of Study Drug (tonabersat 80 mg or placebo) on the day of randomization, with the first dose (two pills) taken in the evening on the day of randomization per the dosing instructions.

2.5 Screen Failures

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

Chapter 3: Randomized Trial Procedures

3.1 Home Procedures

All participants will be instructed verbally regarding medication administration and will be provided with a medication use instruction sheet to take home. Participants should take two pills once daily for 6 months. It is recommended the Study Drug be taken in the evening without food, preferably at bedtime, to reduce the occurrence of side effects. The first dose (two pills) will be taken at home in the evening on the day of randomization.

The initial supply of Study Drug will be provided by the site on the day of randomization. Thereafter, Study Drug supply will be provided at subsequent follow-up visits when needed. Details regarding Study Drug accountability are included in the coordinator manual. See Chapter 4 for additional details regarding Study Drug.

Upon completion of the 6-month visit, participants will stop all use of study drug and return any unused to the site. However, participants will remain enrolled in the trial until completion of the 12-month follow-up visit.

3.2 Text Message Reminders

For participants who agree to receive text message reminders, periodic messages will be sent by the Coordinating Center with a reminder to comply with the Study Drug schedule.

3.3 Study Visits and Phone Contacts

A phone call is completed by site personnel within approximately one week of randomization (5 to 14 days) to emphasize compliance with Study Drug use (including confirmation that the study drug was started on the day of randomization) and answer any questions. Additional phone contacts may be performed as needed.

The schedule of protocol-specified assessment visits is detailed below. If rescue treatment is deemed necessary prior to the primary outcome visit at 6 months, the 6-month visit study procedures also will be performed prior to initiating intraocular medication, PRP or vitrectomy for the first time. Once a patient has received intraocular medication, PRP or vitrectomy, tonabersat will be discontinued but follow-up will continue until completion of the 12-month visit.

3.3.1 Study Visits

Study visits will occur at:

Visit	Target Day/Week	Target Window (around Target Day/Week)	Allowable Window (around Target Day/Week)
1 Week Phone Call	7 days	± 2 days	-2 day/+7 days
1 Month	4 weeks	± 1 week	± 2 weeks
2 Month	8 weeks	± 1 week	± 2 weeks
3 Month	12 weeks	± 1 week	± 2 weeks
4 Month	16 weeks	± 1 week	± 2 weeks
5 Month	20 weeks	± 1 week	± 2 weeks

6 month/Primary Outcome Visit*	24 weeks	± 2 weeks	± 4 weeks
12 month follow-up visit	52 weeks	± 4 weeks	± 6 weeks

*Key visit

The goal will be for all participants to complete all scheduled visits. However, participants who (because of unforeseen circumstances) are unable or unwilling to return for all follow-up visits will be permitted to return for key visits only as an alternative to withdrawal from the study. When a participant is placed into this status as documented, missed visits will not be recorded as protocol deviations (since they would not be recorded as protocol deviations if the participant was dropped from the study).

Additional office visits may occur as needed.

3.3.2 Procedures at Study Visits

Month 1 – 5 Visits

The following procedures will be performed in both groups at each visit:

1. General medical update, including a review of any outstanding and collection of any new adverse events.

2. E-ETDRS visual acuity testing (best corrected) in both eyes.

    - A protocol refraction in the study eye is required at each visit. Refraction in the non-study eye is not required. When a refraction is not performed, the most recently performed refraction is used for the testing.

3. Spectral-Domain OCT using Zeiss Cirrus or Heidelberg Spectralis on the study eye(s) only

    - The same machine type (Cirrus or Spectralis) used at baseline should be used during follow-up.

4. Ocular exam including slit lamp examination, lens assessment, measurement of intraocular pressure and dilated ophthalmoscopy on the study eye(s) only

5. Measurement of blood pressure.

6. Routine central lab samples – Blood & urine Samples (collected only at the 1 and 3-month visits for central lab, does not need to be fasting)

    - Whole blood sample for HbA1c and CBC

    - Serum sample for eGFR, CRP, CMP, cystatin C

    - Urine sample for creatinine & albumin

7. Urine sample for pregnancy test for all females who are premenopausal and are not surgically sterile (performed in clinic at all visits)

8. Compliance assessment

If treatment is planned for the first time at a month 1-5 visit (e.g., anti-VEGF or corticosteroid intravitreous injection, PRP, focal/grid laser, or vitrectomy), the participant will be asked to complete all ocular study procedures from the 6-month visit before the treatment is administered. Standard labs should also be collected pre-treatment, and additional biomarker lab samples, when feasible. If other treatment is given, Study Drug will be discontinued but participation in the study will be continued until completion of the 12-month visit.

Month 6 (Primary Outcome) Visit

The following procedures will be performed in both groups:

1. General medical update, including a review of any outstanding and collection of any new adverse events.

2. E-ETDRS visual acuity testing (best corrected) in both eyes.

    - A protocol refraction in both eyes is required at the 6-month visit.

3. Spectral-Domain OCT using Zeiss Cirrus or Heidelberg Spectralis on both eyes

    - The same machine type (Cirrus or Spectralis) used at baseline should be used during follow-up.

4. Digital fundus photographs on both eyes using the widest approach available (e.g. ultra-widefield imaging device, if available).

5. Ocular exam including slit lamp examination, lens assessment, measurement of intraocular pressure and dilated ophthalmoscopy on both eyes

6. OCT angiography on the study eye(s) only.

7. Physical examination to include:
    - Weight
    - Measurement of blood pressure

8. Blood Draw for Central Lab
    - Whole blood sample for HbA1c and CBC

- Serum samples for eGFR, CRP, CMP, cystatin C, biomarker testing such as Galectin 3, IL-1 β, IL-6, TNFα, and biobanking samples for future use (optional for participant)
    - *Does not need to be fasting.*
    - *If a participant consents to serum biobanking for future use, the sample will be collected from the same tube as the biomarker serum tube (no additional tubes are needed). For participants who do not consent, any remaining serum following completion of study tests will be destroyed by the central laboratory at the end of the study.*
    - ***Dry ice required** for additional serum samples at the 6-month visit; see study lab procedure manual for collection, processing, and shipment procedures.*

9. Urine sample for:
    - Creatinine, albumin, and biomarkers such as MCP1, osteopontin, and IL-18.
        - *Dry ice required for shipment of additional urine sample at the 6-month visit; see study lab procedure manual for collection, processing, and shipment procedures.*
    - Pregnancy test for all females who are premenopausal and are not surgically sterile (performed in clinic at <u>all visits</u>)
10. Compliance assessment

<u>Month 12 Follow-up Visit</u>

The following procedures will be performed in both groups at each visit:

1. General medical update, including a review of any outstanding and collection of any new adverse events.
2. E-ETDRS visual acuity testing (best corrected) in both eyes.
    - A protocol refraction in both eyes is required at the 12-month visit.
3. Spectral-Domain OCT using Zeiss Cirrus or Heidelberg Spectralis on both eyes
    - The same machine type (Cirrus or Spectralis) used at baseline should be used during follow-up.
4. Ocular exam including slit lamp examination, lens assessment, measurement of intraocular pressure and dilated ophthalmoscopy on both eyes
5. Physical examination to include:

- Weight
- Measurement of blood pressure

6. Routine central lab samples – Blood & urine Samples (does not need to be fasting)
    - Whole blood sample for HbA1c and CBC
    - Serum sample for eGFR, CRP, CMP, cystatin C
    - Urine sample for creatinine & albumin
7. Urine sample for pregnancy test for all females who are premenopausal and are not surgically sterile (performed in clinic at all visits)

3.3.3 Compliance Assessment

At the month 1-5 visits, the participant will receive a new supply of medication if they do not have enough to last until the next study visit. The participant will be asked to bring their remaining pills (including empty bottles) to the clinic for compliance assessment. If a visit is missed, the participant may be sent a limited supply via mail and asked to return the remaining used pill bottles using a pre-addressed return package back to the site. Site personnel will count the remaining pills for each returned bottle and enter the number on an eCRF, which will calculate the compliance level. After the pill count at the months 1-5 visits, the partial or unused bottles already assigned will be returned to the participant.

3.4 Early Termination Visit

Study participants who request to withdraw will be asked to have a final closeout visit at which the testing described for the 6-month will be performed.

3.5 Unscheduled Visits

Additional visits may occur as required for usual care of the study participant. Testing procedures at unscheduled visits are at investigator discretion. However, it is recommended that procedures that are performed should follow the standard DRCR Retina Network protocol for each procedure.

3.5.1 Other Treatment Initiation Visit Prior to 6-month Visit

Participants for whom other treatment is planned for the first time at an unscheduled visit prior to the 6-month visit (e.g., anti-VEGF or corticosteroid intravitreous injection, PRP, focal/grid laser, or vitrectomy) will be asked to complete all ocular study procedures on the study eye from the 6-month visit before the treatment is administered. Standard labs should also be collected pre-treatment, and additional biomarker lab samples, when feasible (since dry ice is required for shipment). If other treatment is given for DR, but not DME, Study Drug should be continued. If other treatment is given for DME in the study eye (or both study eyes if two eyes are enrolled),

Study Drug will be discontinued but participation in the study will be continued until completion of the 12-month visit.

3.5.2 Other Treatment Initiation Visit After 6-month Visit

Following completion of the 6-month visit, but prior to the 12-month final visit, other treatment initiation is at the discretion of the investigator. If other treatment is given between 6-12 months, it will be recorded as part of an unscheduled visit at which testing is at investigator discretion.

3.6 Other Treatment for CI-DME Prior to 6-Month Visit

Treatment for CI-DME must not be given **prior to the 6-month visit** unless one of the following criteria have been met or protocol chair approval is obtained:

1) at least 10 letter decrease in visual acuity from baseline presumed to be from DME at a single visit
   or
2) at least a 5 letter decrease in visual acuity from baseline presumed to be from DME at two-consecutive visits at least 21 days apart.

Once criteria are met, other treatment is at the discretion of the investigator as part of usual care. If other treatment for DME is given in the study eye (or both study eyes if two eyes are enrolled), prior to 6 months, Study Drug will be discontinued but participation in the study will be continued until completion of the study at 12 months.

Following completion of the 6-month primary outcome visit, but prior to the 12-month follow-up visit, other treatment for DME is at the discretion of the investigator. If other treatment is given between 6-12 months, the site should record testing and treatment at the unscheduled visit on the study website.

3.7 Participant Access to Study Agent at Study Closure

Any remaining Study Drug must be returned during the 6-month visit. Participants will not be provided study drug during the follow-up period from 6 to 12 months, nor after completion of the 12-month visit. Participants who withdraw from the study prior to the 6-month visit will be asked to return the remaining drug for a final compliance assessment.

Chapter 4: Study Drug

4.1 Study Agent(s) and Control Description

4.1.1 Acquisition

Tonabersat and matching placebo, referred to collectively as Study Drug, will be provided by InflammX Therapeutics, Inc.

The Study Drug will be bottled and labeled with an identifying drug number by InflammX. The Study Drug will be shipped to the central pharmacy responsible for drug distribution to clinical sites according to Study Drug accountability procedures. The packaging for both active and placebo Study Drug will be identical to keep participants and personnel masked. If necessary, Study Drug may also be mailed directly to the participant from the central pharmacy.

4.1.2 Formulation, Appearance, Packaging, and Labeling

All Study Drug dispensed to the participants will be labeled and identified with the study name, drug name, expiration date, drug number, dosing instructions, directions for storage and use, and indication that it is an investigational drug. Placebo packaging will be identical in order to keep study personnel and participants masked. The type of medication (tonabersat vs. placebo) will be tracked by drug number only.

4.1.3 Product Storage and Stability

Study Drug will be stored at the central pharmacy and each clinical site in a room temperature, limited access, secure area. Sites and participants will be instructed to follow manufacturer recommendations for drug storage.

4.1.4 Dispensing Study Drug

At each visit, participants who have not discontinued Study Drug use will be provided a sufficient number of bottles of Study Drug to last through approximately two visits. This quantity ensures enough medication is provided if one follow-up visit is missed. However, additional Study Drug will not be dispensed to the participant if more than one study visit is missed without the investigator first confirming there are no concerns with systemic safety.

4.1.5 Study Drug Return and Compliance Assessment

At each follow-up visit, the participants will be asked to return the prior assigned study drug bottles (including any unused pills, empty bottles, and unopened bottles) to the clinical site for a compliance assessment. The clinical site will check-in the Study Drug and perform a compliance assessment based on the number of pills remaining compared with the number expected, which will be calculated on the study website.

4.1.6 Dosing and Route of Administration

Two pills of Study Drug (80 mg tonabersat or placebo) are taken orally once daily. It is recommended the Study Drug be taken in the evening without food, preferably at bedtime, to reduce the occurrence of side effects. The first dose (two pills) will be taken at home on the day of randomization. If a participant reports intolerable side effects with the 80 mg dose taken as

instructed (two pills at bedtime), the site investigator may down-titrate the participant's dose to a once daily 40 mg dose as an alternative to stopping all study drug.

4.1.7 Duration of Therapy

Participants will continue to take the Study Drug daily for approximately 6 months, unless the criteria for stopping Study Drug are met in Section 6.5.

4.1.8 Study Drug Accountability Procedures

The pharmacy, Coordinating Center, and clinical sites will follow detailed drug accountability procedures. Drug accountability procedures for sites will be detailed in the coordinator manual.

4.2 Unmasking Procedure

In general, all study personnel and participants will remain masked to the treatment assignment throughout the duration of the study. This applies even if the participant's dose is discontinued. In certain circumstances, a site may request that the participant and/or care provider be unmasked to the treatment group assignment during the study if medically necessary. In general, unmasking is only permitted in cases where site/participant knowledge of the treatment group assignment is necessary for the safety or care of the participant. Coordinating Center approval is required, after consultation with the Protocol or Network Chair. The Medical Monitor also will be notified of the request.

