STORM

Clinical Study Protocol

Protocol No.: TTHX-002

Phase: Phase 2

Name of Study: Phase 2 Study to Determine the Safety and Efficacy of the Investigational New Drug TTHX1114(NM141) on the Regeneration and Migration of Corneal Endothelial Cells in Patients undergoing Descemetorhexis without Endothelial Keratoplasty (Descemet Stripping Only)

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 7.0, September 22, 2022**
Amendment 6.0, February 24, 2022
Amendment 5.0, December 22, 2021
Amendment 4.0, July 2, 2021
Amendment 3.0 April 22, 2021
Amendment 2.1: March 5, 2021
Amendment 1.0: January 4, 2021
Version 1.1: November 10, 2020
Version 1.0: October 14, 2020

Approved By:

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David Eveleth, PhD
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.

STORM

"After night comes the dawn. And after every storm there comes clear, open skies."

-Samuel Rutherford

DOCUMENT HISTORY

Document ID	Changes
Version 1.1	The protocol was revised for the following reasons: • To specify that prior exposure to TTHX1114 is exclusionary • To clarify that peripheral endothelial cell count > 1000 cells/mm^2 on specular microscopy does not need to be confirmed by the central reading facility and other considerations (such as CEC morphology) should be considered if the minimum CEC count is not met following discussion with the Medical Monitor • To add a Day 84 visit which will have the same study assessments and Day 168 and Day 336 • To remove the requirement for Pentacam Tomography
Amendment 1	The protocol was revised for the following reasons: • To clarify that guttae in the study eye do not need to be limited to the central 5mm (i.e., people that also have non-confluent peripheral guttae are not excluded) • To exclude all planned post-operative rho-kinase use; rho-kinase use should still be discussed with the Medical Monitor, if needed • To clarify that "prior exposure to TTHX1114 does not include subjects who had participated in Study TTHX-001 who were assigned to placebo or only participated in the Observational Sub-study • To decrease the washout period for hypertonic saline preparations from 14 days to 4 days prior to Study Day 0 • To remove the requirement for bilateral (OU) ocular assessments and require ocular assessments of the Study Eye (SE) • To remove the requirement that Day 0 TTHX1114 administration be performed "immediately" after the DWEK/DSO procedure but should be administered as soon as practicable • To clarify that corneal endothelium tissue and aqueous humor samples will be collected at a subset of sites, where allowed
Amendment 2.1	The protocol was revised for the following reasons: • To add additional treatment groups that will include an additional 16 subjects for a total of up to 40 subjects • Group 3 will consist of 16 additional subjects • Groups 1 and 3a will be comprised of enrolled subjects in Group 1 or Group 3 that, in the opinion of the Investigator (with approval of the Medical Monitor may benefit from an up to 5-week course of TTHX1114 (10ng every 7 days x 5) • To clarify that the efficacy endpoint will include peripheral CEC counts as well as central • To clarify that subjects must be considered suitable candidates for DWEK/DSO and that patients otherwise considering DMEK or DSAEK may be evaluated for eligibility • To remove the criteria of at least 6 months from time of FECD diagnosis • To remove the criteria of at least 1000 CEC/mm^2; if the Investigator feels that the patient has an adequate CEC reserve based other criteria such as CEC characteristics • To clearly state that planned rho-kinase inhibitor use is exclusionary in all treatment groups

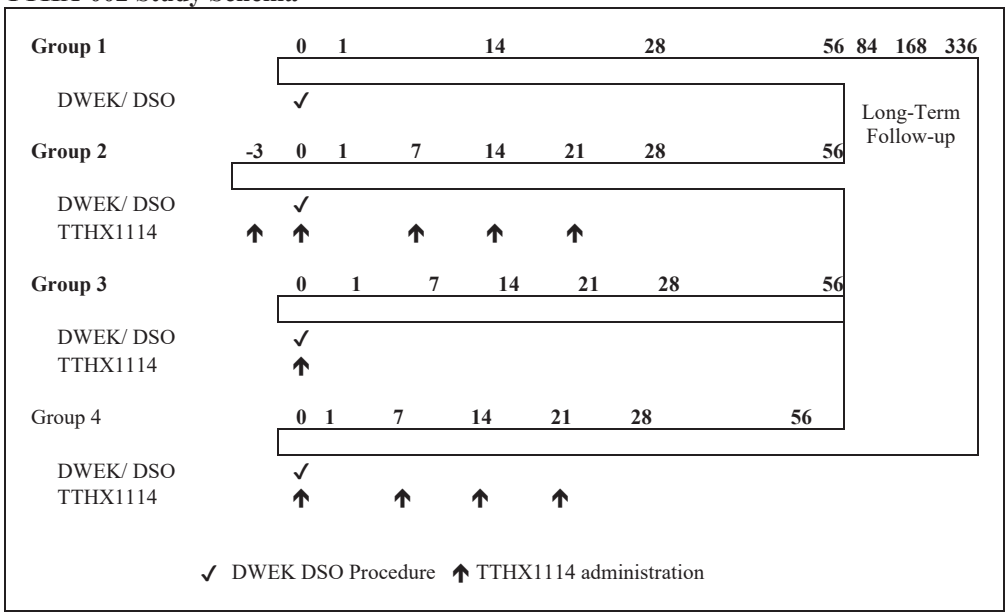
	• To correct the typographical error that PK samples must be collected within 24 hours not 2[sic] hours prior to study treatment administration and to allow collection of the optional genetic sample at any time during the study • To allow CCT to be measured using Pentacam
Amendment 3.0	The protocol was revised for the following reasons: • To add 2 additional dose level following the preliminary safety assessment of the 10ng dose level (in the nonsurgical eye) and the 50ng dose level on the day of surgery ○ The 30ng dose level will be a step-up dose in intrasubject dose escalation • To increase the dose level in Group 3 from 50ng to 100ng on Day 0 • To add an additional treatment group (Group 4) • To increase the dose level in Group 1a and Group 3a from 10ng to 50ng • To remove the exclusion criteria of prior exposure to TTHX1114 as TTHX1114 has only been administered to subjects in clinical trials sponsored by Trefoil and current nonclinical data supports the repeat administration of a total of 5 IC injections (subjects in TTHX-001 would only have received 4 IC injections) • To update Section 2.2.2 with preliminary safety data from Study TTHX-001 (10mcL volume IC injections) and TTHX-002 (50mcL volume IV injections following DWEK/DSO) • Add the rationale for increased volume of IC injections based on observed safety to date and aqueous humor turnover/ physiology • To clarify the criteria by which subjects who had previously participated in Study TTHX-001 may be considered for inclusion and the extent to which they may receive TTHX1114 in Study TTHX-002 • To describe the existing process by which subjects are registered for Study TTHX-002 and are assigned to treatment groups • To describe the existing process by which investigational site will complete Injection Feedback Forms to facilitate the near-to-real-time assessment of DLT and study compliance
Amendment 4.0	The protocol was revised for the following reasons: • Allow treatment of the contralateral eye • Remove the eligibility criteria of no planned ocular surgery in the fellow eye for 3 months • Remove systemic corticosteroids as a prohibited medication • To include baseline and post-treatment macular optical coherence tomography (MAC-OCT) as part of the comprehensive ocular examination (baseline, Day 28, and Day 56) • Add CPTS grading of Stromal Clarity to the Slit Lamp Examination as well as collection of area or edema • Add direction for additional assessments for determining BCVA in subjects who have not recovered to BCVA of ≥20/40 at Day 28 and/or Day 56 • Add Slit Lamp Examination at Day 336 Visit • Add corneal thickness assessment at Day 7 and remove BCVA assessment at Day 7 • Remove IOP assessments after 1 week following the last TTHX1114 injection except for Day 56 • Remove specular imaging from Day 14 and Day 21 and add at Day 84
Amendment 5.0	The protocol was revised for the following reasons: • To remove the preliminary clinical trial safety data and refer the reader to the current version of the Investigator Brochure for the most up to date clinical trial information

