Clinical Study Protocol

Protocol No.: TTHX-001 NCT 04520321

Phase: Phase 1/ Phase 2

Name of Study: A Phase 1/ Phase 2 Study Evaluating the Safety and Efficacy of the Investigational New Drug TTHX1114(NM141) on the Regeneration of Corneal Endothelial Cells in Patients with Corneal Endothelial Dystrophies following Intracameral Delivery

Study Drug Name: TTHX1114(NM141)

IND Number: 128336

Sponsor: Trefoil Therapeutics, Inc.
6330 Nancy Ridge Drive, Suite 103
San Diego, CA 92121

Date of Protocol:
Amendment 1.0: **September 8, 2020**
Original: Version 1.0: June 1, 2020

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9/9/2020

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Chief Executive Officer

Date

This study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP) which have their origins in the Declaration of Helsinki and with other applicable regulatory requirements.

"The only thing worse than being blind is having sight but no vision."

-Helen Keller

DOCUMENT HISTORY

Version	Description of Changes
1.0	N/A
Amendment 1.0	• Define DLT criteria (Section 4.5.1) • Added instruction related to contraception and pregnancy in a subject or partner follow-up (See Section 6.6) • To clarify that visual acuity will be assessed by Best Corrected Visual Acuity (BCVA) not Best Spectacle-corrected Visual Acuity (BSCVA) • Update/ clarify eligibility including: o Exclude a history of repeated elevated IOP o Exclude refractive surgery in the Study Eye o Exclude cataract surgery in the prior 3 months o Exclude Anterior Chamber IOL Placement in the Study Eye o Exclude open or absent posterior capsule, if vitreous present in the anterior chamber unless approved by Medical Monitor o Clarify "either eye" versus "study eye only" criteria o Clarify eligibility related to pregnancy and contraception • Clarify the post-injection IOP must be monitored until less than 5 mmHg greater than pre-injection rather to 2 mmHg • Update PK/ADA sample preparation • Clarify that the comprehensive eye examination will include slit lamp examination and dilated examination of the lens, fundus, and vitreous • Clarify the observational sub-study eligibility based on availability of ocular assessment results

In addition to changes listed above, additional edits may have been made for clarity and consistency.

SYNOPSIS

Study Number:	TTHX-001
Study Name:	A Phase 1/ Phase 2 Study Evaluating the Safety and Efficacy of the Investigational New Drug TTHX1114(NM141) on the Regeneration of Corneal Endothelial Cells in Patients with Corneal Endothelial Dystrophies following Intracameral Delivery
Sponsor:	Trefoil Therapeutics, Inc. 6330 Nancy Ridge Drive, Suite 103 San Diego, CA 92121
Phase	Phase 1/ Phase 2
Objectives	The primary objective is to evaluate the safety and efficacy of TTHX1114(NM141) in patients with corneal endothelial dystrophy. Endpoints include: <u>Primary</u> The change in average ECD in the center of the cornea of the study eye from baseline to Day 56 <u>Secondary</u> The secondary endpoints are defined as the change from baseline at Day 56 for the following variables: [redacted] • Corneal thickness (pachymetry) • Best corrected visual acuity (BCVA) • Corneal edema as measured via slit lamp observations, including grade and area [redacted]
Study Design	This is a prospective, multicenter, randomized, masked, vehicle-controlled, dose-escalation study. The study also includes a non-interventional observational sub-study in which subjects will undergo all (standard) ocular assessments planned for on-study.
Treatment	TTHX1114(NM141) (1 ng, 3 ng, and 10 ng) or placebo (vehicle) will be administered as an intracameral injection on Day 0, Day 7, Day 14, and Day 21.
Eligibility	<u>Inclusion</u> 1. Male or female, 18 years of age or older 2. Able and willing to provide informed consent 3. Fuchs Endothelial Corneal Dystrophy, pseudophakic bullous keratopathy, or endothelial dysfunction/ insufficiency due to surgical intervention diagnosed more than 6 months prior to Study Day 0 in the study eye 4. Intact anterior chamber anatomy (if posterior capsule is open or absent, the absence of vitreous in the anterior chamber must be verified or enrollment approved by the Medical Monitor) in the study eye and no structural abnormalities that would make intracameral injection difficult 5. Able to obtain clear corneal endothelial cell (CEC) morphology and central endothelial cell counts by specular microscopy from both eyes as determined by the central reading facility 6. Central endothelial cell count of < 2000 mm^2 in at least one eye as determined by the central reading facility during the screening period Note: FECD patients with dense central guttae may not be countable and therefore, not eligible 7. Vision of 20/400 or better, in each eye (BCVA of at least 15 letters (per ETDRS]) 8. Subjects who are women of childbearing potential (WOCBP) must be using an acceptable method of birth control [e.g., an Intrauterine Contraceptive Device (IUCD), hormonal contraceptives, or double barrier method] starting at least 1 month prior to Study Day 0 and for the duration of the study. If a female subject is currently abstinent, they must agree to use a double barrier method of birth control

	before they become sexually active. Men who are sexually active with a woman of childbearing potential must agree to use a double barrier method. Exclusion 1. History of any of the following in either eye: a. Ocular cancer (including melanoma) b. Herpetic keratitis c. Corneal transplant (full-thickness or lamellar) d. Documented and repeated elevated IOP in either eye e. Posterior Polymorphous Corneal Dystrophy (PPCD; aka Schlichting dystrophy) f. Uveitis g. Cataract surgery within the 3 months prior to Study Day 0 2. Refractive surgery in the Study Eye, including phakic IOL currently in place **Note**: Potential subjects who have had the phakic IOL placed and removed are allowed. Limbal relaxing incision performed at the time of cataract surgery is allowed 3. Any condition that would impair examination of the anterior chamber structures in the Study Eye 4. Anterior Chamber IOL placement (in the Study Eye) 5. Expected or planned ocular surgery (incisional or laser) in the Study Eye within the 3 months following Study Day 0 (including endothelial keratoplasty) 6. Use of systemic or dermatological cytotoxic chemotherapy within the 1 month prior to or planned use within the 3 months following Study Day 0 7. Use of hypertonic saline eye preparations (e.g.; Muro 128) within the 3 days prior to Study Day 0 (either eye) 8. Active extra-ocular inflammation from any non-infectious or infectious cause (bacterial, viral or fungal) in the Study Eye within the 6 months prior to Study Day 0 **Note**: Mild blepharitis and/or dry eye related inflammation are allowed 9. Treatment with a rho kinase inhibitor within the last 3 months (either eye) 10. Use of cyclosporine ophthalmic emulsion,(e.g.; RESTASIS®) within the 30 days prior to Study Day 0 (either eye) 11. Use of Xiidra® (lifitegrast ophthalmic solution) within the 30 days prior to Study Day 0 (either eye) 12. Systemic (nasal, inhaled, oral, parenteral, or topical) or ophthalmic corticosteroid use in the 30 days prior to Study Day 0 unless approved by the Medical Monitor **Note:** Potential subjects on a stable chronic low-dose that is not expected to change in the 3 months following Study Day 0 may be considered for the study after approval by the Medical Monitor 13. History of significant allergy, hypersensitivity, or intolerance to any drug compound, food, or other substance **Note:** This includes all components and excipients of the study drug 14. Women of childbearing potential who are currently pregnant, breast feeding, planning to become pregnant during the study, or not willing to use highly effective birth control measures (e.g., hormonal contraceptives [oral, implanted, transdermal], or mechanical barrier methods [spermicide in conjunction with a barrier such as a condom or diaphragm], or intra-uterine device [IUD]) during the entire course of the study 15. Current or recent (e.g., the 30 days prior to Study Day 0) participation in any other, interventional clinical research study 16. An employee of the site that is directly involved in the management, administration, or support of this study or be an immediate family member of the same

17. Any other reason (e.g., serious systemic disease or uncontrolled medical condition) that, in the opinion of the Investigator could increase the subject's risk, interfere with the interpretation of the study results, or affect the subject's ability to provide informed consent or comply with the study

Sample Size

	Study Treatment Randomization				
Cohort	**Vehicle** [redacted]	[redacted]	[redacted]	[redacted]	**Total**
1	3	6	-	-	9
2	3	3	6	6	18
3	11	11	11	11	44
Total	17	20	17	17	71

[redacted]

	Sub-Study		Study TTHX-001									
			Screen	Study Day								
	Visit			0		3	7 ±1	14 ±1	21 ±1	28 ±1	56 ±3	90 ± 7
	1	2-4		Pre								
Informed consent	X		X									
Demographics	X		X									
Medical and ocular history	X		X									
Comprehensive ocular examination (including slit lamp examination and dilated exam of the lens, fundus and vitreous)			OU						SE[f]		OU	
Urine pregnancy test (WOCBP)				X							X	
Laboratory testing (Chem, Heme, UA, & HbA1c)				X						X	X	
NEI-VFQ-25[a]				X							X	
Optional blood sample for genetic testing				X								
ADA assay Pre-injection				X					X	X	X	
PK sampling Pre-injection 1.5 hours after injection				X	 X				 X X		X	
Study Treatment IC Injection (SE)					X		X	X	X			
Record Concomitant Medications*	X	X	X	X	X	X	X	X	X	X	X	X
Record Adverse Events					X	X	X	X	X	X	X	X
Ocular Assessments												
Specular microscopy[b] (including automated pachymetry)	OU[c,d]	OU	OU	OU			OU	OU	OU	OU	OU	OU
Pentacam examination				OU							OU	
Best corrected visual acuity[b]	OU[c,d]		OU	SE			SE	SE	SE	SE	SE	SE
Slit lamp biomicroscopy	OU[c,d]			SE			SE	SE	SE	SE		SE
Intraocular Pressure (IOP) measurement	OU[c,d]			SE[e]	SE[e]		SE[e]	SE[e]	SE[e]	SE	SE	SE

OU=both eyes, SE=Study Eye, [a] Perform prior to any other assessments, [b] If subject is to be dilated, perform prior to dilation, [c] 2 timepoints (1 in the early morning and 1 in the late afternoon), may be different days. [d] [redacted], [e] IOP pre-dose and post-dose at least every 30 minutes until normalized (defined as less than 5 mmHg higher than pre-injection IOP), [f] dilated exam only in subjects with vitreous in the AC at baseline
*Including all ocular medications