Chapter 5: Testing Procedures

5.1 Testing Procedures

The testing procedures are detailed in the DRCR Retina Network (or "DRCR.net") procedures manuals. An overview of the equipment and certification requirements for all testing are as follows.

Study Procedures	Equipment Required (if applicable)	Who can Perform
Ocular Exam (including slit lamp examination, lens assessment, and dilated ophthalmoscopy)	Any equipment is acceptable	Certified investigator
Physical examination (height and weight)	Any equipment is acceptable	Does not need to be performed by study certified personnel*
Blood pressure	Proper size blood pressure cuff	Does not need to be performed by study certified personnel*
Intraocular pressure IOP	A Goldmann tonometer should be used if available.	Does not need to be performed by study certified personnel*
Visual Acuity – Refraction	EVA refraction chart and trial frames	Clinical site personnel certified for protocol refraction
Visual Acuity – ETDRS	EVA system (preferred) otherwise ETDRS charts if EVA not available	Clinical site personnel certified for ETDRS
SD-OCT	Zeiss Cirrus or Heidelberg Spectralis	Clinical site personnel certified for specific SD-OCT machine type
Fundus Photography	Optos, if available, otherwise a digital system certified by the central Reading Center	Clinical site personnel certified for fundus photography
OCT angiography	Zeiss AngioPlex, Optovue AngioVue, or additional system approved by central Reading Center	Clinical site personnel certified for specific OCTA machine type
Sample collection and processing	Centrifuge required; collection kit provided by study	Does not need to be performed by study certified personnel*
Sample shipment	Shipping materials provided by study; dry ice required for some shipments	IATA trained personnel*

Pregnancy Urine Test	Urine aliquot from main urine sample and HCG testing kit	Does not need to be performed by study certified personnel*
** Personnel who will be performing procedure must be documented in the Study Staff Delegation Log. The Principal Investigator (PI) is responsible for verifying individual qualifications and training specific to performing each type of procedure and ultimate accuracy and integrity of such data*		

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Chapter 6: Unanticipated Problem and Adverse Event Reporting

6.1 Unanticipated Problems

Site investigators will promptly report all unanticipated problems meeting the criteria below on an eCRF. Sites overseen by the JCHR IRB must report Unanticipated Problems to the IRB within seven (7) calendar days of recognition. For this protocol, an unanticipated problem is an incident, experience, or outcome that meets all the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places participants or others at a greater risk of harm than was previously known or recognized (including physical, psychological, economic, or social harm)

The Coordinating Center also will report to the IRB all unanticipated problems not directly involving a specific site such as unanticipated problems that occur at the Coordinating Center or at another participating entity such as a pharmacy or laboratory. These instances must be reported to the JCHR IRB within seven (7) calendar days of recognition. The Director of the Human Research Protection Program (HRPP) will report to the appropriate regulatory authorities if the IRB determines that the event indeed meets the criteria of an Unanticipated Problem that requires further reporting to fulfill the reporting obligations of the HRPP.

6.2 Adverse Events

6.2.1 Definitions

Adverse Event (AE): Any untoward medical occurrence (including laboratory findings) associated with study procedures, the use of a device, biologic, or drug in humans, including any comparator used, whether or not the event is considered related (i.e., irrespective of the relationship between the adverse event and the drug(s) under investigation) (referred to as *Adverse Reaction* when caused by a drug).

To further clarify, for this protocol an adverse event is considered any untoward medical occurrence, unintended disease or injury, or untoward clinically significant clinical sign (including abnormal laboratory findings) in a research participant that manifests while in the study if it was not present before enrolling in the study, or if it was present before enrolling, it has increased in severity, frequency or type since enrolling in the study.

Medical interventions such as surgeries, diagnostic procedures, and therapeutic procedures are not AEs, but the action taken to treat a particular medical condition. They should be recorded as treatment of the AEs.

Serious Adverse Event (SAE): Any untoward medical occurrence that results in any of the following outcomes:

- Death.
- A life-threatening adverse event; (a non-life-threatening event which, had it been more severe, might have become life-threatening, is not necessarily considered a serious adverse event).
- Inpatient hospitalization or prolongation of existing hospitalization.
- A persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.

An important medical event that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical and surgical intervention to prevent one of the outcomes listed in this definition. Note: If either the Sponsor or investigator believes that the event is serious, the event must be considered serious and evaluated by the Sponsor for expedited reporting. See 21 CFR 312 for more information.

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

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

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

Serious and Unexpected Suspected Adverse Reaction (SUSAR): An adverse event that is a suspected adverse reaction, is serious, and is unexpected. This event requires a submission of an IND Safety Report. See 21 CFR 312 for more information.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

6.2.2 Reportable Adverse Events

For this protocol, a reportable adverse event includes all events meeting the definition of an adverse event, or impacting use of Study Drug (including temporary or permanent discontinuation).

Surgical or medical procedures (e.g., appendectomy, endoscopy) are not reported as AEs per se, but the condition leading to the procedure will be reported as the AE with the procedure being reported as the management of the AE. A procedure (e.g., cosmetic surgery) performed in the absence of a condition meeting the definition of an AE will not be reported on an AE form.

All reportable Adverse Events whether volunteered by the participant, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported on an adverse event form online. Each adverse event form is reviewed by the Medical Monitor to verify the coding and the reporting that is required.

6.2.3 Relationship of Adverse Event to Study (Investigational) Drug or Study Procedure

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

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

Unrelated: The AE is clearly not related to a study drug/intervention/procedure and a likely alternative etiology exists such as an underlying disease, environmental or toxic factors or other therapy.

Unlikely Related: The AE does not follow a reasonable temporal sequence during or after use of study drug/intervention/procedure and a more likely alternative etiology exists such as an underlying disease, environmental or toxic factors, or other therapy.

Possibly Related: The AE occurred in a reasonable time during or after use of study drug/intervention/procedure; but could be related to another factor such as an underlying disease, environmental or toxic factors, or other therapy; and there is a possible, though weak, scientific basis for establishing a causal association between the AE and the study drug/intervention/procedure.

Probably Related: The AE occurred in a reasonable time during or after use of study drug/intervention/procedure; is unlikely to be related to another factor such as an underlying disease, environmental or toxic factors, or other therapy; and there is a plausible, though not strong, scientific basis for establishing a causal association between the AE and the study drug/intervention/procedure.

Definitely Related: The AE occurred in a reasonable time during or after use of study drug/intervention/procedure; cannot be explained by another factor such as an underlying disease, environmental or toxic factors, or therapy; and there is a strong scientific basis for establishing a causal association between the AE and the study drug/intervention/procedure.

Events determined to be *Possibly Related, Probably Related,* or *Definitely Related* will be considered to meet the *reasonable possibility* causality standard as related and necessitate reporting as required (see 21 CFR 312.32 for more information).

6.2.4 Study Drug Severity (Intensity) of Adverse Event

A severity assessment is a clinical determination of the intensity of an event. Thus, a severe adverse event is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious. The severity (intensity) of adverse events will be rated on a five-grade scale as follows:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (e.g., preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living (e.g., bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to the adverse event.