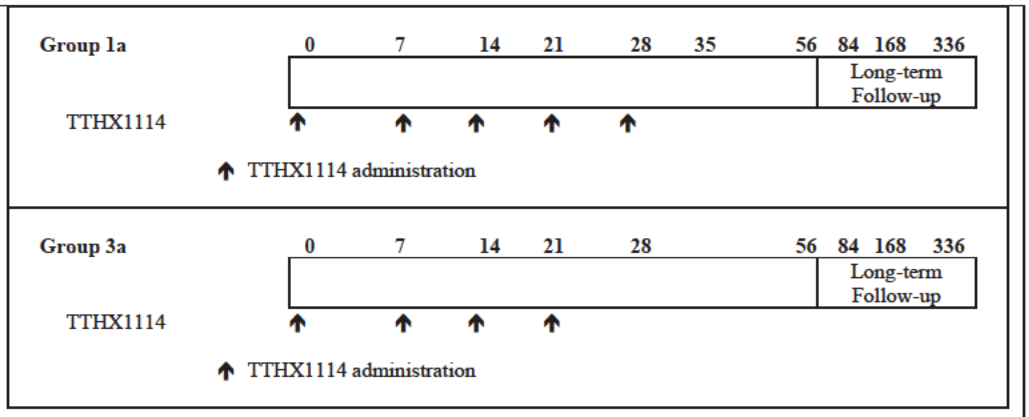
	• To add an Optional Extended Dosing Period OEDP for subjects who have received fewer than 5 TTHX1114 administrations • To add 2 additional dose levels to be explored in dose-escalation • To add additional detail around dose-escalation rules • To add clarification regarding nomenclature related to Study Eye and Fellow Eye • To change the distribution of subjects in each group and decrease the overall number of subjects in the trial • To update the definition of efficacy endpoints to align with the Statistical Analysis Plan • Provide additional guidance related to eligibility regarding allergy history • Clarify that peripheral specular images will be collected at baseline and sent to CIARC for archiving • Clarify that the average IOP measurement will be recorded at each required timepoint • Add definitions for Adverse Events of Special Interest • Update Per Protocol Population definition
Amendment 6.0	The protocol was revised for the following reasons: • To modify the exclusion of prior refractive surgery in the study eye unless approved by the Sponsor • To remove reference to 75ng dose and dose escalation during the OEDP • To clarify that the planned and actual area of descemetorhexis area is 4 to 5mm and to specify that subject with a descemetorhexis area of > 5mm will be excluded from efficacy analyses • To require the reporting of the reasons for changes in study procedures • To add the provision of AS-OCT imaging at least once post-operatively to confirm the size of descemetorhexis area • To clarify that specular microscopy images will be collected at designated timepoint, when possible • To allow alternate methods of measuring CCT within a subject • To provide additional guidance regarding ocular assessments for subjects with delayed recovery • Update Per Protocol Population definition
Amendment 7.0	The protocol was revised to: • Remove reference to the Optional Extended Dosing Period (OEDP) that was not implemented • Include an End of Study (EOS) Visit for all subjects that includes Assessments scheduled for Day 336 and a plasma sample for ADA • Correct the typo in Section 8.3 clarifying that all deaths occurring with 1 year of study treatment (not 28 days) are to be reported as AEs of Special Interest (AESI)

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

SYNOPSIS

Study Number:	TTHX-002
Study Name:	Phase 2 Study to Determine the Safety and Efficacy of the Investigational New Drug TTHX1114(NM141) on the Regeneration and Migration of Corneal Endothelial Cells in Patients undergoing Descemetorhexis without Endothelial Keratoplasty (Descemet Stripping Only) (DWEK/DSO)
Sponsor:	Trefoil Therapeutics, Inc. 6330 Nancy Ridge Drive, Suite 103 San Diego, CA 92121
Phase	Phase 2
Objectives	The primary objective of this clinical trial is to evaluate the safety and efficacy of TTHX1114 in the setting of DWEK/DSO. The efficacy endpoints will include CEC counts/ characteristics, corneal edema, and BCVA following the surgical procedure.

Study Design	Open label, single treatment, with a concurrent non-treatment control in patients with FECD undergoing DWEK/DSO procedure.
	• Group 1 (No TTHX1114)
	  ○ DWEK/DSO: Day 0
	• Group 2: TTHX1114 in combination with DWEK/DSO
	  ○ DWEK/DSO: Day 0
	  ○ TTHX1114: Day (-3), 0, 7, 14, and 21
	• Group 3: TTHX1114 in combination with DWEK/DSO
	  ○ DWEK/DSO: Day 0
	  ○ TTHX1114: Day 0
	• Group 4
	  ○ DWEK/DSO: Day 0
	  ○ TTHX1114: Days 0, 7, 14, 21
	• Group 1a
	  ○ TTHX1114 weekly x 5
	• Group 3a
	  ○ TTHX1114 weekly x up to 4
	TTHX-002 Study Schema
	 

	 All subjects will be followed for long-term safety and durability of response for up to at least 1 year following DWEK/DSO or until subsequent treatment/ surgery of the Study Eye.
Treatment	TTHX1114(NM141) (referred to as TTHX1114) is an engineered FGF-1 (eFGF-1) that has activity at much lower applied concentrations but qualitatively has biological activity identical to that of wtFGF-1. As such, TTHX1114 is expected to have the same mechanism-driven effects as wtFGF-1 with no unexpected activity.
Eligibility	**Inclusion Criteria** 1. Male or female, 18 years of age or older 2. [redacted] To be eligible for the Optional Extended Dosing Period (OEDP); study subjects must have visible central endothelium by specular microscopy on Day 168 as assessed by the Investigator, unless discussed with and approved by the Sponsor. **Exclusion Criteria** 1. Planned post-operative rho-kinase inhibitor use 2. [redacted]

	c. [redacted] 9. 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
Statistical Methods	The Per Protocol Population will be used to assess the primary and secondary efficacy endpoints compared to the control group. The Safety Population set includes all participants who received any TTHX1114 and will be the population for safety outcomes; this population will be compared with the control group. Ocular AEs involving the study eye will be tabulated. Ocular AEs for the fellow eye and non-ocular AEs will be provided as a listing.

Table 1: Schedule of Events Group 1

Study TTHX-002: Group 1									
	Screening (≈28 days	**0***	**1**	**14** (±1)	**28** (±3)	**56** (±3)	**84** (±21)	**168** (±21)	**336** (±21)
Informed Consent	X								
Demographics	X								
Medical and Ocular History	X								
Comprehensive Ocular Examination (including slit lamp examination and dilated exam of the lens, fundus, and vitreous)	SE				SE	SE			
Slit Lamp Biomicroscopy	SE[a]	SE	SE	SE	SE[a]	SE[a]			
Specular Microscopy	SE			SE	SE	SE			SE
Assessment of Corneal Thickness[b]	SE	SE		SE	SE	SE	SE	SE	SE
Best-corrected Visual Acuity	SE	SE	SE	SE	SE	SE	SE	SE	SE
Intraocular Pressure (IOP) Measurement	SE	SE	SE	SE	SE	SE			
Optional Blood for Genetic Testing		X							
V-FUCHS	X				X	X			
DWEK/DSO		X							
Collect Endothelium Tissue sample		X							
Collect Aqueous Humor sample		X							
Concomitant Medications Review	X	X	X	X	X	X			
Assess Adverse Events		X	X	X	X	X			

OU=both eyes, SE=Study Eye, V-FUCHS=Visual Function and Corneal Health Status
Note: Unless otherwise specified assessments to be performed prior to study treatment injection, if applicable
*Day 0 pre-treatment ocular assessments may be obtained within the 7 days prior to Day 0 (if screening visit assessments were within 7 days, they do not need to be repeated)
[a] as part of Comprehensive Eye Examination
[b] measured by AS-OCT (preferred) if AS-OCT not available, Pentacam or Ultrasound Pachymetry is to be used (Note: subjects must be assessed using the same modality throughout the study)

Table 2: Schedule of Events Group 2

Study TTHX-002: Group 2												
	Screening (≈28 days)	Study Day										
		-3	0	1	7 (±1)	14 (±1)	21 (±1)	28 (±3)	56 (±3)	84 (±21)	168 (±21)	336* (±21)
Informed Consent	X											
Demographics	X											
Medical and Ocular History	X											
Comprehensive Ocular Examination	SE							SE	SE			
Slit Lamp	SE[a]	SE	SE	SE	SE	SE	SE	SE[a]	SE[a]			
Specular Microscopy	SE					SE	SE	SE	SE	SE		SE
Assessment of Corneal Thickness[b]		SE				SE	SE	SE	SE	SE	SE	SE
Best-corrected Visual Acuity	SE	SE		SE	SE	SE	SE	SE	SE	SE	SE	SE
Intraocular Pressure (IOP) measurement	SE							SE	SE			
Pre-injection/procedure		SE	SE		SE	SE	SE					
Post-injection [c]		SE	SE		SE	SE	SE					
Urine pregnancy test (WOCBP)	X							X				
Clinical Laboratory Collection		X						X				
Plasma for ADA								X				X
Pre-injection		X										
Plasma for Pharmacokinetics (PK)								X				
Pre-injection		X	X									
1.5 hours after injection			X									
Optional Blood for Genetic Testing		X										
V-FUCHS	X							X	X			
DWEK/DSO			X									
Collect endothelium tissue sample			X									
Collect Aqueous Humor sample			X									
Study Treatment IC Injection (SE)		X	X		X	X	X					
Concomitant Medications Review	X	X	X	X	X	X	X	X	X			
Assess Adverse Events		X	X	X	X	X	X	X	X			