LIST OF ABBREVIATIONS

ADA	Anti-Drug Antibody
AE	Adverse Event
BCVA	Best Corrected Visual Acuity
CEC	Corneal Endothelial Cell
CED	Corneal Endothelial Dystrophies
CIARC	Cornea Image Analysis Reading Center
CRF/ eCRF	Case Report Form/ electronic Case Report Form
CRO	Contract Research Organization
DHHS	Department of Health and Human Services
DLT	Dose limiting toxicity
DMEK	Descemet Membrane Endothelial Keratoplasty
DSEK	Descemet Stripping Endothelial Keratoplasty
ECD	Endothelial Cell Density
EDC	Electronic Data Capture
eFGF-1	engineered FGF-1
ETDRS	Early treatment diabetic retinopathy study
FDA	Food and Drug Administration
FECD	Fuchs Endothelial Corneal Dystrophy
FGF	Fibroblast Growth Factor
GAT	Goldmann Applanation Tonometry
GCP	Good Clinical Practice
HED	Human Equivalent Dose
IC	Intracameral
ICF	Informed consent form
ICH	International Conference on Harmonisation
IOP	Intraocular pressure
IRB	Institutional review board
ITT	Intent to Treat
IUD	Intrauterine device
LOCF	Last Observation Carried Forward
MMRM	Mixed Model Repeated Measures
MRSD	Maximum Recommended Starting Dose
NEI-VFQ	National Eye Institute Visual Function Questionnaire
NOAEL	No Observable Adverse Event Level
PAD	Pharmacologically Active Dose
PK	Pharmacokinetic
PP	Per Protocol
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SE	Study Eye
TM	Trabecular Meshwork
WOCBP	Women of Childbearing Potential
wtFGF-1	Wild type FGF-1

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1. INTRODUCTION

1.1 Corneal Endothelial Dystrophy

Corneal endothelial hypocellularity driven by corneal endothelial cell (CEC) loss due to trauma, surgical damage, or corneal endothelial dystrophies (CED) can have a significant effect on vision and a high impact on quality of life. Corneal endothelial cell loss or low CEC counts contribute to poor outcomes of ocular surgery, including cataract surgery. Fuchs Endothelial Corneal Dystrophy (FECD) is the most common driver of corneal transplantation [1], with 17,468 endothelial keratoplasty procedures performed in the US in 2008 [2]. Corneal endothelial dystrophies have been estimated to affect approximately 4% of the US population over the age of 40 [3] although more recent estimates using claims data and more stringent criteria put the overall prevalence of CED at 0.13% and the prevalence of FECD at 0.078% [4]. Corneal endothelial hypocellularity secondary to surgery or trauma is an infrequent but significant complication that can lead to corneal transplantation and contributes to visual impairment.

1.2 Treatment Options

Beyond symptomatic treatment using various pharmacologic therapies, the only treatment option for patients with CED is the transplantation of CECs either as a whole corneal transplant or transplantation of the endothelial layer via a variety of endothelial keratoplasty procedures, including Descemet Stripping Endothelial Keratoplasty (DSEK) [5] and Descemet Membrane Endothelial Keratoplasty (DMEK) [6]. Even after transplantation, the number of CECs in the transplant continues to decrease [7] and repeat transplants are sometimes needed. Corneal transplantation is an option of last resort as donor tissue is in short supply, the surgery requires a highly skilled surgeon, and there is a significant risk of complications, transplant rejection and long-term failure. Corneal transplantation in patients with CED has a significantly higher long-term failure rate than transplantation for keratoconus and approximately 34% of transplants will fail in the first 10 years (vs 11% for keratoconus) [8].

While the CECs do not repopulate or renew in either normal eyes or in patients with FECD, there is evidence suggesting that cells from the peripheral corneal endothelium can, under some conditions, migrate centrally [9] and that cells of the peripheral corneal endothelium [10-12] or trabecular meshwork (TM) [13, 14] may contain a progenitor population [12]. In addition, while CECs do not seem to regenerate *in vivo*, when explanted they show the ability to proliferate if stimulated by growth factors [15]. Therefore, treatments that increase migration and proliferation of CECs or their progenitor population in situ may regenerate the endothelium and improve barrier function in the central cornea. This in turn should reduce corneal edema, improve vision, and delay (or eliminate) the need for corneal transplantation.

1.3 FGF for the Treatment of Corneal Endothelial Dystrophy

Fibroblast growth factors (FGFs) are a large family of proteins that stimulate the proliferation and migration of a wide variety of cells. FGFs are key developmental growth factors in many tissues including the cornea. Fibroblast growth factor-1 (FGF-1) is the most potent stimulator of proliferation of CECs known and is the rationale for applying FGF-1 to CED.

FGF-1 in its native or wild type form (wtFGF-1) is susceptible to both proteolysis and cysteine oxidation, creating a very short biological half-life and thus making it unsuitable as a therapy. To overcome these deficiencies, FGF-1 was stabilized via the substitution of key amino acids to decrease the flexibility of the protein without introducing any new functionalities of the protein

on a qualitative basis. These engineered FGF-1s (eFGF-1s) would not be expected to have any activities that the wtFGF-1 does not possess as well as an increased affinity for the FGF receptors and/or heparin sulfate proteoglycans.

1.4 Diurnal Variation in Ocular Function

Diurnal variation is a common concern in clinical trials where endpoint measurements may fluctuate throughout the course of the day and are associated the timing of assessments. The effect of diurnal variation in intraocular pressure has been observed in patients with glaucoma [17, 18] and in healthy volunteers [18] and it is recommended that comparative assessments be obtained in the early morning to help avoid variability in intraocular pressure measurements due to diurnal fluctuations [17, 18]. Other ocular assessments, such as corneal thickness, have also been shown to be affected by diurnal variation when assessed in patients with dry eye [19] and in healthy volunteers [20]. Variability within the first 4 hours of waking has been observed in patients with FECD [21]. The degree of diurnal variation in all the ocular assessments planned in this study has not been evaluated in this study population. The Observational Sub-study will collect study endpoints (ocular assessments) in the morning and in the evening and evaluate the effect of diurnal variation on the individual study endpoints. The timing of study assessments in the main study is consistent as early morning assessments at all timepoints; if the diurnal variation effect is determined to be minimal or insignificant, the timing of non-baseline and non-primary endpoint evaluations may be relaxed to accommodate subject scheduling and convenience.

2. BACKGROUND INFORMATION

2.1 Investigational Product TTHX1114(NM141)

Trefoil has developed TTHX1114(NM141) which is an engineered FGF-1 (eFGF-1) containing three stabilizing mutations (including the introduction of an internal disulfide bond that has activity at much lower applied concentrations but qualitatively has biological activity identical to that of wtFGF-1). As such, TTHX1114(NM141) is expected to have the same mechanism-driven effects that wtFGF-1 has with no unexpected activity. Since wtFGF-1 has been clinically tested in several systems including gene therapy, the potential safety issues with eFGF-1 can be anticipated based on those studies.

Please refer to the Pharmacy Manual for study treatment storage, handling, and preparation instructions.

2.2 Summary of Nonclinical Studies and Clinical Trials

2.2.1 Nonclinical Studies

In the rabbit corneal cryoinjury model, a single intracameral injection of TTHX1114(NM141) accelerated the clearing of the cornea for as much as 14 days post injury.

In addition, TTHX1114(NM141) stimulates the proliferation of CECs in both normal human corneas in organ culture and in corneas that show signs of FECD.

In preclinical toxicology testing in the rabbit and the dog, no changes in intraocular pressure (IOP) and no neovascularization was noted at doses up to 100x the highest dose to be evaluated in this study with greater total exposure (more than 4 injections). Following intracameral injection of 30-100x the highest dose to be evaluated in this trial, dose-dependent mild to

moderate vacuolation of the posterior epithelium of the iris was noted. This effect was reversible on cessation of drug treatment.

Please review the Investigator's Brochure for detailed information involving the preclinical development program.

2.2.2 Clinical Trials

This is the first clinical study of TTHX1114(NM141).

2.3 Potential Risks and Benefits

This is the first in man clinical study. Anticipated hypothetical adverse events include, but are not limited to:

- Anterior chamber flare
- Conjunctival inflammation
- Endophthalmitis
- Inflammation of the anterior chamber (e.g.: cornea and/or iris)
- Bruising of external eye structures (especially for subjects on anti-coagulant therapy)

2.4 Rationale for Dose Selection

This study is a dose escalation study that will assess up 3 dose levels of TTHX1114(NM141) ([redacted] in patients with CED. The maximum recommended starting dose (MRSD) for this first-in-human trial was based on the following conservative assumptions:

1) The lowest dose in current toxicology studies of 100 ng via intracameral (IC) injection results in 300 ng/mL Cmax and was a no observable adverse event level (NOAEL) dose
2) The pharmacologically active dose (PAD) was predicted based on the dose that was active in cell culture (the 90% effective concentration [EC90] of approximately 3 ng/mL = 1 ng IC injection)
3) Given the anatomical similarity of the eyes of the toxicology species (rabbits and dogs) and humans, no scaling factor was used to determine the human equivalent dose (HED)
4) Study treatment will be administered in at 10 mcL volume. This volume was chosen so as to be minimally disruptive and is well below the 0.1mL (100mcL) volume used in many intracamerally administered antibiotics.

The 2005 FDA guidance titled *"Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers"* suggests a safety factor for the MRSD of at least 10-fold below the HED, and this should be adjusted taking into account the PAD. The PAD based on tissue culture experiments is 3 ng/mL. An IC dose of 1 ng = 3ng/mL Cmax due to the limited volume of the anterior chamber. Based on these data, the dose range for this trial includes the PAD (1ng/mL) as the low dose and a high dose of 10 ng (=30 ng/mL Cmax) which is a 10-fold safety margin over the NOAEL HED.

2.5 Statement of Compliance

This study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP) which have their origins in the Declaration of Helsinki and with all other applicable regulatory requirements.

2.6 Study Population

The study population will consist of men and women aged ≥ 18 years presenting with corneal endothelial dystrophy/ hypocellularity. Subjects will be recruited from a non-surgical population or post-operative cataract population (at least 3 months post-op). The intent is to enroll subjects with low ECD counts and endothelial cell images clearly quantitatable using specular microscopy imaging. Subjects included should not be expected to progress to transplantation within the study period of 3 months. The population is expected to include a mix of patients with corneal edema and those that do not yet have edema.

3. TRIAL OBJECTIVES AND PURPOSE

The primary objective of this Phase 1/ Phase 2, prospective, masked, randomized, placebo-controlled study is to evaluate the safety and efficacy of TTHX1114(NM141) in patients with corneal endothelial dystrophy.

4. TRIAL DESIGN

This is a prospective, multi-center, randomized, masked, vehicle-controlled, dose-escalation study that will include an observational (no intervention) sub-study. Up to approximately 71 eligible subjects with moderate to severe corneal endothelial dystrophy (defined as ECD < 2000 mm^2) in at least one eye at approximately 10 investigational sites will be enrolled and randomized in the main study after the IND has been approved by the FDA. The Observational Sub-study (See Appendix B) will enroll approximately 25 to 50 subjects and will precede Cohort 1 enrollment and run in parallel to Cohorts 1 and 2. Subjects enrolled in and participating in the Observational Sub-study who are eligible may "roll-over" into the main study provided they meet all of the additional eligibility criteria, there is an open cohort, and they consent.