6.2.5 Study Drug Expectedness

For a serious adverse event that is considered possibly related to Study Drug, the Medical Monitor will classify the event as unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described in the IB.

6.2.6 Coding of Adverse Events

Adverse events will be coded using the MedDRA dictionary. To facilitate coding, the site will enter a preliminary MedDRA code which the Medical Monitor may accept or change (the Medical Monitor's MedDRA coding will be used for all reporting). The Medical Monitor will review the investigator's assessment of causality and may agree or disagree. Both the investigator's and Medical Monitor's assessments will be recorded. The Medical Monitor will have the final say in determining the causality as well as whether an event is classified as a serious adverse event and as a serious unanticipated event.

6.2.7 Outcome of Adverse Event

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

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

- RECOVERED/RESOLVED WITH SEQUELAE – AE/SAE where the subject recuperated but retained pathological conditions resulting from the prior disease or injury. Record the AE/SAE stop date.
- FATAL – A fatal outcome is defined as the SAE that resulted in death. Only the event that was the cause of death should be reported as fatal.
- ONGOING NOT RECOVERED/NOT RESOLVED – An ongoing AE/SAE is defined as an ongoing event with an undetermined outcome.
    - An ongoing outcome will require follow-up by the site in order to determine the final outcome of the AE/SAE.
    - The outcome of an ongoing event at the time of death that was not the cause of death will not be updated.
- ONGOING (MEDICALLY STABLE) – AE/SAE is ongoing, but medically stable. For example, a chronic condition where no further change is expected.

If any reported adverse events are ongoing when a participant completes the study (or withdraws), adverse events classified as suspected, unexpected serious adverse reactions (SUSARs) will be followed until they are either resolved, or have no prospect of improvement or change, even after the participant has completed all applicable study visits/contacts. For all other adverse events, data collection will end at the time the participant completes the study. Note: participants should continue to receive appropriate medical care for an adverse event after their participation in the study ends.

If a participant is lost to follow up and participant outcome cannot be determined, outcome classification will be the last known outcome.

6.3 Timing of Event Reporting

Serious or unexpected adverse events must be reported by the investigator within twenty-four (24) hours via completion of the online serious adverse event form.

Other reportable adverse events will be reported within three (3) days of the investigator becoming aware of the event by completion of an electronic case report form.

The Sponsor will notify the FDA and all participating investigators of any adverse event that is serious, related, and unexpected. Notification will be made no later than fifteen (15) calendar days after the Sponsor becomes aware of the event, and no later than seven (7) calendar days where the event is fatal or life-threatening.

Each principal investigator is responsible for reporting serious study-related adverse events and abiding by any other reporting requirements specific to his/her Institutional Review Board or Ethics Committee. Where the JCHR IRB is the overseeing IRB, sites must report all serious, related adverse events regardless of whether they are expected/anticipated and regardless of whether they are fatal or life-threatening within seven (7) calendar days.

6.4 Safety Oversight

The Medical Monitor will review all adverse events that are reported during the study. SAEs typically will be reviewed within twenty-four (24) hours of reporting. Other AEs typically will be reviewed on a weekly basis. Additionally, the Medical Monitor will review compiled safety data at periodic intervals (generally timed to the review of compiled safety data by the DSMC).

A Data and Safety Monitoring Committee (DSMC) will advise the Coordinating Center regarding the protocol, template informed consent form, and protocol amendments and will provide independent monitoring of adverse events. Cumulative adverse event data are semi-annually tabulated for review by the DSMC. Following each DSMC data review, a summary will be provided to institutional review boards. A list of specific adverse events to be reported to the DSMC expeditiously, if applicable, will be compiled and included as part of the DSMC Monitoring Plan. The DSMC can request modifications to the study protocol or suspension or outright stoppage of the study if deemed necessary based on the totality of safety data available.

6.5 Stopping Criteria

6.5.1 Participant Discontinuation of Study Drug Prior to 6-month Visit

Rules for discontinuing Study Drug use prior to the 6-month primary outcomes visit are described below.

- The investigator believes it is unsafe for the participant to continue to receive the drug. This could be due to the development of a potential side effect of the drug, a new medical condition or worsening of an existing condition; or participant behavior contrary to the indications for use of the drug that imposes on the participant's safety
- The participant requests that the treatment be stopped
- Participant pregnancy
- The study eye is treated with intraocular medication, laser or surgery for DME.

Even if the Study Drug is discontinued, the participant will be encouraged to remain in the study through the final 12-month study visit.

6.5.2 Criteria for Suspending or Stopping Overall Study

If applicable, a formal plan for interim data monitoring for both futility and efficacy will be established in consultation with the Data and Safety Monitoring Committee. In addition, the DSMC may request suspension of study activities or termination of the study if deemed necessary at any time based on the totality of safety data available. Review of serious, unexpected, and related AEs by the Medical Monitor, DSMC, IRB, or the FDA or relevant local regulatory authorities may also result in suspension of further study agent administration. The FDA and study sponsor(s) retain the authority to suspend additional enrollment and study agent for the entire study, as applicable. The study may be discontinued by the Executive Committee (with approval of the DSMC) prior to the preplanned completion of follow-up for all study participants.

6.6 Unmasking Due to Adverse Event

Unmasking due to an adverse event would follow the unmasking procedures described in section 4.2. Coordinating Center approval is required, after consultation with the Protocol or Network Chair. The Medical Monitor also will be notified of the request.

Chapter 7: Miscellaneous Considerations

7.1 Collection of Medical Conditions and Medications

Pre-Existing Condition: Any medical condition that is either present at screening, a chronic disease, or a prior condition that could impact the participant's health during the course of the study (e.g., prior myocardial infarction or stroke) should be recorded.

Medications: All medication for the treatment of chronic pre-existing conditions, medical conditions, and/or adverse events that the participant is currently taking at screening and during the course of the study should be recorded. Nutraceuticals and preventative treatment also should be recorded.

7.2 Prohibited Medications, Treatments, and Procedures

Other treatment for DME will not be permitted prior to the 6-month visit unless protocol criteria in section 3.6 are met or treatment is discussed with and approved by the Protocol Chair or Coordinating Center designee.

7.3 Precautionary Medications, Treatments, and Procedures

There are no known food or drug interactions or precautions for concomitant use with study agents.

7.4 Prophylactic Medications, Treatments, and Procedures

Not Applicable.

7.5 Rescue Medications, Treatments, and Procedures

If criteria are met prior to the 6-month visit, other treatment for DME may be provided as part of usual care. Once a patient has received intraocular medication, laser, or vitrectomy for DME in the study eye, the Study Drug will be discontinued but follow-up will continue until completion of the 12-month visit.

Following completion of the 6-month visit, but prior to the 12-month final visit, other treatment for DME is at the discretion of the investigator.

7.6 Pregnancy Reporting

If pregnancy occurs, study intervention will be discontinued while continuing safety follow-up. The occurrence of pregnancy must be reported to the Coordinating Center within seven (7) days of notification and to the JCHR IRB as an Unanticipated Problem within seven (7) calendar days. An electronic case report form must be completed for all confirmed pregnancies.