OU=both eyes, SE=Study Eye, V-FUCHS=Visual Function and Corneal Health Status
Note: Unless otherwise specified assessments to be performed prior to study treatment injection, if applicable
*Day -3 pre-treatment ocular assessments may be obtained within the 7 days prior to Day 0 (if screening visit assessments were within 7 days, they do not need to be repeated) [a] as part of Comprehensive Eye Examination
[b] measured by AS-OCT (preferred) if AS-OCT not available, Pentacam, or Ultrasound Pachymetry is to be used (Note: subjects must be assessed using the same modality throughout the study)
[c] IOP pre-injection and then post-injection every 30 to 60 minutes until less than at least 5 mmHg higher than pre-injection IOP (Note: IOP may not be measurable post injection on Day 0)
* End of Study Visit (EOS) may be performed any time after Day 168; if the Day 336 visit, if the subject has already completed the study, the subject should be contacted and asked to provide a plasma sample for ADA; any subject who did not have gradeable specular images at their most recent visit should be re-imaged and gradeable images obtained

Table 3: Schedule of Events Group 3

Study TTHX-002: Group 3											
		Study Day									
	Screening (≈28 days)	0*	1	7 (±1)	14 (±1)	21 (±1)	28 (±3)	56 (±3)	84 (±21)	168 (±21)	336* (±21)
Informed Consent	X										
Demographics	X										
Medical and Ocular History	X										
Comprehensive Ocular Examination (including slit lamp examination and dilated exam of the lens, fundus, vitreous and MAC-OCT)	SE						SE	SE			SE
Slit Lamp	SE[a]	SE	SE	SE	SE	SE	SE[a]	SE[a]			SE
Specular Microscopy (incl peripheral images at Screening)	SE						SE	SE	SE		SE
Assessment of Corneal Thickness[b]		SE		SE	SE	SE	SE	SE	SE	SE	SE
Pentacam		SE					SE	SE			
Best-corrected Visual Acuity	SE	SE			SE	SE	SE	SE	SE	SE	SE
Intraocular Pressure (IOP) measurement Pre-injection/procedure Post-injection [c]	SE	 SE SE	SE	SE				SE			
Urine pregnancy test (WOCBP)	X						X				
~~Clinical Laboratory Collection (Chemistry, Hematology, UA, & HbA1c)~~		~~X~~					~~X~~				
Plasma for ADA Pre-injection		X					X				X
Plasma for Pharmacokinetics (PK) Pre-injection 1.5 hours after injection		 X X					X				
Optional Blood for Genetic Testing		X									
V-FUCHS	X						X	X			
DWEK/DSO		X									
Collect endothelium tissue sample		X									
Collect Aqueous Humor sample		X									
Study Treatment IC Injection (SE)		100 ng									
Concomitant Medications Review	X	X	X	X	X	X	X	X			
Assess Adverse Events		X	X	X	X	X	X	X			

OU=both eyes, SE=Study Eye, V-FUCHS=Visual Function and Corneal Health Status
Note: Unless otherwise specified assessments to be performed prior to study treatment injection, if applicable
*Day 0 pre-treatment ocular assessments may be obtained within the 7 days prior to Day 0 (if screening visit assessments were within 7 days, they do not need to be repeated)
[a] as part of Comprehensive Eye Examination
[b] measured by AS-OCT (preferred) if AS-OCT not available, Pentacam, or Ultrasound Pachymetry is to be used (Note: subjects must be assessed using the same modality throughout the study)
[c] IOP pre-injection and then post-injection every 30 to 60 minutes until less than at least 5 mmHg higher than pre-injection IOP (Note: IOP may not be measurable post injection on Day 0)
* End of Study Visit (EOS) may be performed any time after Day 168; if the Day 336 visit, if the subject has already completed the study, the subject should be contacted and asked to provide a plasma sample for ADA; any subject who did not have gradeable specular images at their most recent visit should be re-imaged and gradeable images obtained

Table 4: Schedule of Events Group 4

Study TTHX-002: Group 4											
		Study Day									
	Screening (≈28 days)	0*	1	7 (±1)	14 (±1)	21 (±1)	28 (±3)	56 (±3)	84 (±21)	168 (±21)	336* (±21)
Informed Consent	X										
Demographics	X										
Medical and Ocular History	X										
Comprehensive Ocular Examination (including slit lamp examination and dilated exam of the lens, fundus, vitreous, and MAC-OCT	SE						SE	SE			SE
Slit Lamp	SE[a]	SE	SE	SE	SE	SE	SE[a]	SE[a]			SE
Specular Microscopy (incl peripheral images at Screening)	SE						SE	SE	SE		SE
Assessment of Corneal Thickness[b]		SE		SE	SE	SE	SE	SE	SE	SE	SE
Pentacam		SE					SE	SE			
Best-corrected Visual Acuity	SE	SE			SE	SE	SE	SE	SE	SE	SE
Intraocular Pressure (IOP) measurement	SE		SE				SE	SE			
Pre-injection/procedure		SE		SE	SE	SE					
Post-injection [c]		SE		SE	SE	SE					
Urine pregnancy test (WOCBP)	X						X				
~~Clinical Laboratory Collection (Chemistry, Hematology, UA, & HbA1c)~~							~~X~~				
Plasma for ADA		X					X				X
Pre-injection											
Plasma for Pharmacokinetics (PK)							X				
Pre-injection		X									
1.5 hours after injection		X									
Optional Blood for Genetic Testing		X									
V-FUCHS	X						X	X			
DWEK/DSO		X									
Collect endothelium tissue sample		X									
Collect Aqueous Humor sample		X									
Study Treatment IC Injection (SE)		100 ng		50 ng	50 ng	50 ng					
Concomitant Medications Review	X	X	X	X	X	X	X	X			
Assess Adverse Events		X	X	X	X	X	X	X			

OU=both eyes, SE=Study Eye, V-FUCHS=Visual Function and Corneal Health Status
Note: Unless otherwise specified assessments to be performed prior to study treatment injection, if applicable
*Day 0 pre-treatment ocular assessments may be obtained within the 7 days prior to Day 0 (if screening visit assessments were within 7 days, they do not need to be repeated)
[a] as part of Comprehensive Eye Examination
[b] measured by AS-OCT (preferred) if AS-OCT not available, Pentacam or Ultrasound Pachymetry is to be used (Note: subjects must be assessed using the same modality throughout the study)
[c] IOP pre-injection and then post-injection every 30 to 60 minutes until less than at least 5 mmHg higher than pre-injection IOP (Note: IOP may not be measurable post injection on Day 0)
*End of Study Visit (EOS) may be performed any time after Day 168; if the Day 336 visit, if the subject has already completed the study, the subject should be contacted and asked to provide a plasma sample for ADA; any subject who did not have gradeable specular images at their most recent visit should be re-imaged and gradeable images obtained

Table 5: Schedule of Events Group 1a

Study TTHX-002: Group 1a										
	Study Day									
	0*	**7** (±1)	**14** (±1)	**21** (±1)	**28** (±1)	**35** (±3)	**56** (±3)	**84** (±21)	**168** (±21)	**336** (±21)
Informed Consent										
Comprehensive Ocular Examination (including slit lamp examination and dilated exam of the lens, fundus, and vitreous)	SE				SE	SE	SE			
Slit Lamp	SE	SE	SE	SE	SE[a]	SE[a]	SE[a]			
Specular Microscopy	SE		SE	SE	SE	SE	SE			SE
Assessment of Corneal Thickness[b]	SE		SE	SE	SE	SE	SE	SE	SE	SE
Best-corrected Visual Acuity	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE
Intraocular Pressure (IOP) measurement					SE	SE	SE			
Pre-injection/procedure	SE	SE	SE	SE	SE					
Post-injection [c]	SE	SE	SE	SE	SE					
Urine pregnancy test (WOCBP)	X				X					
~~Clinical Laboratory Collection (Chemistry, Hematology, UA, & HbA1c)~~					~~X~~					
Plasma for ADA Pre-injection	X				X					
Plasma for Pharmacokinetics (PK)					X					
V-FUCHS						X	X			
Study Treatment IC Injection (SE)	X	X	X	X	X					
Concomitant Medications Review	X	X	X	X	X	X	X			
Assess Adverse Events	X	X	X	X	X	X	X			

OU=both eyes, SE=Study Eye, V-FUCHS=Visual Function and Corneal Health Status
Note: Unless otherwise specified assessments to be performed prior to study treatment injection, if applicable
*Day 0 pretreatment assessments may be obtained within the 7 days prior to Day 0 (if screening assessment within 7 days, do not need to be repeated)
[a] as part of Comprehensive Eye Examination
[b] measured by AS-OCT (preferred) if AS-OCT not available, Pentacam, or Ultrasound Pachymetry is to be used (Note: subjects must be assessed using the same modality throughout the study)
[c] IOP pre-injection and then post-injection every 30 to 60 minutes until less than at least 5 mmHg higher than pre-injection IOP