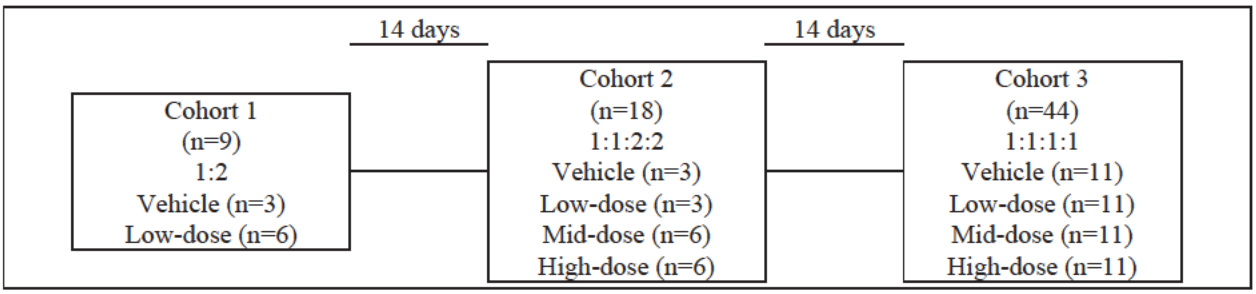
The number of subjects per cohort in the main study and per study treatment is summarized below in Table 1.

Table 1: Study Cohorts and Number of Subjects

	Study Treatment Randomization				
Cohort	Vehicle (placebo)	Low-dose [redacted]	Mid-dose [redacted]	High-dose [redacted]	Total
1	3	6	-	-	9
2	3	3	6	6	18
3	11	11	11	11	44
Total	17	20	17	17	71

The study team will include a masked and an unmasked Medical Monitor. The masked Medical Monitor will discuss eligibility questions with the investigational sites and oversee the daily running of the clinical study. The masked and unmasked Medical Monitors will review clinical data (including adverse events frequency and severity) on an ongoing basis. The unmasked Medical Monitor will review all data and make decisions regarding the opening of subsequent cohorts (See Section 4.4.1). The dose escalation schema is presented in Figure 1.

Figure 1: Study Schema (Dose Escalation)



4.1 Efficacy Endpoints

4.1.1 Primary Endpoint

The primary endpoint is the change in average ECD in the center of the cornea of the study eye from baseline to Day 56; the center of the cornea is defined as the region over the pupil. Each treatment group will be compared to the vehicle group with no correction for multiple comparisons. [redacted]

4.1.2 Secondary Endpoints

The secondary endpoints are defined as the change from baseline at Day 56 for the following variables:

- [redacted]
- Corneal thickness (pachymetry)
- Best corrected visual acuity (BCVA)
- Corneal edema as measured via slit lamp observations, including grade and area

[redacted]

4.1.3 Exploratory Endpoints

[redacted]

4.2 Measures Taken to Minimize/ Avoid Bias

All adverse events will be recorded regardless of suspected causality. Additionally, subjects will be randomized and all members of the study team will be unaware of treatment assignment.

4.2.1 Randomization

Participants will be randomized and assigned to study treatment in order of determination of eligibility. Randomization will be managed centrally. To assure the safety of the subjects and to

escalate dose in a step-wise manner while maintaining masking, central randomization will be structured such that the study is conducted in 3 cohorts, see Figure 1.

4.2.2 Masking

Visually, TTHX1114(NM141) is indistinguishable from vehicle, therefore, the clinician administering the intracameral injection will be masked to study treatment assignment. All study subjects will also be masked to study treatment assignment and masked examiners will perform all ocular examinations, including specular microscopy, slit lamp biomicroscopy, dilated eye exam, intraocular pressure (IOP), and BCVA.

4.2.3 [redacted]

[redacted]

4.2.4 Masked Medical Monitor

As described above, the study will include a masked and an unmasked Medical Monitor. To avoid bias on the part of the unmasked Medical Monitor, all communication with the investigational site staff will be through the masked Medical Monitor.

4.3 Study Treatment

Study treatment (TTHX1114(NM141) [1 ng, 3 ng, and 10 ng] or vehicle/ placebo) will be administered as an intracameral injection on Day 0, Day 7, Day 14, and Day 21. Please refer to Section 6.2 for instructions for study treatment administration. Please refer to the Pharmacy Manual for study treatment storage, handling, and preparation instructions.

4.3.1 TTHX1114(NM141) Drug Product

TTHX1114(NM141) drug product is a solution formulated in phosphate buffered saline manufactured in a contract sterile fill/finish facility in compliance with cGMP regulations. TTHX1114(NM141) will be provided in the following concentrations:

[redacted]

Vehicle is the phosphate buffered saline without active ingredient. Study treatment is supplied in 2 mL sterile, plastic, single-use, snap-cap vials with 1 mL of test article in each vial.

4.3.2 TTHX1114(NM141)/ Vehicle Packaging

The study treatment kit will contain 5 vials of masked study treatment vials (1 for each of the 4 study treatment administrations and 1 back-up vial). [redacted]

4.3.3 TTHX1114(NM141)/ Vehicle Storage

The vials are shipped frozen and must be stored [redacted] in a secure area. Excursions from temperature limits must be reported to the Trefoil (or designee) to determine whether the affected vials can be used. Access to investigational product must be limited to individuals authorized by the Investigator to dispense the study treatment.

4.3.4 TTHX1114(NM141)/ Vehicle Preparation

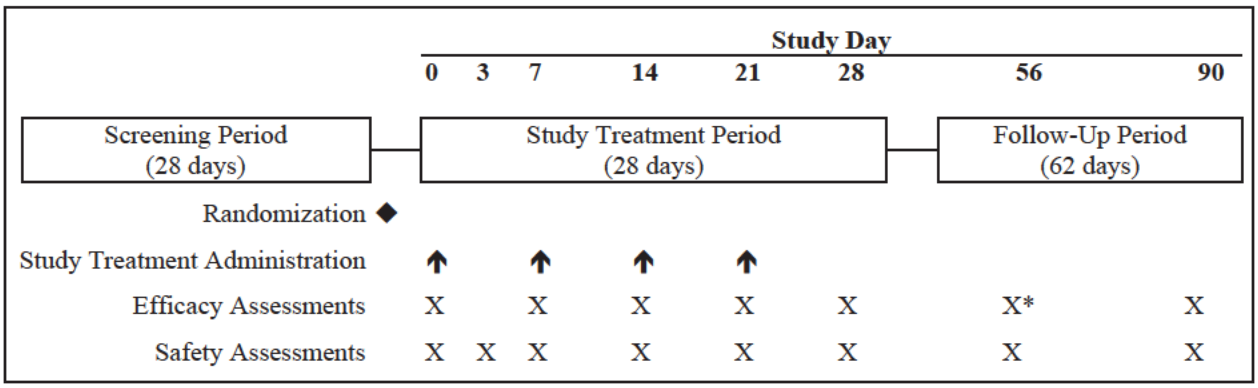
Vials must be thawed prior to study treatment administration and gently mixed by inversion. Thawed vials are stable at room temperature for up to 8 hours. Study treatment preparation should be started (i.e.; allowed to thaw at room temperature NB: do not apply external heat) after the subject has arrived in the clinic to undergo study treatment. All study treatment injections must be administered within 8 hours of the start of thawing of the study treatment vial.

Trefoil will supply a low-volume syringe for each study treatment administration. [redacted]

4.4 Duration of the Study and Study Treatment

Each subject is expected to be on study for approximately 90 days following a 28-day screening period. Study treatment will be administered on Day 0, Day 7, Day 14, and Day 21 as an intracameral injection. Following the last intracameral injection on Day 21, all participants will return to the clinic on Day 28, Day 56 and Day 90 for follow-up assessments.

Figure 2: Study Participant Schema



Not to scale.
See Appendix A for a complete schedule of assessments
* Primary efficacy endpoint assessment

4.4.1 Retreatment Criteria

Subject may continue to receive study treatment (Days 7, 14, and 21) as long as they remain eligible and have not experienced any dose limiting toxicity (DLT) (see Section 4.5.1) below.

4.5 Dose Escalation/ Cohort Review

The unmasked Medical Monitor will review all available clinical data when the last subject of the first cohort of subjects completes the Day 7 examinations before enrollment in the second cohort may start. Then, the unmasked Medical Monitor will review clinical data when the last subject in the second cohort completes the Day 7 examinations before enrollment into the third

cohort may start. If, at any time, the safety profile is deemed unacceptable, treatment will revert to the prior lower-dose level which was deemed safe.

4.5.1 Dose Limiting Toxicities

Potential dose limiting toxicity (DLT) is defined as any of the following events:

- Visual acuity loss of 15 ETDRS letters or more
- > 5 anterior chamber cells observed on slit lamp examination
- > 10 vitreous cells observed on slit lamp examination
- >710 micron corneal thickness or a 150 micron increase from baseline (Day 0 pre-treatment)
- Increase of 2 or more grades in conjunctival hyperemia
    - Grade 0 = No vasodilation (Absent)
    - Grade 1 = Some vasodilation
    - Grade 2 = Extensive vessel vasodilation
    - Grade 3 = Overall vasodilation
- IOP that remains higher than 32 mmHg and not responding to therapy

If any subject experiences any of the events listed above, study treatment must be held for at least 7 days, and the masked Medical Monitor must be contacted to discuss medical management of the subject including possible treatment and additional unscheduled study assessments.

After the 7-day study treatment delay, the subject may receive additional study treatment if the subject remains eligible and all of the potential DLTs the subject experienced have decreased to a level that no longer meets the DLT requirements. Any of the events listed above that continue to meet the DLT requirements after the 7-day delay in study treatment will be considered DLT and the subject will be discontinued from study treatment. Any study treatment delay of greater than 7 days must be discussed with and approved by the masked Medical Monitor.

4.6 Stopping Rules

Stopping rules for the study will be the incidence of unacceptable toxicity. If, at any time, the safety profile is deemed unacceptable, study treatment will revert to the prior lower-dose level(s) deemed safe.

4.7 Investigational Product Accountability

In accordance with 21 CFR 312.61 and 21 CFR 312.62(a), the Investigator

- Will administer the drug only to subjects under the investigator's personal supervision or under the supervision of a subinvestigator responsible to the investigator,
- Shall not supply the investigational drug to any person not authorized under this part to receive it, and
- Will maintain adequate records of the disposition of the drug, including dates, quantity, and use by subjects

If the investigation is terminated, suspended, discontinued, or completed, the investigator shall return the unused supplies of the drug to the sponsor, or otherwise provide for disposition of the unused supplies of the drug under 21 CFR 312.59.