7.7 Participant Compensation

Participant compensation will be specified in the informed consent form.

7.8 Participant Withdrawal

Participation in the study is voluntary, and a participant may withdraw at any time. For participants who withdraw, their data will be used up until the time of withdrawal.

7.9 Confidentiality

For security and confidentiality purposes, participants will be assigned an identifier that will be used instead of their name. Protected health information gathered for this study will be shared with the coordinating center, the Jaeb Center for Health Research in Tampa, FL. De-identified participant information may also be provided to research sites involved in the study.

7.9.1 Contact Information Provided to the Coordinating Center

If approved by the overseeing IRB/ethics board, the Coordinating Center will be provided with contact information for each study participant. Permission to obtain such information will be included in the Informed Consent Form. The contact information will be maintained in a secure database and will be maintained separately from the study data.

When contact information is provided, phone contact from the Coordinating Center will be made if necessary, to facilitate the scheduling of the study participant for follow-up visits. A participant-oriented newsletter and a study logo item may be sent once. For participants who agree to text message reminders, periodic messages will be sent by the Coordinating Center with a reminder to comply with the study medication schedule.

Study participants will be provided with a summary of the study results in a newsletter format after completion of the study by all study participants.

In addition, name and mailing address may be provided to the central pharmacy for Study Drug distribution if needed between visits.

Chapter 8: Statistical Considerations

8.1 Statistical and Analytical Plans

The approach to sample size and statistical analyses are summarized below. A detailed statistical analysis plan will be written and finalized prior to the first enrollment.

8.2 Statistical Hypotheses

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

Null Hypothesis (H_0): There is no difference in the mean change in CST from baseline to 6 months between tonabersat and sham groups.

Alternative Hypothesis (H_a): There is a difference in the mean change in CST from baseline to 6 months between tonabersat and sham groups.

8.3 Sample Size

As a phase 2 study, the primary objective will be to determine if there is a short-term positive effect of tonabersat on central subfield thickness to warrant conduct of a larger randomized trial. A sample size of 128 participants (64 per treatment group) will be randomized for this purpose.

Results of this study will provide information to aid development of a phase 3 trial including duration of drug effect, frequency of resolution of edema, comparison of Study Drug against placebo, and safety evaluation of a novel oral treatment.

8.3.1 Outcome Projections

Data from DRCR Retina Network Protocol AE and Protocol V were used to estimate the standard deviation. Both studies enrolled eyes with CI-DME and good vision (VA of 20/25 or better). Protocol AE randomized eyes to a photobiomodulation (PBM) or placebo device, with the primary outcome at four months. Protocol V randomized eyes to initial aflibercept, focal/grid laser, or observation, with the primary end at two years. In Protocol V, eyes randomized to laser and observation could receive aflibercept if VA worsened during follow-up. The table below shows the standard deviations of CST change adjusted for correlation with baseline CST and truncation of outliers. CST change was truncated to within 3 standard deviations of the overall mean evaluated at the studies' primary end point.

Study	Time	Treatment Group	N	Standard Deviation (95% CI), µm, of CST change, adjusted for correlation with baseline CST	Correlation (95% CI) between CST change and baseline CST
Protocol AE*	Baseline to 4 months	PBM	68	53 (45 to 64)	-0.16 (-0.39 to 0.08)
		Placebo	66	58 (49 to 70)	0.03 (-0.21 to 0.27)
Protocol V**	Baseline to 4 months	Laser	228	47 (43 to 52)	-0.33 (-0.44 to -0.21)
		Observation	223	48 (44 to 53)	-0.35 (-0.46 to -0.23)
	Baseline to 1 year	Laser	223	57 (52 to 63)	-0.56 (-0.64 to -0.46)
		Observation	220	67 (61 to 74)	-0.41 (-0.52 to -0.30)

*In Protocol AE, CST change was truncated to within 13 ± 3 × 58, and eyes that received another treatment for DME (N = 3 (PBM); N = 1 (placebo)) used the last OCT measurement prior to receiving the other treatment.

**In Protocol V, CST change was truncated to within -44 ± 3 × 75.

8.3.2 Sample Size Estimates

The sample size estimate was calculated for the primary outcome of mean change in CST from baseline to 6 months. The table below shows the total number of eyes required to reject the null hypothesis of a zero-mean difference in a two-sided superiority test with 90% power, a 5% type I error rate, and a 5% loss to follow-up under various true mean difference and standard deviation assumptions.

True Mean Difference, µm	Effective Standard Deviation*, µm			
	45	**50**	**55**	**60**
20	228	280	338	404
30	104	**128**	152	182
40	60	72	88	104

*Effective standard deviation = standard deviation in CST change adjusted for correlation with baseline CST and truncation of outliers.

Assuming an effective standard deviation of 50 microns and a true difference in mean CST of 30 microns, the required total sample size is 128 eyes to achieve 90% power with 5% type I error rate and a 5% loss to follow up. A total of 128 participants with at least one eligible eye will be recruited. If both eyes from a participant are eligible then both eyes will be included in the analysis and random effects will be used to model the potential correlation between two eyes from the same participant. As this power calculation is based on one eye per participant, the power is expected to be greater than this projection.

8.4 Outcome Measures

Outcomes marked with an asterisk (*) will include descriptive statistics by treatment group only. Outcomes without an asterisk will include a point estimate and 95% confidence interval of the treatment effect (mean difference, odds ratio, or risk difference) but only the primary and secondary outcomes may include p-values. P-values will not be reported for exploratory outcomes.

Primary Outcome:

- Change in CST from baseline to 6 months

Secondary Outcomes:

- Change in retinal volume from baseline to 6 months
- CST below the OCT machine- and sex-specific threshold for CI-DME at 6 months and a 10% or more decrease in CST from baseline to 6 months (composite outcome)
- Change in ETDRS BCVA from baseline to 6 months
- A decrease in ETDRS BCVA of 10 or more letters from baseline to 6 months, or 5 or more letters from baseline to 6 months and the previous study visit.
- Receiving other treatment for DME prior to the 6-month visit*

Exploratory Outcomes:

- Changes in DR on fundus photos from baseline to 6 months of
  - 1-step or more worsening*
  - 2-steps or more worsening
  - 1-step or more improvement*
  - 2-steps or more improvement
- Changes on OCTA from baseline to 6 months in
  - Vessel density
  - Nonperfusion area
- Treatment compliance from baseline to 6 months (percentage of prescribed study pills taken)*
- Changes in blood and urine samples from baseline to 6 months of
  - eGFR
  - CRP
  - HbA1c
  - Cystatin C
  - TSH
  - Galectin 3
  - IL-1 β
  - IL-6

- TNFα
- Urine creatinine
- Urine albumin
- Urine MCP1
- Urine osteopontin
- Urine IL-18

8.5 Analysis Datasets and Sensitivity Analyses

The analyses of the primary, secondary, exploratory, and safety outcomes will follow the intention-to-treat (ITT) principle, analyzing all randomized participants according to their random group assignment, regardless of treatment received. A sensitivity analysis for the primary outcome will provide additional information on the magnitude of the treatment effect using a per-protocol analysis (PPA) cohort if the PPA criteria would exclude more than 10% of randomized participants. The ITT analysis is considered primary; if the ITT and PPA analysis are inconsistent, an exploratory analysis will evaluate possible differences.