Table 6: Scheduled of Events Group 3a

Study TTHX-002: Group 3a									
	Study Day								
	0*	7 (±1)	14 (±1)	21 (±1)	28 (±1)	56 (±3)	84 (±21)	168 (±21)	336* (±21)
Informed Consent	X								
Comprehensive Ocular Examination (including slit lamp examination and dilated exam of the lens, fundus, and vitreous)	SE				SE	SE			SE
Slit Lamp	SE	SE	SE	SE	SE[a]	SE[a]			SE
Specular Microscopy	SE				SE	SE	SE		SE
Assessment of Corneal Thickness[b]	SE		SE	SE	SE	SE	SE	SE	SE
Best-corrected Visual Acuity	SE	SE	SE	SE	SE	SE	SE	SE	SE
Intraocular Pressure (IOP) measurement					SE	SE			
Pre-injection/procedure	SE	SE	SE	SE					
Post-injection [c]	SE	SE	SE	SE					
Urine pregnancy test (WOCBP)					X				
~~Clinical Laboratory Collection (Chemistry, Hematology, UA, & HbA1c)~~									
Plasma for ADA	X								X
Pre-injection									
Plasma for Pharmacokinetics (PK)									
V-FUCHS					X	X			
Study Treatment IC Injection (SE)	X	X	X	X					
Concomitant Medications Review	X	X	X	X	X	X			
Assess Adverse Events	X	X	X	X	X	X			

OU=both eyes, SE=Study Eye, V-FUCHS=Visual Function and Corneal Health Status
Note: Unless otherwise specified assessments to be performed prior to study treatment injection, if applicable
*Day 0 pretreatment assessments may be obtained within the 7 days prior to Day 0 (if screening assessment within 7 days, do not need to be repeated)
[a] as part of Comprehensive Eye Examination
[b] measured by AS-OCT (preferred) if AS-OCT not available, Pentacam, or Ultrasound Pachymetry is to be used (Note: subjects must be assessed using the same modality throughout the study)
[c] IOP pre-injection and then post-injection every 30 to 60 minutes until less than at least 5 mmHg higher than pre-injection IOP
*End of Study Visit (EOS) may be performed any time after Day 168; if the Day 336 visit, if the subject has already completed the study, the subject should be contacted and asked to provide a plasma sample for ADA; any subject who did not have gradeable specular images at their most recent visit should be re-imaged and gradeable images obtained

LIST OF ABBREVIATIONS

ADA	Anti-Drug Antibody
AE	Adverse Event
AS-OCT	Anterior Segment Optical Coherence Tomography
BCVA	Best Corrected Visual Acuity
CCT	Central Corneal Thickness
CEC	Corneal Endothelial Cell
CED	Corneal Endothelial Dystrophies
CIARC	Cornea Image Analysis Reading Center
Con Med	Concomitant Medication
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
DWEK/ DSO	Descemet Stripping Without Endothelial Keratoplasty/Descemet Stripping Only
ECD	Endothelial Cell Density
EDC	Electronic Data Capture
eFGF-1	engineered FGF-1
ETDRS	Early treatment diabetic retinopathy study
FDA	Food and Drug Administration
FE	Fellow Eye
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
NOAEL	No Observable Adverse Event Level
OEDP	Optional Extended Dosing Period
PAD	Pharmacologically Active Dose
PK	Pharmacokinetic
PP	Per Protocol
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SE	Study Eye
TM	Trabecular Meshwork
V-FUCHS	Visual Function and Corneal Health Status
WOCBP	Women of Childbearing Potential
wtFGF-1	Wild type FGF-1

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1. INTRODUCTION

[redacted]

1.2 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.

1.3 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].

[redacted]

1.3.1 Descemetorhexis without Endothelial Keratoplasty (DWEK)

[redacted]

2. BACKGROUND INFORMATION

2.1 Investigational Product TTHX1114(NM141)

[redacted]

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

[redacted]

2.2.2 Clinical Trials

This is the second clinical trial of TTHX1114. The first clinical trial (Study TTHX-001) is a randomized, placebo-controlled, masked study. As clinical safety data becomes available, the Investigator Brochure (IB) will be updated and provided to all Investigators involved in clinical trials of TTHX1114. Please refer to the current version of the IB for the most up to date safety information related to clinical trials of TTHX1114.

Please refer to the current version of the Investigator Brochure for the most current data related to TTHX1114 clinical trials.

2.3 Potential Risks and Benefits

[redacted]

- [redacted]

2.4 Rationale for Dose Selection

[redacted]

[redacted]

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 with a confirmed diagnosis of FECD who will undergo DWEK/DSO.

3. TRIAL OBJECTIVES AND PURPOSE

The primary objective of this clinical trial is to evaluate the safety and efficacy of TTHX1114 in the setting of DWEK/DSO. The primary efficacy endpoint will include CEC counts/ characteristics and BCVA following the surgical procedure.

4. TRIAL DESIGN

This is an open-label, multi-center study in which up to approximately 50 subjects with FECD who are scheduled for DWEK/ DSO will be enrolled. Study subjects will be assigned to Study Treatment Group based on the Group(s) open at the time of treatment of the primary (first) eye; if a subject elects to have an eligible second (contralateral) eye treated, that "fellow" eye will be treated based on the Group(s) open at the time. The number of subjects per group in the study and per study treatment is summarized below in Table 7.

Note: the study was amended to allow treatment of the contralateral eye. To avoid ambiguity the eye that was treated first will be referred to as the Study Eye (SE); the contralateral eye will be referred to as the Fellow Eye (FE).

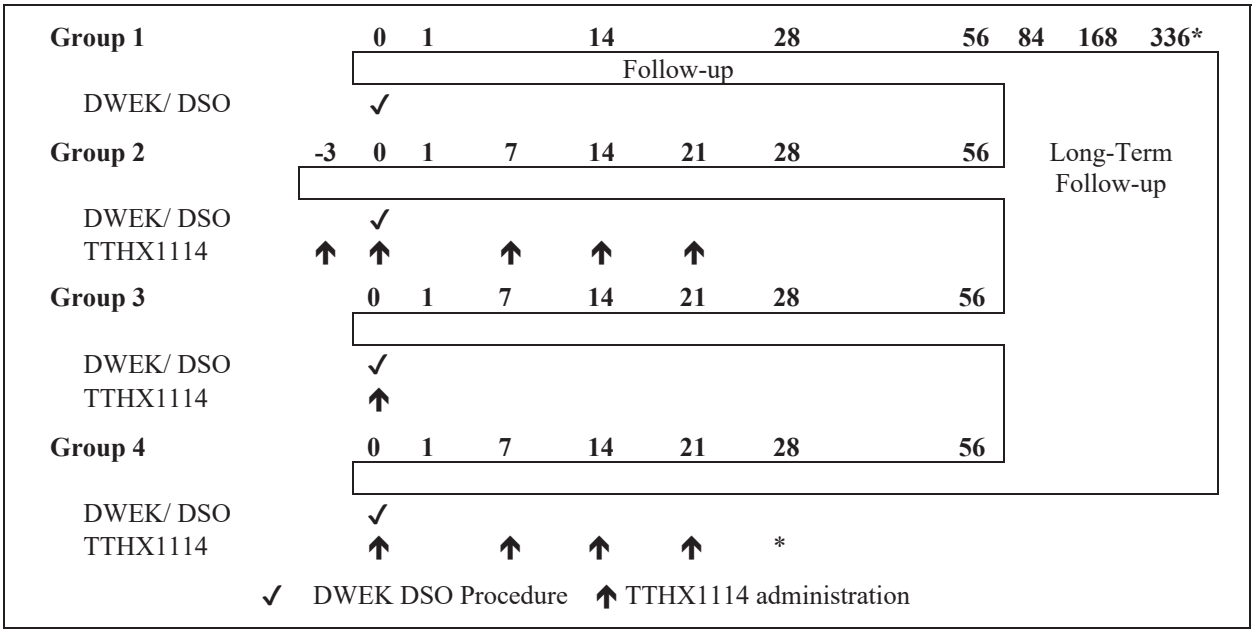
Table 7: Study Groups and Number of Subjects

	TTHX1114 Regimen		
	Day	**Dose**	**Number of Subjects**
Group 1	N/A	N/A	**8** (Closed after 1 enrolled)
Group 2	Day -3 Day 0 Day 7 Day 14 Day 21	10ng 50ng 10ng 10ng 10ng	**8-16** (Closed after 9 enrolled)
Group 3	Day 0	100ng	**20**
Group 4	Day 0 Day 7 Day 14 Day 21	100ng 30 or 50ng 30 or 50ng 30 or 50ng	**20**
Group 1a	Weekly x 5	30 or 50ng	**NA**
Group 3a	Weekly x4	30 or 50ng	**NA**
			up to 50

Subject will be consented and screened for eligibility during the screening period prior to the scheduled DWEK/DSO procedure.