All thawed and partially used study treatment vials will be refrozen and stored. Unused and partially used study treatment vials will be returned to Trefoil (or designee) at the end of the Study Treatment Period.

4.8 Investigational Product Ordering and Resupply Request

Investigational sites will be provided with a subject-specific study treatment kit containing 5 vials at the time of randomization. In the event of loss or damage, the investigational site must contact the central study team and request replacement study treatment vial(s) for the specific subject. The drug depot will ship replacement masked study treatment vials corresponding with the subject's treatment assignment.

4.9 Maintenance of and Procedure for Breaking the Randomization Code

The randomization list will be maintained by the study team. An unmasked biostatistician (or designee) will provide the randomization list and masked Study Treatment Kit IDs to the study team.

In the event that unmasking a subject to treatment assignment is considered necessary prior to the database lock, the Investigator is instructed to contact the masked Medical Monitor to discuss the reasons for requesting unmasking. If the reason for requesting unmasking is for the management of adverse events or a decision to continue study treatment, the adverse events should be managed as medically appropriate and the subject should be discontinued from study treatment without unmasking. If unmasking is determined to be required, the masked Medical Monitor will contact the unmasked Medical Monitor and the masked Medical Monitor will communicate with the Investigator and reveal the subject's study treatment assignment.

4.10 Data to be Entered into the Database

Endothelial cell count/ density measured by the central reading facility is the primary efficacy endpoint for this study. The data generated by the central reading facility will be reconciled (Subject ID, date, time, timepoint) with the clinical database and the results will be uploaded into the database under a Quality and Data Transfer Specifications Agreement. Uploaded data will then be 100% quality checked (e.g.; sourced data verified) with the data at the central reading facility.

Other data and results generated by the investigational site will be data entered directly into the electronic data capture (EDC) system. All data in the EDC will be monitored and critical variables will be 100% source data verified.

5. SELECTION AND WITHDRAWAL OF SUBJECTS

Patients will be primarily selected on the basis of diagnosis of Corneal Endothelial Dystrophy and corneal endothelial cell (CEC) hypocellularity. Eligibility may be based on the pathology noted in one or both eyes. If both eyes meet ocular eligibility criteria, the eye with the lower CEC count will be designated as the study eye (SE); if only 1 eye meets the eligibility criteria, it will be the SE.

5.1 Inclusion Criteria

1. Male or female, 18 years of age or older
2. Able and willing to provide informed consent

3. Fuchs Endothelial Corneal Dystrophy, pseudophakic bullous keratopathy, or endothelial dysfunction/ insufficiency due to surgical intervention diagnosed more than 6 months prior to Study Day 0 in the study eye
4. Intact anterior chamber anatomy (if posterior capsule is open or absent, absence of vitreous in the anterior chamber must be verified or enrollment approved by the Medical Monitor) in the study eye and no structural abnormalities that would make intracameral injection difficult
5. Able to obtain clear corneal endothelial cell (CEC) morphology and central CEC counts by specular microscopy from both eyes as determined by the central reading facility
6. Central endothelial cell count of < 2000 mm^2 in at least one eye as determined by the central reading facility during the screening period

   Note: FECD patients with dense central guttae may not be countable and therefore, not eligible
7. Best Corrected Visual Acuity (BCVA) of at least 15 letters (per Early Treatment of Diabetic Retinopathy Study [ETDRS] testing) (approximately 20/400), in each eye
8. If female, are non-pregnant, non-lactating Subjects who are women of childbearing potential (WOCBP) must be using an acceptable method of birth control [e.g., an Intrauterine Contraceptive Device (IUCD), hormonal contraceptives, or double barrier method] starting at least 1 month prior to Study Day 0 and for the duration of the study. If a female subject is currently abstinent, they must agree to use a double barrier method of birth control before they become sexually active. Men who are sexually active with a woman of childbearing potential must agree to use a double barrier method.

5.2 Exclusion Criteria

1. History of any of the following in either eye:
   a. Ocular cancer (including melanoma)
   b. Herpetic keratitis
   c. Corneal transplant (full-thickness or lamellar)
   d. Documented and repeated elevated IOP in either eye
   e. Posterior Polymorphous Corneal Dystrophy (PPCD; aka Schlichting dystrophy)
   f. Uveitis
   g. Cataract surgery within the 3 months in prior to Study Day 0
2. Refractive surgery in the study eye, including phakic IOL currently in place
   Note: Potential subjects who have had the phakic IOL placed and removed are allowed. Limbal relaxing incision performed at the time of cataract surgery is allowed
3. Any condition that would impair examination of the anterior chamber structures in the Study Eye
4. Anterior Chamber IOL placement (in the Study Eye)
5. Expected or planned ocular surgery (incisional or laser) in the Study Eye within the next 3 months (including endothelial keratoplasty)
6. Use of systemic or dermatological cytotoxic chemotherapy within the 1 month prior to or planned use within the 3 months following Study Day 0

7. Use of hypertonic saline eye preparations (e.g.; Muro 128) within the 3 days prior to Study Day 0 (either eye)
8. Active extra-ocular inflammation from any non-infectious or infectious cause (bacterial, viral or fungal) in the Study Eye within the 6 months prior to Study Day 0

   Note: Mild blepharitis and/or dry eye related inflammation are allowed
9. Treatment with a rho kinase inhibitor within the last 3 months (either eye)
10. Use of cyclosporine ophthalmic emulsion (e.g.; RESTASIS®) within the 30 days prior to Study Day 0 (either eye)
11. Use of Xiidra® (lifitegrast ophthalmic solution) within the 30 days prior to Study Day 0 (either eye)
12. Systemic (nasal, inhaled, oral, parenteral, or topical) or ophthalmic corticosteroid use in the 30 days prior to Study Day 0 unless approved by the Medical Monitor

    Note: Potential subjects on a stable chronic low-dose that is not expected to change in the 3 months following Study Day 0 may be considered for the study after approval by the Medical Monitor
13. History of significant allergy, hypersensitivity, or intolerance to any drug compound, food, or other substance

    Note: This includes all components and excipients of the study drug
14. Women of childbearing potential who are currently pregnant, breast feeding, planning to become pregnant during the study, or not willing to use highly effective birth control measures (e.g., hormonal contraceptives [oral, implanted, transdermal], or mechanical barrier methods [spermicide in conjunction with a barrier such as a condom or diaphragm], or intra-uterine device [IUD]) during the entire course of the study
15. Current or recent (e.g., the 30 days prior to Study Day 0) participation in any other, interventional clinical research study
16. An employee of the site that is directly involved in the management, administration, or support of this study or be an immediate family member of the same
17. Any other reason (e.g., serious systemic disease or uncontrolled medical condition) that, in the opinion of the Investigator could increase the subject's risk, interfere with the interpretation of the study results, or affect the subject's ability to provide informed consent or comply with the study

5.3 Subject Withdrawal Criteria

Subjects will be discontinued from continued study treatment if any of the following occur:

- Intolerable side effects
- The Investigator feels it is in the subject's best interest
- Non-compliance
- Pregnancy

Subjects who are discontinued from study treatment are expected to remain on the study and complete all appropriate safety and efficacy assessments.

Subjects may withdraw from the trial at any time and for any reason.

If a subject withdraws from the trial, attempts should be made to contact the subject to determine the reason(s) for discontinuation while respecting the subject's privacy. All procedures and evaluations required by the final study visit should be completed in the event of early withdrawal. All subjects who discontinue the trial due to an adverse event must be followed until the event has resolved, returned to baseline, or stabilized (if resolution is not anticipated).

6. TREATMENT OF SUBJECTS

This study will be conducted at qualified clinical investigational sites. Sites will be selected based on the ability to safely conduct the study and where Trefoil can be assured of patient safety and the ability to effectively monitor. Investigational sites should monitor health websites such as (https://www.cdc.gov/coronavirus/2019-ncov/index.html) for up-to-date information and resources.

Investigational site staff are instructed to follow their institutional infection control standard procedures and to consider additional measures during periods of community transmission of conditions such as COVID-19. These additional measures may include:

- Contacting subjects the day before a scheduled appointment
    - To determine if they are experiencing any signs or symptoms of possible infections, such as
        - Fever
        - Cough
        - Shortness of breath
        - Sore throat
    - To determine if the subject has tested positive for COVID-19, been in contact with someone who is known or suspected COVID-19, or traveled to an area of risk
- Rescheduling subjects who are experiencing respiratory symptoms and instructing them to seek appropriate medical attention
- Reminding subject to contact the investigational site if they develop respiratory symptoms on the morning before a scheduled examination or if they have had changes to their health status

Upon arrival at the investigational site, subjects should be

- Assessed for fever
- Provided with PPE (e.g.; face masks)

Equipment that is meant to be used on more than one person should be draped to prevent possible contamination and appropriately cleaned before and after each use. Additional measures should also be considered such as:

- Posting guidelines
- Remind subjects of the need for social distancing/ consider having subjects wait outside of the investigational site (e.g.; in their vehicles until ready to be seen)
- Non-essential individuals accompanying study subjects should not permitted in patient areas

- Provide supplies for respiratory hygiene and cough etiquette, including 60-95% alcohol-based hand sanitizer (ABHS), tissues, no-touch receptacles for disposal, face masks, and tissues at healthcare facility entrances, waiting rooms, patient check-ins, etc.
- Provide respiratory hygiene supplies such as tissue to cover cough and hands-free receptacle for refuse disposal
- Remind subjects to wash hands and/ or use hand sanitizer

Study visits and assessments should be rescheduled at the discretion of the Investigator when there is a perceived increased risk to study subjects and/or investigational site staff.

6.1 Subject Screening and Enrollment

Potential study participants will be asked to sign an Informed Consent Form (ICF) prior to any study assessments. Screening should be completed within 28 days of Study Day 0; non-qualifying screening assessments (e.g.; specular microscopy) and screening assessments outside this window may be repeated. Screening assessments will include:

- Assessment of study eligibility,
- Medical and ocular history,
- BCVA assessment,
- Comprehensive ocular examination (slit lamp biomicroscopy and dilated examination of lens, fundus, and vitreous),
- Specular microscopy (including images of the central cornea),

If the subject is deemed preliminarily eligible by the Investigator, specular microscopy images of the central cornea will be sent to central image reading facility. If the reading center confirms that ECD is at least one eye is < 2000 cells/mm^2, a space is available in a cohort, and the subject meets all of the additional eligibility criteria, the subject will be randomized and additional baseline assessments obtained.