- ITT Analysis Cohort: all randomized participants irrespective of treatment received, analyzed according to treatment assignment
- Safety Analysis Cohort: all randomized participants irrespective of treatment received, analyzed according to treatment assignment
- PPA Analysis Cohort: participants who complete the 6-month visit, achieve at least 75% compliance with the assigned Study Drug when required to be taken (prior to protocol-allowed discontinuation), and do not receive any other treatment for DME

8.6 Analysis of the Primary Outcome

The primary outcome of change in CST from baseline to 6 months is a continuous variable. The mean difference between treatment groups will be estimated using a linear mixed model with adjustment for baseline CST. Participant-level random intercept effects will model the potential correlation within participants having two study eyes. The assumptions of linearity, normality, and homoscedasticity will be verified using graphical methods. If serious violations occur, a generalized linear model (such as gamma or lognormal distributions for skewed data) or non-parametric method may be considered to estimate the mean treatment group difference. Missing data for eyes not treated with another DME treatment will be imputed using Markov chain Monte Carlo (MCMC) multiple imputation (MI). The imputation model will stratify by treatment group and include baseline CST and changes in CST from baseline to all monthly visits. For eyes that receive treatment for DME (see section 3.6) prior to the 6-month visit, OCT measurements after treatment will be considered missing for imputation, and the primary analysis will use change from baseline to the last OCT measurement prior to treatment (last observation carried forward, LOCF). Change in CST from baseline to 6 months/LOCF will be truncated at the overall mean ±3 SD of the observed values so that statistical outliers do not disproportionately impact the analysis (imputed values not used to truncate and truncated values not used to impute). Descriptive statistics for each treatment group will include the mean, standard deviation, median and interquartile range using the truncated data from eyes that completed the

6-month visit or received DME treatment prior to the 6-month visit (no imputed values). The estimated treatment effect will include the adjusted mean difference, 2-sided 95% confidence interval, and p-value using all MI/LOCF data. OCTs can be obtained from either Spectralis or Cirrus images. Values from these machines cannot be used interchangeably. OCT values will be converted to a common value for reporting and analyses as described in the SAP.

8.7 Analysis of Secondary and Exploratory Outcomes

The analysis of all secondary and exploratory outcomes will use the ITT analysis cohort. Unless otherwise specified, eyes that receive treatment for DME (see section 3.6) will use LOCF values for analysis. Missing data for eyes that did not receive treatment for DME will be imputed with multiple imputation for the secondary outcomes but not for the exploratory outcomes. The imputation models will be stratified by treatment group and include baseline CST (the randomization stratification factor), the baseline value of the outcome and changes in the outcome from baseline to all monthly visits. All values after DME treatment will be considered missing for the imputation and then overwritten with LOCF values for analysis. Continuous outcomes will be truncated at the overall mean ±3 SD so that statistical outliers do not disproportionately impact the analysis. Truncation will be performed after imputation (where applicable) and truncation limits will be determined from the observed values (imputed values not used to truncate and truncated values not used to impute).

All secondary outcomes will be summarized with descriptive statistics by treatment group, but only some will have inferential statistics on treatment effect. Descriptive statistics will be computed using observed 6-month/LOCF data only (no imputed values). For continuous outcomes, means with standard deviations, and/or medians with interquartile ranges of the truncated data will be reported. For binary outcomes, frequencies with percentages will be reported. Treatment effects will include mean differences and odds ratios that adjust for baseline levels of the outcome and baseline CST (the randomization stratification factor). Treatment effects will be estimated using linear or generalized linear models with multiple imputation for missing data (where applicable). For eye-level outcomes, random intercepts will model the potential correlation among participants having two study eyes. For participant-level outcomes, a participant-level model will be used.

For each continuous outcome with a mean difference estimated from a linear model, the assumptions of linearity, normality, and homoscedasticity will be verified using graphical methods. If serious violations occur, a generalized linear mixed model or non-parametric method may be considered to estimate the mean difference. The adjusted mean difference between treatment groups and two-sided 95% CI will be reported.

For each binary outcome with an odds ratio estimated from a generalized linear model with a logistic link function, the adjusted odds ratio between treatment groups, and two-sided 95% CI will be reported. Additionally, the risk difference between treatment groups and two-sided 95% CI will be estimated at the mean levels of other covariates in the model, with the variance estimated using the delta method.[19]

P-values will be reported for two-sided tests of superiority on the pre-specified secondary outcomes only. P-values will not be reported for exploratory outcomes. The false discover rate (FDR) of secondary outcomes will be controlled at .05 using the Benjamini-Hochberg adaptive

FDR procedure.[20] The steps to determine if a null hypothesis for a secondary outcome is rejected or not using the adaptive FDR procedure is outlined in the SAP.

Secondary Outcomes:

- Change in retinal volume from baseline to 6 months is a continuous variable. The mean difference between treatment groups will be estimated using a linear mixed model with adjustment for baseline retinal volume, baseline CST, and participant-level random intercepts.

- CST below the OCT machine- and sex-specific threshold for CI-DME at 6 months and a 10% or more decrease in CST from baseline to 6 months is a binary variable. The odds ratio between treatment groups will be estimated using a generalized linear mixed model for a binary outcome with a logit link function and adjustment for baseline CST and participant-level random intercepts. Missing data will not be imputed because DME indicators are OCT machine- and sex-specific.

- Change in ETDRS BCVA from baseline to 6 months is a continuous variable. The mean difference between treatment groups will be estimated using a linear mixed model with adjustment for baseline ETDRS BCVA, baseline CST, and participant-level random intercepts.

- A decrease in ETDRS BCVA of 10 or more letters from baseline to 6 months, or of 5 or more letters from baseline to 6 months and the previous study visit is a binary variable. The odds ratio between treatment groups will be estimated using a generalized linear mixed model for a binary outcome with a logit link function and adjustment for baseline ETDRS BCVA, baseline CST, and participant-level random intercepts.

- Receiving any other treatment for DME prior to 6 months is a binary variable. The odds ratio between treatment groups will not be estimated with a model or tested with a hypothesis, but descriptive statistics will be used to summarize receipt of other DME treatment in each treatment group. Descriptive statistics will only use data from eyes that received other treatment for DME or completed the 6-month visit without receiving other treatment for DME.

Exploratory Outcomes:

- A 1-step or more worsening of DR and a 1-step or more improvement of DR on fundus photos from baseline to 6 months are binary variables. The odds ratios between treatment groups will not be estimated with a model or tested with a hypothesis, but descriptive statistics will be used to summarize DR worsening and improvement in each treatment group.

- A 2-step or more worsening of DR and a 2-step or more improvement of DR on fundus photos from baseline to 6 months are binary variables. The odds ratios between treatment groups will be estimated using generalized linear mixed models for a binary outcome with

a logit link function and adjustment for baseline DR, baseline CST, and participant-level random intercepts.