The study schema is presented in Figure 1 and Figure 2.

Figure 1: Study Treatment Schema



*Subjects who have not sufficiently recovered by Day 28, may receive an additional dose of TTHX1114 following discussion with the Medical Monitor
* End of Study Visit (EOS) may be performed any time after Day 168; if the Day 336 visit has already been completed subjects should be contacted and asked to provide a plasma sample for ADA; any subject who did not have gradeable specular images at their most recent visit should be re-imaged and gradeable images obtained

Eligible subjects who do not wish to receive investigational product or who do not have a qualifying Fellow Eye will be assigned to Treatment Group 1.

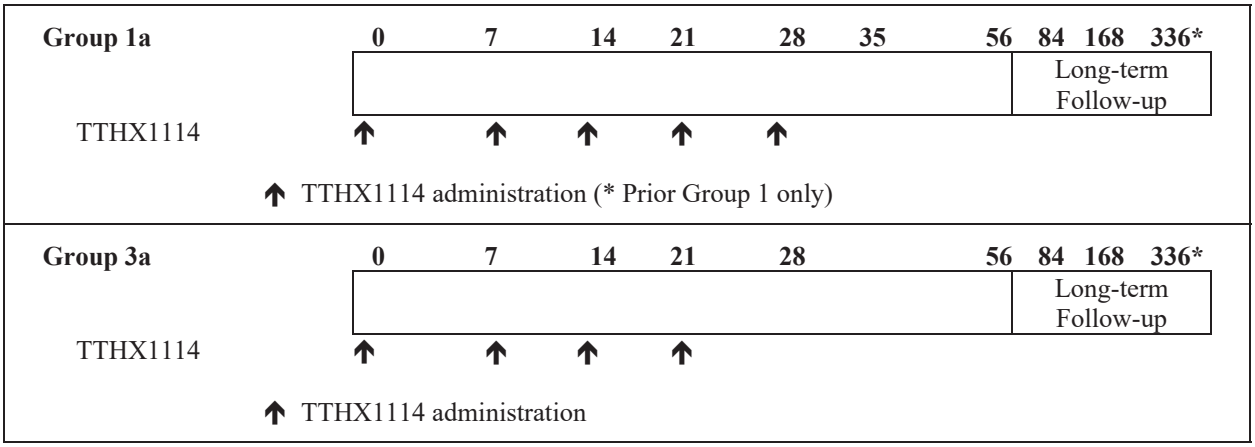
Following preliminary safety and tolerability assessment of the 50ng IC injection in Group 2, Trefoil may elect to open Group 3 and Group 4

- Group 3 in which eligible subjects will be given a "1 and done" dosing regimen in which TTHX1114 will be administered as a 100ng/100mcL volume injection at the time of DWEK/DSO

- Group 4 in which eligible subjects will be given a 100ng/100mcL volume injection at the time of DWEK/DSO and then weekly injections of 30 or 50ng (30 or 50mcL) x3. Subjects in Group 4, who have not sufficiently recovered by Day 28, may receive an additional dose of TTHX1114 following discussion with the Medical Monitor

Following preliminary safety and efficacy evaluation in subjects who received TTHX1114, Trefoil may open enrollment in Groups 1a and 3a in which TTHX1114-naive subjects in Group 1 and subjects in Group 3 may be allowed to "roll-over" to a TTHX1114 regimen of up to 5 weekly injections of TTHX1114. To be eligible for Group 1a or Group 3a, subjects must continue to be eligible for the study (e.g., have a suitable fellow eye and not have had any severe suspected adverse reactions.

Figure 2: Group 1a and Group 3a Study Treatment Schema "Roll-over"



* End of Study Visit (EOS) may be performed any time after Day 168; if the Day 336 visit has already been completed subjects should be contacted and asked to provide a plasma sample for ADA; any subject who did not have gradeable specular images at their most recent visit should be re-imaged and gradeable images obtained

Study Treatment Group 1:

Study subjects in Group 1 will undergo Descemetorhexis without Endothelial Keratoplasty (DWEK/ DSO) on Study Day 0 and will not receive any TTHX1114. Subjects in this group will function as a control arm and following DWEK/ DSO will be seen in follow-up on:

- Day 1
- Day 14
- Day 28
- Day 56

Assessments conducted at follow-up visits will include routine ocular assessments.

Study Treatment Group 2:

Approximately 48 to 72 hours prior to DWEK/ DSO (Day -3), study subjects will receive a "conditioning dose" (10ng [10mcL]) of TTHX1114. The conditioning dose is intended to provide anti-apoptotic protection for the existing cells. On Day 0, subjects will undergo DWEK/ DSO and will receive TTHX1114 (50ng [50mcL]) at the time of the surgical procedure. As described above, the immediate post-surgical anterior chamber should easily accommodate the additional volume.

Study subjects will then return for follow-up on Day 1 (the day after DWEK/DSO) and subsequent study treatment administration on:

- Day 7
- Day 14
- Day 21

Follow-up visits will occur on:

- Day 28
- Day 56

Study Treatment Group 3:

On Day 0, subjects will undergo DWEK/ DSO and will receive TTHX1114 (100ng [100mcL]) at the time of the surgical procedure. As described above, the immediate post-surgical anterior chamber should easily accommodate the additional volume.

Study subjects will then return for follow-up on Day 1 (the day after DSO) and subsequent follow-up visits will occur on:

- Day 7
- Day 14
- Day 21
- Day 28
- Day 56

Study Treatment Group 4:

On Day 0, subjects will undergo DWEK/ DSO and will receive TTHX1114 (100ng [100mcL]) at the time of the surgical procedure and then weekly injections x 3. Subjects who have not sufficiently recovered by Day 28, may receive an additional dose of TTHX1114 following discussion with the Medical Monitor.

Study subjects will then return for follow-up on Day 1 (the day after DWEK/DSO) and subsequent study treatment administration on:

- Day 7
- Day 14
- Day 21

Follow-up visits will occur on:

- Day 28*
- Day 56

* Subjects who have not sufficiently recovered by Day 28, may receive an additional dose of TTHX1114 following discussion with the Medical Monitor. In this context, Medical Monitor refers to Sponsor (or designee). If the Sponsor (or designee) agrees that the study subject can receive the 5th TTHX1114 administration, all assessments scheduled for Day 21 should be repeated on Day 28 and an unscheduled visit conducted on or around Day 35 when all assessments scheduled for Day 28 should be conducted.

Study Treatment Group 1a:

TTHX1114-naive subjects in Group 1 may be allowed to “roll-over” to a TTHX1114 regimen of up to 5 weekly injections of TTHX1114. To be eligible for Group 1a, subjects must

- continue to be eligible for the study (e.g., have a suitable fellow eye and not have had any severe suspected adverse reactions)
- not have achieved adequate restoration of the endothelium (anatomic recovery) by the anticipated timepoint following the DWEK/DSO procedure (e.g., Day 56 or 84)
    - Note: the trigger for roll over to Group 1a may be earlier if the time to anatomic recovery observed in Group 2 or 3 is sooner

Subjects who had previously participated in Group 1 will receive up to 5 weekly study treatment administrations.

- Day 0
- Day 7
- Day 14
- Day 21
- Day 28

Follow-up visits will occur on:

- Day 35
- Day 56

Study Treatment Group 3a:

Subjects in Group 3 may be allowed to "roll-over" to a TTHX1114 regimen of up to 4 weekly injections of TTHX1114. To be eligible for Group 3a, subjects must

- continue to be eligible for the study (e.g., have a suitable fellow eye and not have had any severe suspected adverse reactions)
- not have achieved adequate restoration of the endothelium (anatomic recovery) by the anticipated timepoint following the DWEK/DSO procedure (e.g., Day 56 or 84)
    - Note: the trigger for roll over to Group 3a may be earlier if the time to anatomic recovery observed in Group 2 or 3 is sooner

Subjects who had previous participated in Group 3 will receive up to 4 weekly study treatment administrations.

- Day 0
- Day 7
- Day 14
- Day 21

Follow-up visits will occur on:

- Day 28
- Day 56

Long-term Follow-up (All Treatment Groups)

A subjects will undergo long-term follow-up for up to 1 year following the later of DWEK/DSO or the first TTHX1114 administration.

- Long-term Follow-up visits will occur on: Day 84
- Day 168
- Day 336/ End of Study Visit

For subjects who have had both eyes treated on study, the Day 168 and Day 336 visits for both eyes may be combined calculated based on the date of surgery of the second eye. Day 336 is considered the End of Study Visit and includes a Comprehensive Eye Examination.