If both eyes qualify, the eye with the lowest visible cell count (per the central reading facility) will be designated as the Study Eye (SE). If both eyes have the same visible cell count, the right eye will be designated the SE.

All potential study subjects will be provided with a unique Subject ID Number (Subject ID) at the time of providing written informed consent. The Subject ID will consist of a unique 6-digit number (3-digit site number and a 3-digit subject number, separated by a hyphen [-]).

Figure 3: Subject ID Numbering

Subject ID Number						
Site Number				Subject Number		
9	9	9	-	9	9	9

For example, Subject ID 101-001 = The first participant consented at Site 101; 103-003 = The third participant consented at Site 103

Once a Subject ID number has been issued, it cannot be reused.

Subjects enrolled in and participating in the Observational Sub-study who are eligible may "roll-over" into the main study provided they meet all of the additional eligibility criteria, there is an open cohort, and they consent. Subjects who "roll-over" into the main study do not need to complete the remaining visits in the Observational Sub-study, and Observational Sub-study assessments may be used as screening assessments for the main study as long as they were performed within the protocol-specified timeframe.

Potential subjects may be rescreened or have repeat eligibility assessments, if appropriate.

6.2 Study Treatment Administration/ Intracameral Injection

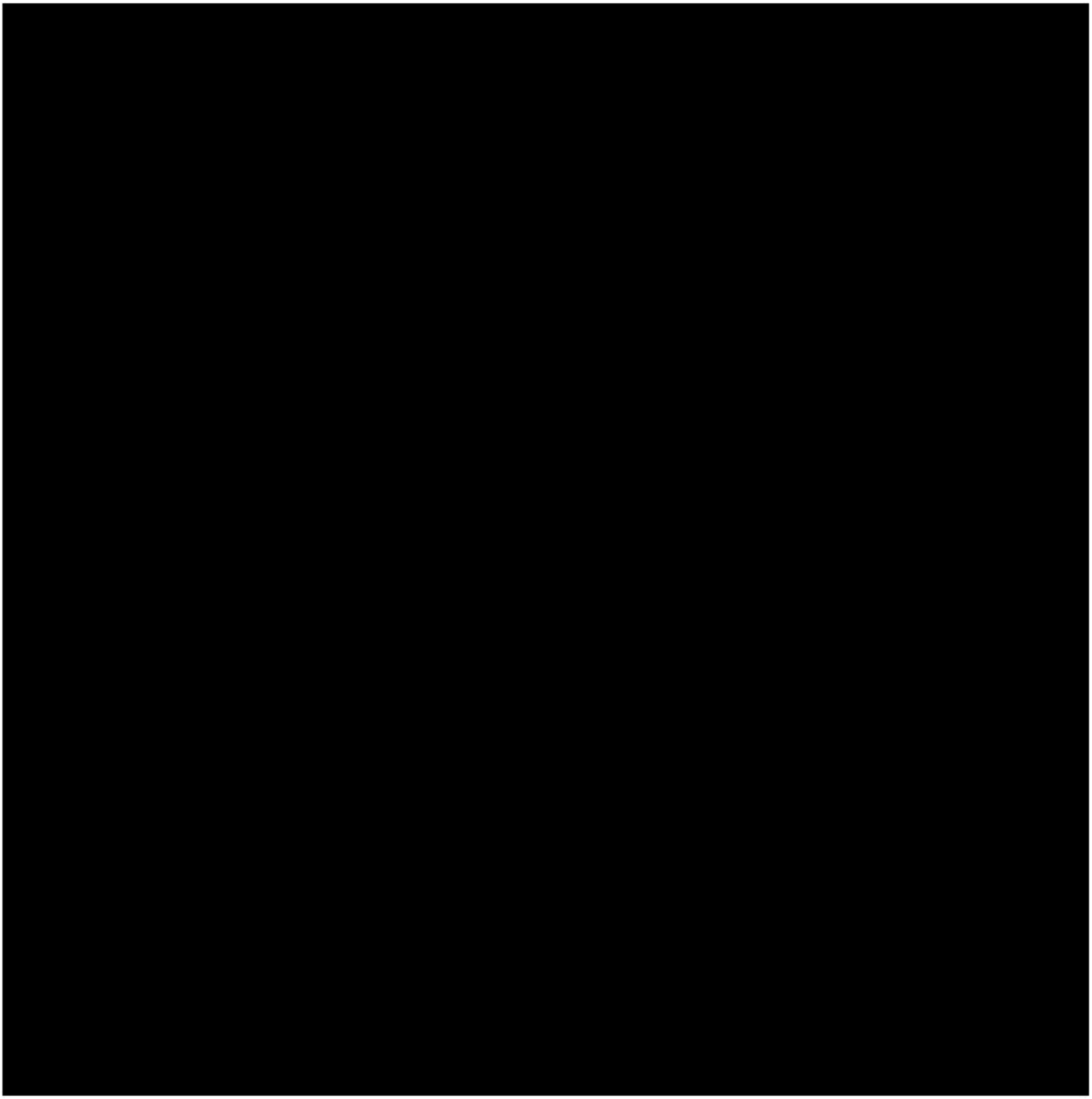
This procedure can be performed by a qualified and trained investigator at the slit lamp microscope or in a procedure room setting. Subsequent injections should be performed in the same setting.

Materials:

Procedure:

After appropriate sterile prepping and draping,

1. 



6.3 Concomitant Medications and Treatments

6.3.1 Concomitant Medications

Subjects are expected to receive concomitant medications for the management of concurrent medical conditions. Medications taken during the 28 days prior until 28 days following the last study treatment administration will be recorded. Artificial tears are allowed during the study but other ocular medications must be discussed with the masked Medical Monitor.

6.3.2 Prohibited Medications

The following medications are prohibited:

- Rho-kinase inhibitors (topical or systemic, e.g.; Fasudil, Netarsudil [Rhopressa])
- Systemic or dermatological cytotoxic chemotherapy
- Hypertonic sodium chloride ointment and drops including Muro 128
- Systemic (nasal, inhaled, oral, parenteral, or topical) or ophthalmic corticosteroids
  Note: Inhaled corticosteroids are acceptable as long as the subject is on stable low doses and the dose is not expected to change for the duration of the study
- Cyclosporine ophthalmic emulsion
- Xiidra ® (lifitegrast ophthalmic solution)

Subjects requiring the use of prohibited medications must be discussed with the masked Medical Monitor.

6.3.3 Pregnancy Testing and Contraception

Women of child-bearing potential (WOCBP) are defined as a premenopausal female capable of becoming pregnant. Women who are not WOCBP are those who have not had a menstrual cycle for at least 2 years or who have had hysterectomy, bilateral tubal ligation, or bilateral oophorectomy. Sexually active female subjects who are WOCBP must use at least 1 highly-effective method of contraception or at least 2 less effective methods of contraception (including 1 barrier method), starting at least 1 month prior to Day 0, and male subjects who are sexually active with women of childbearing potential must agree to use a double-barrier method of contraception. Per *ICH M3(R2) Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals*, highly-effective methods are defined as "those, alone or in combination, that result in a low failure rate (i.e., less than 1% per year) when used consistently and correctly." FDA-approved contraceptive methods and estimated failure rates are summarized in Table 2.

Table 2: FDA-Approved Methods of Contraception and Estimated Failure Rates

Method	Failure Rate
Highly-Effective*	
• Sterilization surgery for women	Less than 1%
• Sterilization implant for women	Less than 1%
• Sterilization surgery for men	Less than 1%
• IUD Copper	Less than 1%
• IUD with Progestin	Less than 1%
• Implantable Rod	Less than 1%
Less-Effective	
• Shot/ Injection	6%
• Oral Contraceptives "The Pill" *	9%
• Patch	9%
• Vaginal Contraceptive Ring	12%
• Diaphragm with Spermicide	12-24%
• Cervical Cap with Spermicide	17-23%
• Male Condom	18%
• Female Condom	21%
• Spermicide Alone	28%

*(including Combined, Extended/ Continuous Use Combined Pill, and the "Mini Pill", Progestin only)
Adapted from FDA Birth Control Guide www.FDA.gov/birthcontrol

The contraceptive plan for each subject should be discussed with the Investigator and will be determined at the Investigator's discretion including the use of a double-barrier (e.g.; male condom, female diaphragm with spermicide) in male subjects.

6.4 Study Procedures

6.4.1 Informed Consent

A sample Informed Consent Form (ICF) will be provided by Trefoil and will be in accordance with 21 CFR 312 Part 50 Subpart B Section 50.25. The sample ICF will be revised by the investigational site to include site-specific information and required language, as needed. The Investigator ensure IRB/IEC review and approval of ICF.

Written informed consent will be obtained from all potential subjects prior to any study-specific assessments are conducted. Informed consent will be obtained in accordance with *21 CFR Part 50 Protection of Human Subjects*.

Alternative therapies will be discussed with each potential participant including other pharmacologic therapies, investigational treatments (if available), or watchful waiting. Informed consent will include a HIPAA release/ waiver.

6.4.2 Demographics

Demographic information will be recorded, including:

- Age
- Sex
- Race
- Ethnicity

The subject's height and weight will be measured and recorded. Height should be measured without shoes and weight should be measured with minimal street clothing. If the Investigator feels that the subject is a reliable source, self-reported height and weight may be recorded.

6.4.3 Medical History and Ocular History

During the Screening/ Pretreatment Period, the Investigator (or designee) will review the medical history of all potential subjects to document all relevant clinically significant baseline medical conditions, concomitant medication use, and to determine study eligibility. Medical History Assessment will include review of all available medical records and patient interview. Assessment of all eligibility criteria should be evidenced in the study records. Medical history will include a detailed disease history regarding onset, severity, and history of prior therapy.

In addition to the data collected in demographics and medical history, specific information will be collected regarding each subject's ocular history, including:

- Present condition
    - Duration (date/ age of onset)
    - Single or both eyes involved
    - Associated symptoms
- Other corneal dystrophies
- Contact lens use and history
- Past ocular surgery (including LASIK)

- History of ocular trauma
- Family history of ocular conditions
- Allergies
- Diabetes
- Hypertension
- Autoimmune disease

Additional relevant medical history will also be recorded.

6.4.4 Comprehensive Ocular Examination

A comprehensive ocular examination will be performed at Screening and Day 56 and will include slit-lamp biomicroscopy and a dilated examination of the lens, fundus, and vitreous.

6.4.5 Specular Microscopy

Specular microscopic images will be obtained of the center of the cornea at every visit. The center of the cornea is defined as the region of the cornea above the pupil. If a subject is to be dilated at a visit, specular microscopy must be performed prior to dilation.