- Change in OCTA vessel density and change in OCTA nonperfusion area from baseline to 6 months are continuous variables. The mean differences between treatment groups will be estimated using linear mixed models with adjustment for the baseline level of the variable, baseline CST, and participant-level random intercepts.

- Treatment compliance from baseline to 6 months is a participant-level continuous variable. The mean difference between treatment groups will not be estimated with a model or tested with a hypothesis, but descriptive statistics will be used to summarize compliance in each treatment group.

- The exploratory outcomes change in eGFR, CRP, HbA1c, Cystatin C, TSH, Galectin 3, IL-1 β, IL-6, TNFα, urine creatinine, urine albumin, urine MCP1, urine osteopontin, and urine IL-18 from baseline to 6 months are all participant-level continuous variables. The mean differences between treatment groups will be estimated using linear models with adjustment for baseline values of the outcome and baseline CST.

8.8 Safety Analysis

The analysis of all safety outcomes will use the ITT cohort, analyzing all randomized participants by treatment assignment, irrespective of treatment received. Safety outcomes will be summarized with descriptive statistics only and will be reported for the 6-month (primary outcome visit) and 12-month follow-up safety visit (study treatment discontinued after 6 months). No formal hypothesis test comparing safety outcomes between treatment groups will be performed. All reportable adverse events (AEs) will be categorized as ocular or systemic. All events will be tabulated by treatment group in a listing of each reported Medical Dictionary for Regulatory Activities (MedDRA) term and summarized over each MedDRA System Organ Class. For each ocular AE, the number of events and the number of eyes that had the event will be reported separately for study and non-study eyes. For each systemic AE, the number of events and the number of participants that had the event will be reported. In addition to the listings of all AEs, the following safety outcomes will be tabulated.

Ocular Safety Outcomes (at 6 and 12 months)

- At least 1 ocular AE

- At least 1 serious ocular AE

- At least 1 serious ocular AE for each MedDRA System Organ Class reported

- At least 1 ocular AE thought by investigator to be related to study treatment

- Frequencies of ocular AEs thought by investigator to be related to study treatment

Systemic Safety Outcomes (at 6 and 12 months)

- At least 1 systemic AE
- At least 1 serious systemic AE
- At least 1 serious systemic AE for each MedDRA System Organ Class reported
- At least 1 systemic AE thought by investigator to be related to study treatment
- Frequencies of systemic AEs thought by investigator to be related to study treatment (systemic side effects)
- Frequencies of headache, drowsiness, or dizziness
- A change in renal function defined as
    - eGFR reduced from ≥ 60 to < 60
    - eGFR reduced from ≥ 30 to < 30
    - Dialysis
    - Renal transplant
- An increase in liver function tests defined as greater than 3 times the upper limit of the normal range by sex:
    - ALT: male >123 U/L, female >99 U/L
    - AST: male >120 U/L, female >96 U/L
- A change in any blood count analyte from a normal to abnormal range
- A change in any metabolic panel analyte from a normal to abnormal range

Other Safety Outcomes at 12 months

- Change in CST from 6 to 12 months
- Change in retinal volume from 6 to 12 months
- CST below the OCT machine- and sex-specific threshold for DME at 12 months and a 10% or more decrease in CST from 6 to 12 months
- Change in ETDRS BCVA from 6 to 12 months
- Receiving other treatment for DME between the 6 and 12-month visit
- Changes in blood and urine samples from 6 to 12 months

- eGFR
- CRP
- HbA1c
- Cystatin C
- TSH
- Galectin 3
- IL-1 β
- IL-6
- TNFα
- Urine creatinine
- Urine albumin
- Urine MCP1
- Urine osteopontin
- Urine IL-18

8.9 Intervention Adherence

The clinical site will perform a compliance assessment each time Study Drug is returned (approximately 1-month intervals). Treatment compliance will be calculated as one minus the proportion of pills remaining out of the number expected to have been taken.

8.10 Protocol Adherence and Retention

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

8.11 Baseline Descriptive Statistics

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

8.12 Planned Interim Analyses

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

8.13 Sub-Group Analyses

Subgroup analyses/assessments of effect modification (interaction) will be conducted for the primary outcome. Since there is no strong prior rationale for potential subgroup effects, these analyses will be considered exploratory and hypothesis generating, rather than definitive. It is recognized that the study is not powered to detect subgroup effects and that lack of significance is not necessarily an indication that subgroup effects do not exist. Additionally, interpretation of the analyses will depend on whether the primary analysis demonstrates a significant treatment group difference; in the absence of such a difference, subgroup analyses will be interpreted with caution. The general approach for these exploratory analyses will be to add an interaction term for the subgroup factor by treatment into the primary analysis model.

Subgroup analyses will use observed data (no imputation) from participants who complete the primary outcome visit without missing CST data. Exploratory subgroup analysis will evaluate the effects of the following baseline factors:

- Baseline CST: continuous and by the randomization stratification factor of baseline CST relative to the CI-DME CST values above the threshold (<75 μm above threshold, 75 to <175 μm above threshold, ≥175 μm above threshold)
- Hemoglobin A1c: continuous and <7.5% vs. ≥7.5%
- Sex
- Race/ethnicity

Subgroup analyses will only be conducted if there are at least 20 eyes in each subgroup for each treatment group. There are no data to suggest that the treatment effect will vary by sex or race/ethnicity. However, both these factors will be evaluated in exploratory subgroup analyses as mandated by NIH guidelines. Subgroup factors will be analyzed as continuous/quasi-continuous ordinal variables where possible.

8.14 Multiple Comparison/Multiplicity

The primary analysis consists of one comparison of the primary outcome evaluated at the $\alpha = .05$ significance level. For the secondary outcomes, p-values will be reported but the decision to reject or fail to reject the null hypotheses will control for a false discovery rate of $q = .05$ using the Benjamini-Hochberg adaptive procedure.[20] A rejection of the null hypothesis for a secondary outcome indicates that the outcome is of interest and warrants further investigation. For exploratory outcomes, no p-values or hypothesis tests will be reported.

8.15 Additional Considerations

This phase 2 study is being conducted to determine whether the conduct of a phase 3 trial has merit based on an anatomic outcome and to provide information on outcome measures needed to design the trial. If the results of this study support proceeding with a phase 3 trial after evaluation of all the data and discussion within DRCR, information from this study will contribute to designing the phase 3 trial. Data on which outcomes to target, standard deviations of outcomes to target, potential stratification factors and patient compliance (for example, the proportion of enrolled participants that are randomized and the proportion of randomized participants who comply with medication adherence post-randomization) will aid in the design of the phase 3 trial.

Chapter 9: Data Collection and Monitoring

9.1 Case Report Forms and Other Data Collection

The main study data are collected on electronic case report forms (eCRFs). When data are directly collected in electronic case report forms, this will be considered the source data. For any data points for which the eCRF is not considered source (e.g., lab results that are transcribed from a printed report into the eCRF), the original source documentation must be maintained in the participant's study chart or medical record. This source must be readily verifiable against the values entered into eCRF. Even where all study data are directly entered into the eCRFs at office visits, evidence of interaction with a participant must be recorded (e.g., office note, visit record, etc.).