End of Study Visit

An End of Study Visit (EOS) will be performed on all subjects. The EOS may be conducted any time after Day 168, during the EOS assessments scheduled for Day 336 will be performed and a plasma sample for ADA analysis will be collected. If the subject has already completed the study, the subjects should be contacted and asked to provide a plasma sample for ADA. Any subject who did not have gradeable specular images at their most recent visit should be re-imaged and gradable images obtained. It is important that gradeable specular images are collected for all subjects at the subjects' last visit.

4.1 Efficacy Endpoints

Efficacy endpoints in this study include:

- The number of subjects in each group with 20/40 or better BCVA at Day 28
- Central corneal thickness at Day 28

The following additional endpoints will also be assessed:

- [redacted]

4.2 Measures Taken to Minimize/ Avoid Bias

All adverse events will be recorded regardless of suspected causality. Additionally, subjects will be assigned to the open treatment group at the time of determination of eligibility dependent upon the subject's desire to receive investigational product. All subjects in all treatment groups are required to meet all eligibility criteria. All primary and secondary efficacy endpoints will be source data verified or provided by an independent 3rd party (e.g., central reader).

4.3 Study Treatment

[redacted]

4.3.1 TTHX1114 Drug Product

[redacted]

4.3.2 TTHX1114 Storage

[redacted]

4.3.3 TTHX1114 Preparation

[redacted]

4.4 Duration of the Study and Study Treatment

Each subject is expected to be on study for approximately 1 year following DWEK/DSO.

	Main Study		Optional Extended Dosing Period	
	# of injections	Duration of Treatment	# of injections	Duration of Treatment
Group 1	-	-	-	-
Group 1a	5	over 28 days	-	-
Group 2	5	over 24 days		
Group 3	1	one time	4	over 21 days
Group 3a	4	over 21 days	-	-
Group 4	4 to 5*	over 21 to 28 days	1*	one time

*Subjects who received 5 injections in Group 4 are NOT eligible for OEDP

4.5 Stopping Rules

Stopping rules for the study will be the incidence of unacceptable toxicity. This is an open-label study, and the frequency and severity of all adverse events will be monitored on an ongoing basis.

4.6 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.

4.7 Data to be entered into the Database

The majority of data collected will be entered into the database via the EDC system. Data from 3rd parties (e.g., central labs or reading facilities) will be imported into the clinical database, these data include:

- Endothelial cell counts and characteristics assessed by the central reading center
- Pharmacokinetic measurements
- Anti-drug antibody assay results
- Clinical laboratory testing results

Imported data will be reconciled (e.g., 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 with the data at the source.

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

Potential subjects will be identified on the basis of having a confirmed diagnosis of FECD and being a suitable candidate for DWEK/DSO. Most potential subjects will come directly from the patients receiving care at the investigational site. All subjects must be suitable for DWEK/DSO procedure independent of the clinical trial.

Subjects who participated in Study TTHX-001 and who subsequently were scheduled for DWEK/DSO may be enrolled in Study TTHX-002 if they meet all eligibility criteria.

Subjects who participated in Study TTHX-001 and did not receive active study treatment (i.e., were assigned to the placebo treatment or only participated in the Observational Sub-study) may be included in any treatment group in Study TTHX-002.

Subjects who participated in Study TTHX-001 and received any dose level active study treatment (i.e., received 4 IC injections of TTHX1114) may be included in Group 3 of Study TTHX-002. Note: No subject can receive more than 5 cumulative administrations of TTHX1114. Subjects who received TTHX1114 in Study TTHX-001 can receive 1 additional dose in Group 3 but WILL NOT be eligible to roll-over to Group 3a.

5.1 Inclusion Criteria

1. Male or female, 18 years of age or older
2. [redacted]

5.2 Exclusion Criteria

1. Planned post-operative rho-kinase inhibitor use
   [redacted])

[redacted]

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

[redacted]

[redacted]

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 and https://www.aao.org/coronavirus) 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 approximately 28 days of Study Day 0 (unless approved by the Medical Monitor); 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 (including slit lamp biomicroscopy and dilated examination of lens, fundus, and vitreous)
- Specular microscopy (including images of the central and peripheral cornea)

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	-	2	0	1

For example, Subject ID 101-201 = The first participant consented at Site 101; 103-203 = The third participant consented at Site 103

Once a Subject ID number has been issued, it cannot be reused. Potential subjects may have repeat eligibility assessments (if appropriate) or be rescreened, following discussion with the Medical Monitor (or designee).

Subjects who had participate in Study TTHX-001 will receive a new Subject ID number for identification in Study TTHX-002. Subjects who "roll-over" to Group 1a or Group 3a will retain the same Subject ID number.

6.1.1 Descemetorhexis without Endothelial Keratoplasty (DWEK)/ Descemet Stripping Only (DSO)

Patients with FECD who have a planned DWEK/DSO or DMEK procedure will be evaluated for eligibility in this study. As the DWEK/DSO procedure is not a study procedure DWEK/DSO will be done according to institutional policy with a descemetorhexis area of 4 to 5mm. The method of surgery many include the use of the Sinskey Hook (with or without forceps) or an irrigation/aspiration (I&A) tip. The method of surgery will be at the discretion of the Investigator and Investigators will be selected based on their demonstrated expertise (e.g., historical number of procedures and relative success rate). The Lead Investigator may assist in the selection of investigational sites and the vetting of Investigators. Only qualified and vetted ophthalmologists will perform the surgical procedure on subjects enrolled in this study to minimize operator variability. Subjects with a descemetorhexis area of > 5mmwillbe excluded from efficacy analyses but will be included in the safety analyses.

AS-OCT should be performed at least 1 time following DSO (e.g., Day 28) to confirm the size of the descemetorhexis area, especially in subjects with delayed recovery.

6.1.2 The Study Eye (SE)

The Study Eye (SE) will be determined by the Investigator prior to the clinical trial as eligibility for the study required a planned DWEK/DSO procedure planned for the medical management of the patient. The SE is defined as the eye that was selected by the treating physician for surgical intervention of DWEK/DSO and the eye to which TTHX1114 is administered (if applicable).

If a study subject wishes to undergo DWEK/DSO in the Fellow Eye after the SE has recovered, the subject may be re-enrolled in Group 3 or Group 4 and the contralateral eye may be treated. "Recovery" is based on the Investigator discretion following the primary efficacy endpoint evaluation on Day 28 (e.g., BCVA of at least 20/40 or have a measured CCT of less than a 10% increase from baseline). Planned treatment of all contralateral eyes must be discussed with and approved by the Sponsor (or designee) prior to treatment.

6.1.3 Subject Registration

Prior to enrollment, study subjects in all treatment groups must be registered and approved for enrollment. At a minimum, the investigational site will complete the Subject Registration Form (Subject ID number, planned date of DWEK/DSO, etc.) and an eligibility checklist (including prior participation in Study TTHX-001 or Study TTHX-002, if applicable). Following registration, approval of enrollment, and treatment group assignment, investigational product will be requested and shipped to the investigational site.

6.2 Study Treatment Administration/ Intracameral Injection

This procedure can be performed by a qualified and trained ophthalmologist who is familiar with DWEK/DSO and who has been trained on TTHX1114 administration.

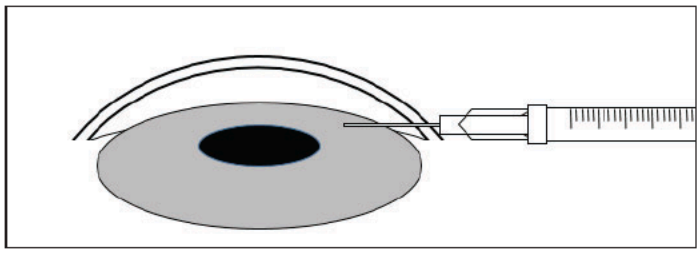
Materials:

[redacted]

Procedure Study Day 0:

[redacted]

Figure 4: Intracameral Injection Entry Point



[redacted]

6.2.1 Timing and Scheduling of TTHX1114 Administration

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted] moved earlier based on the observed time to response in the subjects in Group 2

6.2.2 Subsequent TTHX1114 Administrations

Following the Day 0 TTHX1114 administration, subsequent weekly administrations will occur approximately every 7 days ±1 day. If a study treatment administration cannot be administered within the 6 to 8 days following the prior administration, the study treatment may be delayed, and the scheduling of all remaining administrations adjusted to ensure that TTHX1114 is not administered fewer than 5 days after the prior administration.

6.2.3 [redacted]

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted])

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

[redacted]

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 unless approved by the Medical Monitor:

- [redacted]

Subjects requiring the use of prohibited medications must be discussed with the 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 8.

Table 8: 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

All study procedures/ visits should be performed at the designated timepoint (within the protocol-specified window, if applicable). If an assessment/ visit cannot be collected/ performed within the protocol-specified parameters, it should be collected as soon as practicable, and the actual date/ time/ reason recorded.