Endothelial cell density, percent hexagonality and the coefficient of variation will be assessed from specular microscope images. A centralized reading center with experienced readers [redacted] will be responsible for the choice and certification of equipment and training of technicians as well as reading images and conducting image analysis.

Common sources of variability in specular microscopy are:

- difficulty in returning to same location on the cornea at each visit
- poor image quality (less than 100 countable cells)
- technician error
- improper reader analysis
- maintaining equipment calibration/alignment

All individuals responsible for on-study image capture must be certified by the reading center.

To address differences in location of the image within a given area of the cornea, three acceptable images should be taken at each location, at each visit. The density from at least one preferred image should be determined.

Prior to obtaining baseline images (Day 0), the technician will obtain an initial set of images for an evaluation of image quality. Training (or retraining) will be performed as necessary and include the following important points:

A preferred image has:

- Distinct cells,
- At least 100 identifiable/ countable cells as a minimum (150 cells preferred), and
- Cells that can be grouped in a uniform area.

To capture a good image:

- Make sure the subject is comfortable,
- Instruct the subject to blink,
- Instruct subject not to move and to open eyes wide,

- Instruct subject to focus on the fixation light,
- If necessary, use the manual setting.
  Note: Use of the manual setting may require additional training

A calibration grid may be obtained from the specular microscope manufacturer.

6.4.6 Best corrected Visual Acuity (BCVA)

Wherever possible best corrected visual acuity (BCVA), **should be measured between 07:00 AM and 11AM,** (with the exception of the Observational Sub-Study Visit 1 PM visit which is to be measured after noon). Site staff should follow their standard procedures for conducting BCVA using Early Treatment Diabetic Retinopathy Study (ETDRS) testing. BCVA at a single site should be done consistently throughout the study using the same equipment, lighting conditions etc.

BCVA testing should precede any examination requiring contact with the eye, including measurement of IOP, and should be performed prior to instillation of any of any dye or drops to dilate or anesthetize the eye. Refraction should be performed prior to measurement of BCVA and the appropriate correction worn in a trial frame during visual acuity testing.

6.4.7 Slit Lamp Biomicroscopy

The slit lamp biomicroscopy examination will be performed at every visit with the slit lamp using a beam of 1.0 mm height and 1.0 mm width with the beam at maximum luminance and using the high powered lens. The subject will be seated during the examination. This procedure should be conducted in the same manner for all subjects and will include an assessment of each of the following:

- Lens
- Corneal Edema
  - Assessed in the central region
- Corneal Opacity
- Conjunctiva - Bulbar Injection
- Sclera - Ciliary Flush
- Anterior Chamber Cells
- Anterior Chamber Flare
- Evaluation of the intra-ocular lens (IOL) and the posterior capsule (for subjects with pseudophakic IOL)

These assessments will be graded according to the criteria described in Table 3.

Table 3: Grading of Slit Lamp Biomicroscopy Assessments

Grade	Corneal Edema (central)	Corneal Opacity	Conjunctiva Bulbar Injection	Sclera Ciliary Flush	Conjunctival Hyperemia (vasodilation)
0	Absent	Absent	Absent	Absent	Absent
1	Mild	Mild	Mild	Mild	Some
2	Moderate	Moderate	Moderate	Moderate	Moderate
3	Severe	Severe	Severe	Severe	Excessive
Grade	**Anterior Chamber Cells**		**Anterior Chamber Flare**		
0	No cells seen		None		
0.5+	1 - 5 cells		-		
1+	6 - 15 cells		Faint		
2+	16 - 25 cells		Moderate (iris and lens detail clear)		
3+	26 - 50 cells		Marked (iris and lens details hazy)		
4+	> 50 cells		Intense (fibrin or plastic aqueous)		

6.4.8 Pachymetry

Automated pachymetry will be performed to measure the corneal thickness. The average and standard deviation (if available) will be recorded for the central cornea. There are no restrictions on the type of pachymeter used in collection of data for this study although automated reading from the specular microscope is preferred; the same methodology must be used consistently within a subject. For those pachymeters that are not self-calibrating, calibration must be performed on a quarterly basis and documented in the calibration log in the study regulatory binder.

6.4.9 Intraocular Pressure Measurement

IOP must be recorded using Goldmann Applanation Tonometry (GAT). At least two measurements will be obtained and recorded (a third measurement will be obtained if there is a $\geq$ 3 mmHg difference between the first two measurements). Post-dose IOP will be measured at least every 30 minutes until normalized (defined as less than 5 mmHg higher than pre-injection IOP).

6.4.10 Urine Pregnancy Test

WOCBP must be tested prior to the start of study treatment and after the completion of study treatment to confirm a continued non-pregnant state. Commercially available urine dip-stick pregnancy tests are acceptable.

6.4.11 Clinical Laboratory Assessments

Samples for clinical laboratory assessments will be obtained and transmitted to a central laboratory. The laboratory parameters to be tested are summarized below in Table 4.

All clinically significant laboratory results that are outside of the normal ranges are to be reported as AEs. An abnormal lab value should be deemed clinically significant if either of the following conditions is met:

- The abnormality suggests a disease and/or organ toxicity that is new or has worsened from baseline as determined by the Investigator and in consultation with Medical Monitor consult at the Investigator's request

- The abnormality is of a degree that requires additional active management, e.g., change of dose, discontinuation of the drug, close observation, more frequent follow-up assessments, or further diagnostic investigation

Therefore, a clinically significant laboratory value is one that suggests a new disease process, an exacerbation or worsening of an existing condition, or requires further action(s) to be taken.

If a clinically significant laboratory result is found on a laboratory test (e.g., hematology), the Investigator should determine if an underlying condition is the reportable AE instead of the individual laboratory parameters (e.g., anemia instead of decrease in red blood cell count, hematocrit, reticulocyte and hemoglobin levels).

Table 4: Clinical Laboratory Assessments

Hematology	
• Hematocrit • Hemoglobin • White blood cell (WBC) count with differential • Platelet count	
Serum Chemistry	Urinalysis
• Albumin • Alkaline phosphatase • ALT (alanine amino transferase)/ SGPT • AST (aspartate amino transferase)/ SGOT • Bicarbonate/CO2 • Bilirubin • Blood urea nitrogen • Calcium • Chloride • Creatinine • HbA1-c • LDH (Lactate dehydrogenase) • Potassium • Sodium • Total protein • Uric acid	• Specific gravity • Ketones • pH • Protein • Glucose • Blood • Urobilinogen • Bilirubin • Leukocyte esterase • Microscopic examination (as applicable)

6.4.12 Plasma for PK and ADA

Blood samples will be collected for pharmacokinetic (PK) and anti-drug antibody (ADA) analysis. Samples will be centrifuged and/or prepared per instructions from the analytic laboratory. Pre-injection samples should be collected within the 2 hours prior to study treatment administration. PK samples should be collected within ± 5 minutes of the nominal timepoint or as soon as practicable.

See Study TTHX-001 Laboratory Manual for blood processing instructions.

6.4.13 National Eye Institute Visual Function Questionnaire (NEI-VFQ-25)

The NEI-VFQ-25 is a reliable and validated 25-item version of the 51-item NEI-VFQ [16]. The subject will complete the NEI-VFQ-25 at Day 0 (pre-injection) and Day 56. The NEI-VFQ-25 is to be completed prior to any other procedures at these visits. Upon completion of the

questionnaire, the study coordinator will check the questionnaire for completeness. Any omissions or ambiguous answers will be clarified by the subject prior to leaving the clinic. The NEI-VFQ-25 should be self-administered, however, trained investigational site staff will administer the questionnaire to subjects who are unable to self-administer the questionnaire.

6.4.14 Pentacam Tomography

Pentacam® tomography must be performed during screening and at Study Day 56. The Pentacam images must meet the internal software quality metric to be acceptable.

6.4.15 Optional Blood Sample for Genetic Testing

Subjects with a diagnosis of FECD will be asked to provide an optional blood sample for genetic testing. Subjects will be asked to provide specific consent for this optional genetic testing. This sample will be completely de-identified prior to transmittal to the testing facility. This sample is optional and participation in neither the Observational Sub-study nor the interventional portion is conditional on the consent to provide this sample for genetic testing.

6.5 Study Visits

All study visits/ assessments will be conducted by qualified investigational site staff. Study visits/ assessments are to be conducted within the protocol-specified window for each visit/ assessment. If an assessment is not able to be collected within the protocol-specified window, it should be collected as soon as practicable and the actual date/ time recorded. Comparative assessments should be collected at the same time at each timepoint, when possible (unless otherwise directed). If an onsite visit is not possible, as many assessments as feasible can be conducted remotely.

6.5.1 Screening Period/Baseline Assessments

Screening assessments may be performed anytime during the Screening Period unless otherwise specified.

Screening

- Informed consent
- Eligibility assessment
- Demographics
- Medical and Ocular History
- Record Concomitant Medication Use
- BCVA
    - Both eyes
    - Between 07:00 AM and 11:00 AM
- Specular Microscopy (including automated pachymetry)
    - Both eyes
- Comprehensive Ocular Examination
    - Slit-lamp biomicroscopy
    - Dilated examination of lens, fundus, and vitreous
    - Both eyes

Note: Subjects enrolled in and participating in the Observational Sub-study who are eligible may “roll-over” into the main study provided they meet all of the additional eligibility criteria,

there is an open cohort, and they consent. Observational Sub-study assessments may be used as screening assessments for the main study as long as they were performed within the protocol-specified timeframe.

6.5.2 Day 0

Pre-Injection

- Record Concomitant Medication Use
- Urine pregnancy (WOCBP)
- Obtain samples for laboratory testing:
  - Collect samples for Chemistry, Hematology, Urinalysis, HbA1c, per the TTHX-001 Laboratory Manual
  - Collect optional blood sample for genetic testing (if consented)
- Obtain sample for ADA
- Obtain sample for PK
- NEI-VFQ-25
- Best corrected visual acuity (BCVA)
  - Study eye
  - Between 07:00 AM and 11:00 AM
- Specular Microscopy (including automated pachymetry)
  - Both eyes
- Slit Lamp Biomicroscopy
  - Study eye
- IOP
  - Study eye
- Pentacam examination
  - Both eyes
- Confirm eligibility

Post-Injection

- IOP
  - Study eye only
  - At least every 30 minutes after injection until normalized (within 5 mmHg of pre-injection measurement)
- Record Adverse Events and Concomitant Medication Use

6.5.3 Day 3

- Record Adverse Events and Concomitant Medication Use. This visit may be conducted by phone.