Data from central vendors (laboratory, reading centers) will be provided directly to the Coordinating Center. The site will also have access to print the lab results from the study website. Reading center grading will not be made available to the site.

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

9.2 Study Records Retention

Study documents should be retained for a minimum of 3 years following the NIH grant cycle for which the last visit was completed or 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product, whichever is later. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of JCHR, if applicable. It is the responsibility of JCHR to inform the investigator when these documents no longer need to be retained.

9.3 Quality Assurance and Monitoring

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

A risk-based monitoring (RBM) plan will be developed and revised as needed during the study, consistent with the FDA "Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring" (August 2013). Study conduct and monitoring will conform with 21 Code of Federal Regulations (CFR) 312. This plan describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

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

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

Coordinating Center representatives or their designees may visit the study facilities at any time to maintain current and personal knowledge of the study through review of records, comparison with source documents, observation and discussion of the conduct and progress of the study. The investigational site will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by JCHR, and inspection by local and regulatory authorities.

9.4 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or procedure requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. A significant (or major) deviation is any deviation that departs from the established materials in such a way that it poses an increase in the risk to subjects, adversely affects the welfare, rights, or safety of the research subjects, or negatively influences the scientific study integrity. As a result of significant deviations, corrective and preventive actions are to be developed by the site and implemented promptly.

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

Chapter 10: Ethics/Protection of Human Participants

10.1 Ethical Standard

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

10.2 Institutional Review Boards

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

10.3 Informed Consent Process

10.3.1 Consent Procedures and Documentation

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

The participants should have the opportunity to discuss the study with their family members and/or their personal physicians(s) before deciding whether to participate in the study. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.3.2 Participant and Data Confidentiality

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

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

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

Separately from any research data, the coordinating center for the study, the Jaeb Center for Health Research (JCHR) in Tampa, Florida, will be provided with participant contact information to aid in study retention efforts. Study participant's contact information will be securely stored and will be destroyed at the end of the study.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at JCHR. This will be stored separately from the participant's contact or direct identifying information. For research purposes, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by JCHR research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at JCHR.

To further protect the privacy of study participants, a Certificate of Confidentiality is available from the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.3.3 Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored at the Jaeb Center for Health Research. After the study is completed, the de-identified, archived data will be made publicly available, for use by other researchers including those outside of the study. OCT/OCTA scans and fundus photographs will also be made publicly available. Permission for future use of the data will be included in the informed consent.

In addition, the optional serum samples will be stored indefinitely and the de-identified samples made available to collaborators for future use. Data generated from optional serum samples will be analyzed in the context of this study but may also be explored in aggregate with data from other studies. The availability of a larger dataset will assist in identification and characterization of important biomarkers and pathways to support future biological discoveries.

Chapter 11: References

1. International Diabetes Federation. Diabetes facts and figures: IDF Diabetes Atlas Ninth edition 2019. https://idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html. Accessed 23 June 2021.
2. Varma R, Bressler NM, Doan QV, et al. Prevalence of and risk factors for diabetic macular edema in the United States. *JAMA Ophthalmol.* 2014;132(11):1334-1340.
3. National Eye Institute. Diabetic retinopathy data and statistics. 2020; https://www.nei.nih.gov/learn-about-eye-health/outreach-campaigns-and-resources/eye-health-data-and-statistics/diabetic-retinopathy-data-and-statistics. Accessed 22 September 2021.
4. Early Treatment Diabetic Retinopathy Study Research Group. Photocoagulation for diabetic macular edema: Early Treatment Diabetic Retinopathy Study report no. 4. *Int Ophthalmol Clin.* 1987;27(4):265-272.
5. Diabetic Retinopathy Clinical Research Network. Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology.* 2010;117(6):1064-1077 e1035.
6. Sampat KM, Garg SJ. Complications of intravitreal injections. *Curr Opin Ophthalmol.* 2010;21(3):178-183.
7. Hutton DW, Stein JD, Bressler NM, et al. Cost-effectiveness of Intravitreous Ranibizumab Compared With Panretinal Photocoagulation for Proliferative Diabetic Retinopathy: Secondary Analysis From a Diabetic Retinopathy Clinical Research Network Randomized Clinical Trial. *JAMA Ophthalmol.* 2017;135(6):576-584.
8. Diabetic Retinopathy Clinical Research Network, Wells JA, Glassman AR, et al. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. *N Engl J Med.* 2015;372(13):1193-1203.
9. Chen H, Zhang X, Liao N, et al. Enhanced Expression of NLRP3 Inflammasome-Related Inflammation in Diabetic Retinopathy. *Invest Ophthalmol Vis Sci.* 2018;59(2):978-985.
10. Mugisho OO, Green CR, Kho DT, et al. The inflammasome pathway is amplified and perpetuated in an autocrine manner through connexin43 hemichannel mediated ATP release. *Biochim Biophys Acta Gen Subj.* 2018;1862(3):385-393.
11. Zhu D, Zhu H, Wang C, Yang D. Intraocular soluble intracellular adhesion molecule-1 correlates with subretinal fluid height of diabetic macular edema. *Indian J Ophthalmol.* 2014;62(3):295-298.
12. Loukovaara S, Piippo N, Kinnunen K, Hytti M, Kaarniranta K, Kauppinen A. NLRP3 inflammasome activation is associated with proliferative diabetic retinopathy. *Acta Ophthalmol.* 2017;95(8):803-808.
13. Mugisho OO, Rupenthal ID, Paquet-Durand F, Acosta ML, Green CR. Targeting connexin hemichannels to control the inflammasome: the correlation between connexin43 and NLRP3 expression in chronic eye disease. *Expert Opin Ther Targets.* 2019;23(10):855-863.
14. Nasir Mat Nor M, Rupenthal ID, Green CR, Acosta ML. Connexin Hemichannel Block Using Orally Delivered Tonabersat Improves Outcomes in Animal Models of Retinal Disease. *Neurotherapeutics.* 2020;17(1):371-387.
15. Levy B. Upstream regulation of the Inflammasome (NLRP3) Pathway of Inflammation in Retinal Disease. In: Ocunexus Therapeutics; 2019.
16. Unpublished Data. Paper presented at: Ocunexus Innovation Showcase2019.

17. Price GW, Chadjichristos CE, Kavvadas P, et al. Blocking Connexin-43 mediated hemichannel activity protects against early tubular injury in experimental chronic kidney disease. *Cell Commun Signal.* 2020;18(1):79.
18. InflammX Therapeutics Inc. Investigator's Brochure Edition No. 1: InflammX. In.
19. Billingsley P. *Probability and Measure.* 2nd ed ed. New York John Wiley and Sons; 1986.
20. Benjamini Y, Hochberg Y. On the Adaptive Control of the False Discovery Rate in Multiple Testing With Independent Statistics. *Journal of Educational and Behavioral Statistics.* 2000;25(1):60-83.