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* and may be obtained any time prior to the start of study-specific assessments.

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:

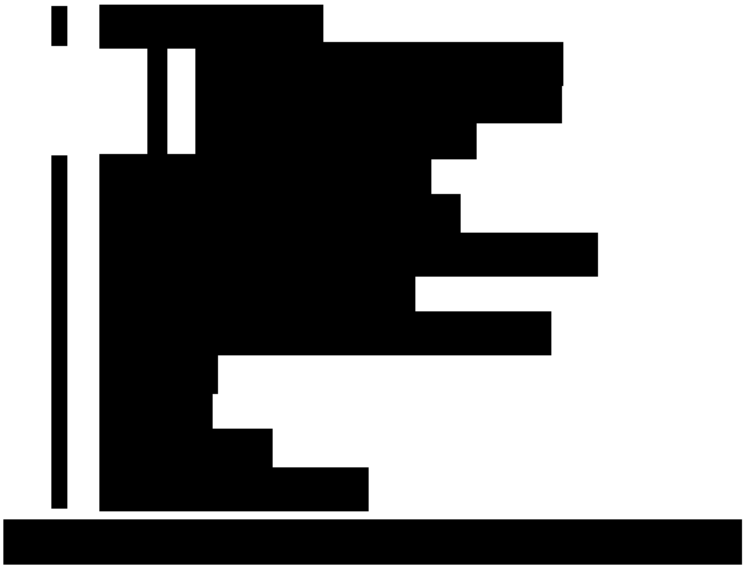
- Age
- Sex
- Race
- Ethnicity

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:



6.4.4 Comprehensive Ocular Examination

A comprehensive ocular examination will include slit-lamp biomicroscopy and a dilated examination of the lens, fundus (retina, optic disc, macula, fovea, and posterior pole), and vitreous.

The comprehensive ocular examination should also include baseline macular optical coherence tomography (Mac-OCT). As BCVA is the primary efficacy measurement in the study, it is important to identify any macular pathology or other confounding co-morbidities.

6.4.5 Slit Lamp Biomicroscopy

The slit lamp biomicroscopy examination will be performed 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:

These assessments will be graded according to the criteria described in Table 9.

Table 9: Grading of Slit Lamp Biomicroscopy Assessments

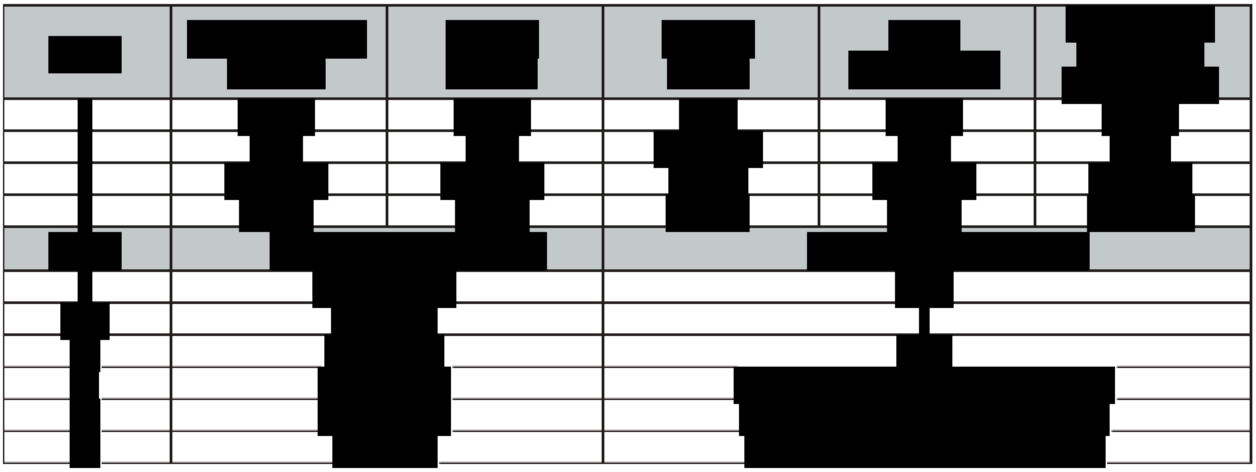
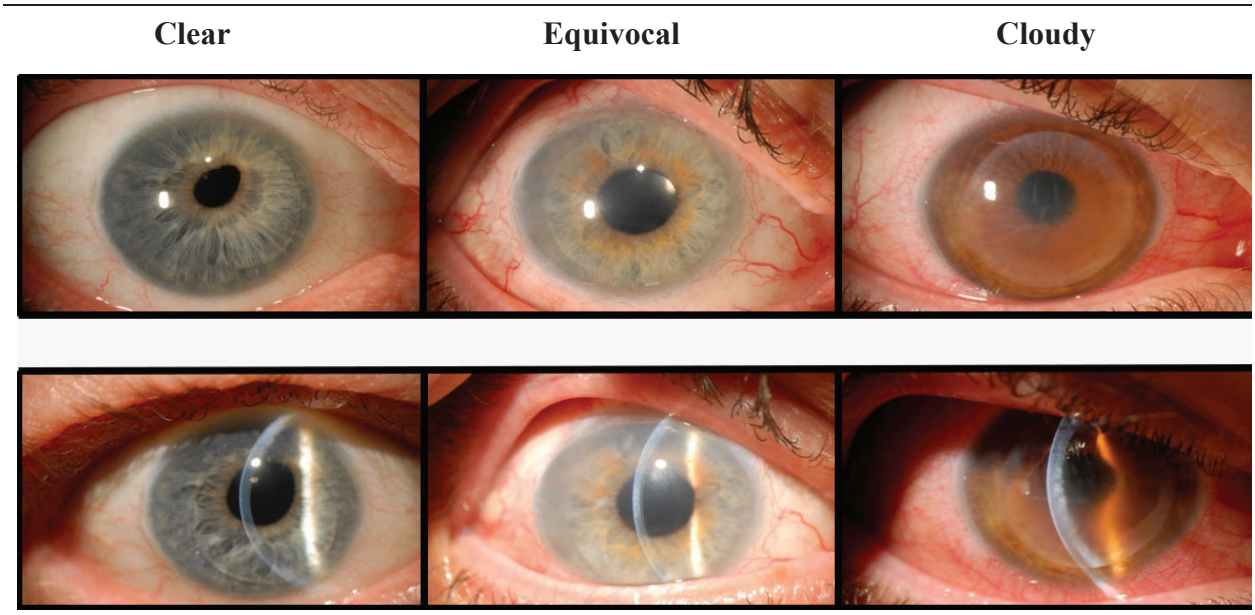


Figure 5: CPTS Stromal Clarity Grading Scale



Lass et al. *Cornea* 34:601-08, 2015
Images courtesy George Rosenwasser MD

6.4.6 Specular Microscopy

Specular microscopy images will be obtained of the periphery and center of the cornea. 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 should be performed prior to dilation. To address differences in location of the image within a given area of the cornea, five acceptable images should be taken at each required timepoint. Peripheral images will be collected at the screening visit; central images will be collected at prior to treatment and whenever possible following treatment. Due to the nature of corneal edema following DSO, specular microscopy images may not be able to be obtained at all post-operative timepoint prior the deturgescence of the cornea. Attempts should be made to collect images at all timepoints; if images are unable to be obtained, the reason for not being able to collect images will be recorded. If specular microscopy images are obtainable, it is expected that images will remain obtainable at all subsequent timepoints. Prior to the end of each specular microscopy session, the technician should review the images to ensure that the captured images are of the highest quality obtainable. Subjects with inadequate images may be required to return to the investigational site for repeat imaging as an unscheduled visit.

Endothelial cell density, percent hexagonality and the coefficient of variation will be assessed from specular microscope images.

Cornea Image Analysis Reading Center (CIARC) at University Hospitals Cleveland Medical Center will function as the centralized reading center and will be responsible for the certification of equipment and training of technicians as well as reading images and conducting image analysis. If more than one specular microscope is installed at a site, the same specular microscope will be used for all visits for a given patient.

Baseline images and on-study central images will be sent to CIARC; baseline images will be archived, and on-study images will be assessed for efficacy.

6.4.7 Central Corneal Thickness Assessment

Anterior segment optical coherence tomography (AS-OCT) is the preferred method of measuring corneal thickness. [redacted]

6.4.8 Best-corrected Visual Acuity (BCVA)

Best corrected visual acuity (BCVA), **should be measured between 07:00 AM and 11AM,** [redacted]

6.4.9 Intraocular Pressure Measurement

Intraocular pressure (IOP) will be measured by Goldmann Applanation Tonometry. At least two measurements will be obtained and recorded (a third measurement will be obtained if there is a ≥ 3 mmHg difference between the first two measurements). [redacted]

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 Delayed Recovery

Subjects with delayed recovery should undergo additional ocular assessments at Day 28, Day 56, or at unscheduled visits. These assessments may include:

- Additional; methods of BCVA (See Section 6.4.8)
- Central Corneal Thickness (by the primary or alternative method)
- AS-OCT
- Pentacam
- etc.