6.5.4 Day 7 ± 1

- BCVA
  - Study eye only
  - Between 07:00 AM and 11:00 AM
  - Pre-injection

- Specular Microscopy (including automated pachymetry)
    - Both eyes
    - Pre-injection
- Slit Lamp Biomicroscopy
    - Study eye only
    - Pre-injection
- IOP
    - Study eye only
    - Pre-injection
    - At least every 30 minutes after injection until normalized (within 5 mmHg of pre-injection measurement)
- Study Treatment IC Injection (SE)
- Record Adverse Events and Concomitant Medication Use

6.5.5 Day 14 ± 1

- BCVA
    - Study eye only
    - Between 07:00 AM and 11:00 AM
    - Pre-injection
- Specular Microscopy (including automated pachymetry)
    - Both eyes
    - Pre-injection
- Slit Lamp Biomicroscopy
    - Study eye only
    - Pre-injection
- IOP
    - Study eye only
    - Pre-injection
    - At least every 30 minutes after injection until normalized (within 5 mmHg of pre-injection measurement)
- Study Treatment IC Injection (SE)
- Record Adverse Events and Concomitant Medication Use

6.5.6 Day 21 ± 1

- Obtain Sample for ADA
    - Pre-injection
- Obtain Sample for PK
    - Pre-injection
    - 1.5 hours after injection
- BCVA
    - Study eye only
    - Between 07:00 AM and 11:00 AM
    - Pre-injection
- Specular Microscopy
    - Both eyes

  - Pre-injection
- Slit Lamp Biomicroscopy
  - Study eye only
  - Pre-injection
- IOP
  - Study eye only
  - Pre-injection
  - At least every 30 minutes after injection until normalized (within 5 mmHg of pre-injection measurement)
- Study Treatment IC Injection (SE)
- Record Adverse Events and Concomitant Medication Use

6.5.7 Day 28 ± 1

- Obtain samples for Laboratory Testing (Chemistry, Hematology, Urinalysis, HbA1c)
- Obtain sample for ADA
- BCVA
  - Study eye only
  - Between 07:00 AM and 11:00 AM
- Specular Microscopy (including automated pachymetry)
  - Both eyes
- Slit Lamp Biomicroscopy
  - Study eye only
- IOP
  - Study eye only
- Record Adverse Events and Concomitant Medication Use

6.5.8 Day 56 ± 3

- Urine pregnancy (WOCBP)
- Obtain samples for laboratory testing (Chemistry, Hematology, Urinalysis, HbA1c)
- Obtain sample for ADA
- Obtain sample for PK
- NEI-VFQ-25
- BCVA
  - Study eye only
  - Between 07:00 AM and 11:00 AM
- Specular Microscopy (including automated pachymetry)
  - Both eyes
- Comprehensive Ocular Examination
  - Slit-lamp biomicroscopy
  - Dilated examination of lens, fundus, and vitreous. Note: dilation should be performed after BCVA and measurement of IOP.
  - Both eyes
- IOP
  - Study eye only
- Pentacam examination

  - Both eyes
- Record Adverse Events and Concomitant Medication Use

6.5.9 Day 90 ± 7

- BCVA
  - Study eye only
  - Between 07:00 AM and 11:00 AM
- Specular Microscopy (including automated pachymetry)
  - Both eyes
- Slit Lamp Biomicroscopy
  - Study eye only
- IOP
  - Study eye only
- Record Adverse Events and Concomitant Medication Use

6.5.10 Early Termination Visit

If a subject is discontinued from study treatment early or withdraws from the study, he/she should be assessed approximately 28 days following the last study treatment administration; if the subject is unable to be evaluated approximately 28 days following the last study treatment administration, he/she should be evaluated as soon as practicable (at least by telephone). All assessments scheduled for Day 56 should be performed at the Early Termination Visit.

6.5.11 Unscheduled Visit

Study subjects may be seen at any time in between scheduled study visits at the discretion of the Investigator and any relevant study assessments performed. The date and reason for the unscheduled visit will be recorded as well as the results of the assessments.

6.6 Pregnancy

Subjects will be instructed to notify the Investigator as soon as possible after becoming pregnant or learning of the pregnancy of a partner. If a subject or partner of a subject becomes pregnant during treatment or up to 120 days following the last study drug administration, the Investigator is instructed to notify Trefoil (or designee) within 24 hours of learning of the pregnancy.

If the subject becomes pregnant while receiving investigational product, the investigational product will be permanently discontinued. Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy. Other appropriate pregnancy follow-up procedures should be considered if indicated.

If the partner of a study participant becomes pregnant, the Investigator is instructed to obtain a Pregnant Partner Release Form and collect relevant information regarding the partner and the pregnancy. The Investigator will discuss the risks and concerns of investigational drug exposure to a developing fetus and counsel the subject and/or pregnant partner (or ensure that such counseling is provided).

Pregnancies will be followed through the outcome of the pregnancy. Newborns should be followed for a minimum of 8 weeks. The Investigator will complete a Pregnancy Surveillance Form and report the information regarding the pregnancy, outcome, and status of the newborn, as appropriate.

7. ASSESSMENT OF EFFICACY

As described above, the primary efficacy endpoint will be endothelial cell count/ density as measures by specular microscopy. Other efficacy assessments include:

- NEI-VFQ-25
- BCVA
- Pentacam examination
- Slit lamp biomicroscopy
- Pachymetry
- IOP

The primacy efficacy timepoint is Day 56. Please refer to Appendix A for the schedule of events.

8. ASSESSMENT OF SAFETY

8.1 Adverse Events

Investigators will collect information related to adverse events (AEs) throughout this clinical trial. The terms and definitions are consistent with *Guidance for Industry and Investigators Safety Reporting Requirements for INDs and BA/BE Studies, FDA 2012*.

- All AEs occurring in all subjects following exposure to study treatment until 30 days after the last study treatment will be reported.
- AEs will be followed until at least the end of the study.
- AEs considered related to the study treatment by the Investigator (SARs) and SAEs will be followed until resolution, return to baseline, or stabilization (if resolution is not expected).

Changes in the subject's medical condition that occur prior to the first exposure to study treatment will be reported as medical history. At each post-treatment visit, subjects will be asked about any possible adverse events in a non-leading manner. An example of a non-leading method of eliciting AE information is "*Have there been any changes in your health since you were here last*?" Subjects should also be asked about the severity and/or persistence of any AEs that were ongoing at the time of their last visit.

8.1.1 Adverse Events Definitions

8.1.1.1 Adverse Events (21 CFR 312.32(a))

An adverse event (AE) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug and does not imply any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

8.1.1.2 Suspected Adverse Reaction (21 CFR 312.32(a))

Suspected adverse reaction means 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.

8.1.1.3 Unexpected (21 CFR 312.32(a))

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

8.1.1.4 Serious Adverse Event (21 CFR 312.32(a))

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/ birth defect

Important medical events 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 or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.2 Adverse Events Reporting

8.2.1 Adverse Event Term

Adverse events must be reported using standard medical terminology. The use of abbreviations (standard and nonstandard) should be avoided to help ensure a clear understanding of the event. An example of a standard abbreviation that may have several meanings is "MI" which could

mean "myocardial infarction" or "mitral insufficiency." All AE terms will be coded using a standardized dictionary (i.e., Medical Dictionary for Regulatory Activities [MedDRA]).

- Generally, when reporting a well-known and understood condition, it is preferable to report the overall diagnosis rather that the individual signs and symptoms; the exception to this rule in this study is when the subject experiences an injection site reaction.
- The term "intermittent" should be avoided as the duration and incidence of events helps in understanding the safety profile of the study drug.

8.2.2 Adverse Event Severity

Adverse events will be reported at the highest experienced. Adverse events severity will be graded according to the criteria described in Table 5.

Table 5: Severity Grading Guideline for Adverse Events

Grade	Description
1	Mild; Asymptomatic or mild symptoms; Clinical or diagnostic observations only; Intervention not indicated
2	Moderate; Minimal, local or noninvasive intervention indicated; Limiting age-appropriate instrumental ADL*
3	Severe or medically significant; Hospitalization or prolongation of hospitalization indicated; Disabling; Limiting self-care ADL**

Adapted from NCI-CTCAE V5.0
Note: A Semi-colon indicates 'or' within the description of the grade.
*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

8.2.3 Adverse Event Duration

The start date (the date that the event was first noticed) and the end date (the date that the event had completely resolved or returned to baseline) will be recorded. If the exact date is not known, the best estimate should be reported.

All SARs will be followed until stabilization or resolution.

8.2.4 Adverse Event Causality

Where the Investigator assessment of the relationship of the AE to investigational product rests on medical judgment, the determination must be made with the appropriate involvement of the Investigator, or, if the Investigator is not a physician, a designated sub-Investigator who is a physician. Assessment of causality should be performed in accordance with the definition of SAR in Section 8.1.1.2.

Using the following criteria, Investigators will assess whether there is a reasonable possibility that the study treatment (drug or procedure) caused or contributed to the AE.

Related
There is a "reasonable possibility" to suggest a causal relationship between the study treatment and the adverse event based on evidence.

- The Investigator should use the following criteria when assessing causality:
- Is the AE a known side effect/ adverse reaction to the study treatment or other therapies in this treatment class?
- Is there a reasonable temporal relationship between the start of the AE and study treatment administration?
- Did the AE improve when the study treatment was stopped?
- Did the AE recur when the study treatment was resumed, if applicable?
- Can the AE be easily attributed to a comorbid condition or concomitant medication/ treatment?

Not Related
There is NOT a "reasonable possibility" to suggest a causal relationship between the study treatment and the adverse event based on evidence.

8.2.5 Adverse Event Seriousness

Please report SAE criteria using the following guidelines:

Death
Report if you suspect that the death was an outcome of the adverse event, and include the date if known.

Life-threatening
Report if suspected that the patient was at substantial risk of dying at the time of the adverse event, or use or continued use of the device or other medical product might have resulted in the death of the patient.

Hospitalization (initial or prolonged)
Report if admission to the hospital or prolongation of hospitalization was a result of the adverse event. Emergency room visits that do not result in admission to the hospital should be evaluated for one of the other serious outcomes (e.g., life-threatening; required intervention to prevent permanent impairment or damage; other serious medically important event).

Disability or Permanent Damage
Report if the adverse event resulted in a substantial disruption of a person's ability to conduct normal life functions, i.e., the adverse event resulted in a significant, persistent or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities and/or quality of life.

Congenital Anomaly/Birth Defect
Report if you suspect that exposure to a medical product prior to conception or during pregnancy may have resulted in an adverse outcome in the child.

Other Serious (Important Medical Events)
Report when the event does not fit the other outcomes, but the event may jeopardize the patient and may require medical or surgical intervention (treatment) to prevent one of the other outcomes. Examples include allergic brochospasm (a serious problem with breathing) requiring

treatment in an emergency room, serious blood dyscrasias (blood disorders) or seizures/convulsions that do not result in hospitalization. The development of drug dependence or drug abuse would also be examples of important medical events.