Additional ocular assessments should be reported as an Unscheduled Visit (See Section 6.5.5)

6.4.12 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 10.

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 10: 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)

Following the assessment of systemic exposure (pharmacokinetics) and review of clinical laboratory results in Study TTHX-001, Trefoil may discontinue collection of clinical laboratory samples.

6.4.13 Plasma for PK and ADA

Blood samples will be collected for pharmacokinetic (PK) and anti-drug antibody (ADA) analysis at selected sites. Samples will be centrifuged and/or prepared per instructions from the analytic laboratory. Pre-injection samples should be collected within the 24 hours prior to study treatment administration when possible. PK samples should be collected within ±5 minutes of the nominal timepoint or as soon as practicable.

Following the assessment of systemic exposure (pharmacokinetics) at the 50ng dose level, Trefoil may discontinue collection of PK samples at dose levels ≤ 50ng. Pre- and post-exposure ADA samples will continue to be collected regardless of observed PK exposure. A plasma sample will be obtained at the end of the study for ADA analysis

See Study TTHX-002 Laboratory Manual for blood processing instructions.

[redacted]

[redacted]

6.4.16 Visual Function and Corneal Health Status (V-FUCHS)

The V-FUCHS is a 15-item questionnaire designed to measure patient-reported visual disability in Fuchs endothelial corneal dystrophy (FECD). 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 V-FUCHS should be self-administered, however, trained investigational site staff will administer the questionnaire to subjects who are unable to self-administer the questionnaire. Collection of V-FUCHS 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 (if applicable).

6.4.17 [redacted]

[redacted]

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/ visit is not able to be collected/ conducted within the protocol-specified window, it should be collected/ performed 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 / baseline assessments may be performed anytime during the Screening Period, unless approved by the Medical Monitor, except for Informed Consent (which must be obtained prior to any protocol-specific assessments).

6.5.2 On-study Visits

The frequency of on-study visits will vary depending on the treatment group to which the study subject belongs.

All study drug administration dates must be at least 5 days after the prior study drug administration day (if applicable). All other visits will be scheduled based on the number of days since Day 0. If a study drug administration cannot be administered within the protocol window, it should be administered as soon as practicable.

Worksheets will be provided describing which assessments are required at each visit for each treatment group.

6.5.3 Long-term Follow-up

Following the Day 56 evaluations, all subjects will continue to be followed for long-term efficacy, durability of responses, and SAE/SAR resolution. Follow-up visits will occur approximately 3, 6, and 12 months after the DWEK/DSO procedure or until subsequent treatment in the Study Eye for FECD. Subjects will also be seen as part of their regular health care, all relevant ocular assessments collected during this period will be recorded.

6.5.4 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 28 should be performed at the Early Termination Visit if Day 28 visit has not already been completed, otherwise assessments scheduled for Day 56 should be collected.

6.5.5 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 relevant assessments.

6.5.6 End of Study Visit

An End of Study Visit (EOS) will be performed on all subjects. The EOS may be conducted any time after Day 168, during the EOS assessments scheduled for Day 336 will be performed and a plasma sample for ADA analysis will be collected. If the subject has already completed the study, the subjects should be contacted and asked to provide a plasma sample for ADA. Any subject who did not have gradeable specular images at their most recent visit should be re-imaged and gradable images obtained.

- Comprehensive Eye Examination
- Specular Image Collection
- Assessment of Corneal Thickness
- Assessment Best Corrected Visual Acuity
- Plasma sample collected for ADA

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 efficacy endpoints will be visual acuity and endothelial cell count/ density as measures by specular microscopy. Other efficacy assessments include:

- Slit lamp biomicroscopy assessments
- Central corneal thickness
- IOP

The primary efficacy timepoint is Day 28.

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 28 days after each study treatment will be reported.
- 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 11.

Table 11: Severity Grading Guideline for Adverse Events

Grade	Description
Mild	Asymptomatic or mild symptoms; Clinical or diagnostic observations only; Intervention not indicated
Moderate	Minimal, local or noninvasive intervention indicated; Limiting age-appropriate instrumental ADL*
Severe	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.

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 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:

[redacted]

If adverse events of special interest (AESI) are identified during the study, Trefoil will notify Investigators and provide instructions for reporting. Deaths occurring within **1 year** 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.

8.4 Adverse Events of Special Interest

[redacted]

9. STATISTICS

9.1 Sample Size

The sample size for this study was determined by clinical and practical rather than statistical considerations. Sixteen subjects in the active treatment group compared to 8 subjects in the non-treatment control group were planned during the study to provide a preliminary estimate of safety and efficacy.

9.2 Analyses

The study includes a concurrent non-treatment control group. The active treatment group will be compared to the control group. In addition, descriptive statistics will be used to tabulate and summarize other efficacy variables and safety outcomes. Baseline and demographic characteristics will be presented. Continuous variables will be summarized by descriptive statistics (sample size, mean/ standard deviation, median, minimum and maximum). Discrete variables will be summarized by frequencies and percentages. Ocular AEs involving the study eye will be summarized by presenting the number and percentage of subjects having any ocular AE. Any other information collected (such as severity or relationship to study drug and intracameral injection) will be listed as appropriate.

9.3 Analysis of the Primary Efficacy Variable

A full Statistical Analysis Plan will be created prior to database lock. All post-hoc analyses will be identified and described in the Clinical Study Report. When possible, statistical testing will be applied.

9.3.1 Analysis Populations and Sets

The analysis sets for this clinical study are defined in accordance with *ICH E9 Guidance for Industry Statistical Principles for Clinical Trials*. The Full Analysis Set is intended to be the population which is as complete as possible and as close as possible to the intention-to-treat ideal. This is an open label study and not randomized, therefore the Full Analysis Set will include only subjects who have received any study treatment and will be the same as the Safety Population. Analysis sets are described below in Table 12.

Table 12: Analysis Sets

Analysis Set	Description of Population	Reference
Full Analysis Set*	• Received any study treatment (at least 1 complete or partial dose)	ICH E9 (5.2.1)
Per Protocol Set	• Met all eligibility criteria • Have received all study treatments per protocol • Underwent DWEK/DSO of 4 to 5mm • Have sufficient data collected for the accurate assessment of study endpoints	ICH E9 (5.2.2)

*The Full Analysis Set will be used for the Safety Population

The Per Protocol Population will be used to assess the primary and secondary efficacy endpoints compared to the control group.

The Safety Population set includes all participants in participants who received any TTHX1114 and will be the population for safety outcomes; this population will be compared with the control group. Ocular AEs involving the study eye will be tabulated. Ocular AEs for the fellow eye and non-ocular AEs will be provided as a listing.

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 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. 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: ETDRS to Snellen Conversion

ETDRS letters	Approximate Snellen Visual Acuities*				Decimal Fraction	Log MAR
Expected Score	1 meter	4 meters	6 meters	20 feet		
34-38	1/10	4/40	6/60	20/200	0.10	+1.0
39-43	1/8	4/32	6/48	20/160	0.125	+0.9
44-48	1/6.25	4/25	6/38	20/125	0.16	+0.8
49-53	1/5	4/20	6/30	20/100	0.20	+0.7
54-58	1/4	4/16	6/24	20/80	0.25	+0.6
59-63	1/3.15	4/12.6	6/20	20/62.5	0.32	+0.5
64-68	1/2.5	4/10	6/15	20/50	0.40	+0.4
69-73	1/2	4/8	6/12	20/40	0.50	+0.3
74-78	1/1.6	4/6.4	6/10	20/32	0.625	+0.2
79-83	1/1.25	4/5	6/7.5	20/25	0.80	+0.1
84-88	1/1	4/4	6/6	20/20	1.00	0.0
89-93	1/0.8	4/3.2	6/5	20/16	1.25	-0.1
94-98	1/0.625	4/2.5	6/3.75	20/12.5	1.60	-0.2
99-100	1/0.5	4/2	6/3	20/10	2.00	-0.3

*Snellen fraction determined from the lowest line read with one or fewer mistakes. This is similar to allowing one mistake per line. If the participant makes more than one mistake on the 20/800 line and on all subsequent lines, then the Snellen fraction should be recorded as 20/800. Note: the actual score may vary from the expected sources listed.

Modified from: Ferris III, F.L., Kassoff, A., Bresnick, G.H., Bailey, I., 1982. New visual acuity charts for clinical research. Am. J. Ophthalmol. 94 (1), 91–96

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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 as well as all subsequent versions provided to me 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)