(https://www.fda.gov/safety/reporting-serious-problems-fda/what-serious-adverse-event)

8.3 Serious Adverse Events and Adverse Events of Special Interest Reporting

Adverse events that meet the definition of serious require accelerated reporting. Investigators must report all Serious Adverse Events (SAEs) regardless of causality immediately (within 24 hours of learning of the event). SAEs will be reported by submitting a Serious Adverse Events Report (SAER) to:

If adverse events of special interest (AESI) are identified during the study, Trefoil will notify Investigators and provide instructions for reporting. Deaths occurring within 30 days of study treatment and AESIs will be reported to Trefoil in the same time frame as SAEs.

Trefoil will be responsible for expedited safety reporting to regulatory authorities and principal investigators where clinical trials involving investigational product are being conducted.

9.

9.3.1 Analysis Populations and Sets

[redacted]

9.3.2 [redacted]

[redacted]

10. QUALITY CONTROL AND QUALITY ASSURANCE

10.1 Data Quality Assurance

This study will be conducted and monitored in accordance with GCP and the Study Monitoring Plan (SMP).

10.1.1 Monitoring

Monitoring of this study will include a masked and an unmasked Medical Monitor, onsite and remote data review and verification. Site monitors will review CRFs to ensure that the collected data accurately reflects the individual subject's experience. Site monitors will also monitor protocol compliance. Protocol deviations include any departure from the study protocol whether intentional or inadvertent. The Medical Monitor (or designee) will periodically review all deviations and determine is corrective action (e.g.; investigational site staff retraining) is required. Important deviations will include subjects who:

- Were enrolled and did not satisfy the entry criteria
- Developed withdrawal criteria during the study but were not withdrawn
- Received the wrong treatment or incorrect dose or an excluded concomitant

Important protocol deviations will be reported to the Medical Monitor(s) in an expedited manner.

10.1.2 Database Management and Quality Control

Reported data will be systematically reviewed and monitored throughout the study. Review will include an assessment of completeness and quality of data. A subset of data variables in the database and CRFs will be source data verified using a risk-based approach as described in the Study Monitoring Plan (SMP). Trefoil (or designee) will issue data queries and/or request for clarification to the investigational site staff as needed.

Additional investigational site training will be provided as needed.

11. ADMINISTRATIVE

11.1 Protocol Approval and Amendment

The Trefoil (or designee) will submit all protocols and protocol amendments to the FDA and relevant regulatory authorities, as applicable. The Investigator will ensure local IRB/IEC review and approval of the protocol and protocol amendments prior to implementation at his/her investigational site.

If an amendment substantially alters the study design or increases the potential risk to the subject:

- The consent form must be revised and submitted to the IRB/IEC for review and approval/ favorable opinion
- The revised form must be used to obtain consent from subjects currently enrolled in the study if they are affected by the amendment
- The new form must be used to obtain consent from new subjects prior to enrollment

If the revision is an administrative letter, Investigators must inform their IRB/IEC.

11.2 Premature Termination of the Study

The study may be prematurely terminated if the Trefoil becomes aware of conditions or events that suggest a possible hazard to subjects or at the discretion of Trefoil. Reasons for premature study termination may include, but are not limited to:

- An unacceptable risk to the subjects,
- Failure to enroll subjects at an acceptable rate, or
- The study objectives have been met

If the study is prematurely terminated, Trefoil will provide instructions regarding a wind-down plan that will include a notification to subjects, IRB/IEC, and relevant regulatory agencies as well as a process for the continuation and/or discontinuation of study treatment and safety follow-up for all active subjects. Subjects who may have already completed the study may be contacted, if applicable, for additional safety assessments.

11.3 Confidentiality

All records identifying the subject will be kept as confidential as possible within the law. Subject names and other personally identifiable information (PII) will not be supplied to the sponsor. Data and/or samples collected from subjects will be identified only by a unique Subject ID Number. If the subject's name appears on any other material collected (e.g., pathologist report) or other study materials (e.g., biopsy tissue slides), all PII must be redacted before being supplied to the Trefoil (or designee). Study data stored on a computer will be stored in accordance with local data protection laws and regulations. Subjects will be informed in writing that representatives of the Trefoil, IRB/IEC, or regulatory authorities may inspect their medical records to verify the information collected. All personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws and regulations. The Investigator will maintain a list to enable subjects' records to be identified in accordance with applicable laws and regulations.

If the results of the study are published, the subject's identity will remain confidential.

12. DATA HANDLING AND RECORD KEEPING

12.1 Case Report Forms and Source Documentation

Per 21 CFR 312.62(b) Investigators shall retain required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurse's notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study. Source documents may be in the local native language but all data reported in the data collections forms will be in English.

12.1.1 Data Collection

CRFs should be completed for each subject included in the study and should reflect the latest observations on the subjects participating in the study and should be completed as soon as possible during or after the assessment. The Investigator must confirm that all data entries in the CRF are accurate and correct.

Data about all study drug dispensed or administered to the subject and any dosage changes will be recorded.

12.2 Access to Source Data

Per 21 CFR 312.68, the Investigator shall upon request from any properly authorized officer or employee of FDA, at reasonable times, permit such officer or employee to have access to, and copy and verify any records or reports made by the investigator pursuant to 21 CFR 312.62. The Investigator must notify the Trefoil (or designee) promptly of any inspections by regulatory authorities as soon as possible and forward promptly copies of inspection reports.

Access to source data extends to the Sponsor (or designee) who will review study records and directly compare them with source documents, discuss the conduct of the study with the Investigator, and verify that the facilities remain acceptable.

12.3 Data Processing

All data will be entered into the data collection forms by the investigational site staff. Ongoing data review will be performed during the conduct of the study including checks for consistency and logic of data. Queries and requests for clarification will be generated by the Sponsor (or designee) and transmitted to the investigational site staff. The collections forms and/or database will be updated as needed while maintaining a clear audit trail.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The versions of the coding dictionary will be provided in the Clinical Study Report.

12.4 Archiving Study Records

Per 21 CFR 312.62(c), Investigators shall retain records required to be maintained under this part for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the

application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. However, documents may be retained for a longer period if required by the applicable legal requirements.

13. PUBLICATION POLICY

By signing the study protocol signature page, the Investigator agrees with the use of results of the study for the purposes of national and international registration, publication and information for medical and pharmaceutical professionals. If necessary, Regulatory Authorities will be notified of the Investigator's name, address, qualifications and extent of involvement.

An Investigator shall not publish any data (poster, abstract, paper, etc.) without discussion with and approval by Trefoil.

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Appendix A: Schedule of Events

	Sub-Study		Study TTHX-001										
	Visit		Screen	Study Day									
				0		3	7 ±1	14 ±1	21 ±1	28 ±1	56 ±3	90 ± 7
	1	2-4		Pre									
Informed consent	X		X										
Demographics	X		X										
Medical and ocular history	X		X										
Comprehensive ocular examination (including slit lamp examination and dilated exam of the lens, fundus and vitreous)			OU						SE[f]		OU		
Urine pregnancy test (WOCBP)				X							X		
Laboratory testing (Chem, Heme, UA, & HbA1c)				X						X	X		
NEI-VFQ-25[a]				X							X		
Optional blood sample for genetic testing				X									
ADA assay Pre-injection				X					X	X	X		
PK sampling Pre-injection 1.5 hours after injection				X	 X				 X X		X		
Study Treatment IC Injection (SE)					X		X	X	X				
Record Concomitant Medications*	X	X	X	X	X	X	X	X	X	X	X	X	
Record Adverse Events					X	X	X	X	X	X	X	X	
Ocular Assessments													
Specular microscopy[b] (including automated pachymetry)	OU[c, d]	OU	OU	OU			OU	OU	OU	OU	OU	OU	
Pentacam examination				OU							OU		
Best corrected visual acuity[b]	OU[c, d]		OU	SE			SE	SE	SE	SE	SE	SE	
Slit lamp biomicroscopy	OU[c, d]			SE			SE	SE	SE	SE		SE	
Intraocular Pressure (IOP) measurement	OU[c, d]			SE[e]	SE[e]		SE[e]	SE[e]	SE[e]	SE	SE	SE	

OU=both eyes, SE=Study Eye, [a] Perform prior to any other assessments, [b] If subject is to be dilated, perform prior to dilation, [c] 2 timepoints (1 in the early morning and 1 in the late afternoon), may be different days. [d] The OSS AM assessments can be used for screening, [e] IOP pre-dose and post-dose at least every 30 minutes until normalized (defined as less than 5 mmHg higher than pre-injection IOP) [f] dilated exam only in subjects with vitreous in the AC at baseline
*Including all ocular medications

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PROTOCOL SIGNATURE PAGE

TREFOIL DISCLOSURE STATEMENT

This document contains information that is confidential and proprietary to Trefoil Therapeutics Inc (Trefoil). This information is being provided to you solely for the purpose of evaluating or conducting a clinical study for Trefoil. You may disclose the contents of this document only to study personnel under your supervision who need to know the contents for this purpose and to your Institutional Review Board (IRB), otherwise the contents of this document may not be disclosed without the prior authorization from Trefoil. The foregoing shall not apply to disclosure required by governmental regulations or laws. Any supplemental information that may be added to this document also is confidential and proprietary to Trefoil and must be kept in confidence in the same manner as the contents of this document.

All documentation for this study that is supplied to me and that has not been previously published will be kept in the strictest confidence. This documentation includes this study protocol, Investigator's Brochure, electronic data capture system, and other scientific data. I have read and understood and agree to abide by all the conditions and instructions contained in this protocol and agree to:

- Conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the sponsor, except when necessary to protect the safety, rights, or welfare of subjects
- Personally, conduct or supervise the described investigation
- Personally, administer all study treatment intracameral injections or only allow qualified individuals who have been approved by Trefoil to administer study treatment intracameral injections
- Inform any patients, or any persons used as controls, that the drugs are being used for investigational purposes and I will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met
- Report to the sponsor adverse experiences that occur in the course of the investigation(s) in accordance with 21 CFR 312.64. I have read and understand the information in the protocol and investigator's brochure, including the potential risks and side effects of the drug
- Ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments
- Maintain adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection in accordance with 21 CFR 312.68
- Ensure that an IRB that complies with the requirements of 21 CFR Part 56 will be responsible for the initial and continuing review and approval of the clinical investigation. I also agree to promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others. Additionally, I will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects
- Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements in 21 CFR Part 312

Responsible Investigator

Signature Date

Name/ Title (Printed)