Descemet Endothelial Thickness Comparison Trials (DETECT I & II)

Protocol Number: 1.0

National Clinical Trial (NCT) Identified Numbers: NCT05289661, NCT05275972

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Sponsor: Stanford University

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
5.1	Clarifications made to Inclusion/Exclusion Criteria	
6.1.1	Instruction to send donor tissue or media for fungal culture	
6.1.1	Addition of DMEK graft insertion tools	
6.1.2	Changed prednisolone to dexamethasone 0.1% for DETECT II	In order to better match placebo, we have changed back to our original plan to treat patients in DETECT II with dexamethasone
6.2.6	Add fill volume (6.5mL into a 10mL droptainer) of Ripasudil	Ripasudil can only be used for 28 days after opening the bottle, per our BUD testing results. We are using a 10mL droptainer to match the placebo (artificial tear) bottle. A fill volume of 6.5mL significantly reduces Ripasudil waste.

Throughout	Removal of AS-OCT imaging	

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: Descemet Endothelial Thickness Comparison Trial (DETECT) I & II

Study Description: Descemet Endothelial Thickness Comparison Trial (DETECT) I is a multi-center, outcome assessor-masked, placebo-controlled clinical trial randomizing 160 patients in a 2x2 factorial design. The purpose of this study is to determine differences in visual outcomes between two types of corneal transplant surgeries, ultrathin Descemet stripping automated endothelial keratoplasty (UT-DSAEK) and Descemet membrane endothelial keratoplasty (DMEK), and to determine the effect of rho-kinase inhibitors on endothelial cell loss. Patients presenting to Oregon Health & Science University, Stanford University, University of Pennsylvania, University of California Davis, or to Dartmouth-Hitchcock Medical Center with endothelial dysfunction who are good candidates for both types of endothelial keratoplasty performed in this study will be eligible for inclusion. Participants will be randomized to one of four treatment groups:

1) UT-DSAEK plus topical ripasudil 0.4%
2) UT-DSAEK plus topical placebo
3) DMEK plus topical ripasudil 0.4%
4) DMEK plus topical placebo

Descemet Endothelial Thickness Comparison Trial (DETECT) II is a multi-center, outcome assessor-masked, placebo-controlled clinical trial randomizing 60 patients with Fuchs endothelial dystrophy to DMEK versus Descemet Stripping Only (DSO) with adjunctive Ripasudil. Patients presenting to Oregon Health & Science University, Stanford University, University of Pennsylvania, University of California Davis, or to Dartmouth-Hitchcock Medical Center with mild Fuchs endothelial dystrophy will be

eligible for inclusion. Participants will be randomized 1:1 to one of two treatment groups:

5) DMEK plus topical placebo
6) DSO plus topical ripasudil 0.4%

The enrollment period is 24 months.

Objectives:

Primary Objectives

Specific Aim 1: To determine whether DMEK has superior post-operative best spectacle corrected visual acuity (BSCVA) compared with UT-DSAEK or DSO with a similar safety profile.

a. *We hypothesize that the DMEK group will have improved visual acuity compared with UT-DSAEK at all time points.*
b. *We hypothesize that the DMEK group will have improved visual acuity compared with DSO at all time points.*

Specific Aim 2: To determine the benefit of adjuvant rho-kinase inhibitors endothelial cell loss in patients who received UT-DSAEK and DMEK

a. *We hypothesize that endothelial cell loss will be higher after DMEK than UT-DSAEK.*
b. *We hypothesize that endothelial cell loss will be lessened among those receiving ripasudil, after controlling for the pre-operative ECD in DMEK and UT-DSAEK.*

Secondary Objectives

- Specific Aim 1: To determine whether DMEK has superior post-operative BSCVA at secondary time points
- Specific Aim 2: To assess the effect of surgery type on endothelial cell loss at all time points
- Specific Aim 1: To assess the safety profile of each surgery type
- Specific Aim 2: To determine if rho-kinase inhibitors affect endothelial cell loss in patients undergoing endothelial keratoplasty at secondary time points
- To determine the effect of study interventions on quality of life
- To determine the effect of study interventions on adverse events
- To assess the relative role of light scatter on visual acuity
- To assess the relative role of higher-order aberrations on visual acuity
- To assess the relative role of graft thickness on visual acuity
- To assess the cost-effectiveness of each intervention
- To assess the effect of surgery type on refractive outcomes

Endpoints:

Primary Endpoints:

DETECT I
- Best spectacle-corrected visual acuity (BSCVA) at 12 months
- Endothelial cell loss at 12 months

DETECT II
- Best spectacle-corrected visual acuity (BSCVA) at 12 months

Secondary Endpoints:
- Specific Aim 1: BSCVA at 3, 6, and 24 months
- Specific Aim 1: Complications over the course of the entire study period, including re-bubble rate, graft rejection, and primary graft failure
- Specific Aim 2: Endothelial cell density at 3, 6, and 24 months
- National Eye Institute Visual Function Questionnaire (VFQ) post-operatively at 3 months and 12 months as compared to baseline
- Adverse events over entire study period
- Corneal haze and higher-order aberrations as measured by Pentacam at baseline and post-operatively at 3, 6, 12, 24 months
- Graft thickness as measured by optical coherence tomography and pachymetry on donor tissue pre-operatively and post-operatively at 6 and 12 months
- Cost-effectiveness analysis at 24 months
- Manifest refraction at baseline and post-operatively at 3, 6, 12, 24 months

Study Population:

160 participants with moderate to severe Fuchs (DETECT I) and 60 participants with mild Fuchs (DETECT II) will be enrolled at Oregon Health & Science University, Stanford University, University of Pennsylvania, University of California Davis, and at Dartmouth-Hitchcock Medical Center. We anticipate participants from each enrollment site will match the demographic makeup of the city the site is located in. Therefore, our study population will be mostly White (non-Hispanic), Hispanic, African-American, and Asian based on the most recent census data for each enrollment area. There is evidence that endothelial dystrophy is more common in women, and the previous DETECT-Therapeutic Exploratory Study (TES) enrolled 58% women. We anticipate a similar gender makeup in this study. In the DETECT-TES the median age of study participants was 67, with an interquartile range of 64 to 71; we expect a similar age range in this study.

Phase:

N/A

Description of Sites/Facilities Enrolling Participants:

Participants will be enrolled at five sites in the United States: Oregon Health & Science University (Portland, OR), Stanford University (Palo Alto, CA), University of Pennsylvania (Philadelphia, PA), University of California Davis (Davis, CA), and Dartmouth-Hitchcock Medical Center (Lebanon, NH). Patients will be enrolled at the Cornea Clinic in each hospital.

Description of Study Intervention:

DETECT I

Study participants will all undergo endothelial keratoplasty followed by an adjuvant topical medication post-operatively. Participants will be randomized to one of four treatment groups in this 2x2 factorial design study:

		Endothelial Keratoplasty	
		UT-DSAEK	**DMEK**
Adjuvant Topical Medication	**Ripasudil 0.4%**	UT-DSAEK + 0.4% ripasudil	DMEK + 0.4% ripasudil
	Placebo	UT-DSAEK + placebo	DMEK + placebo

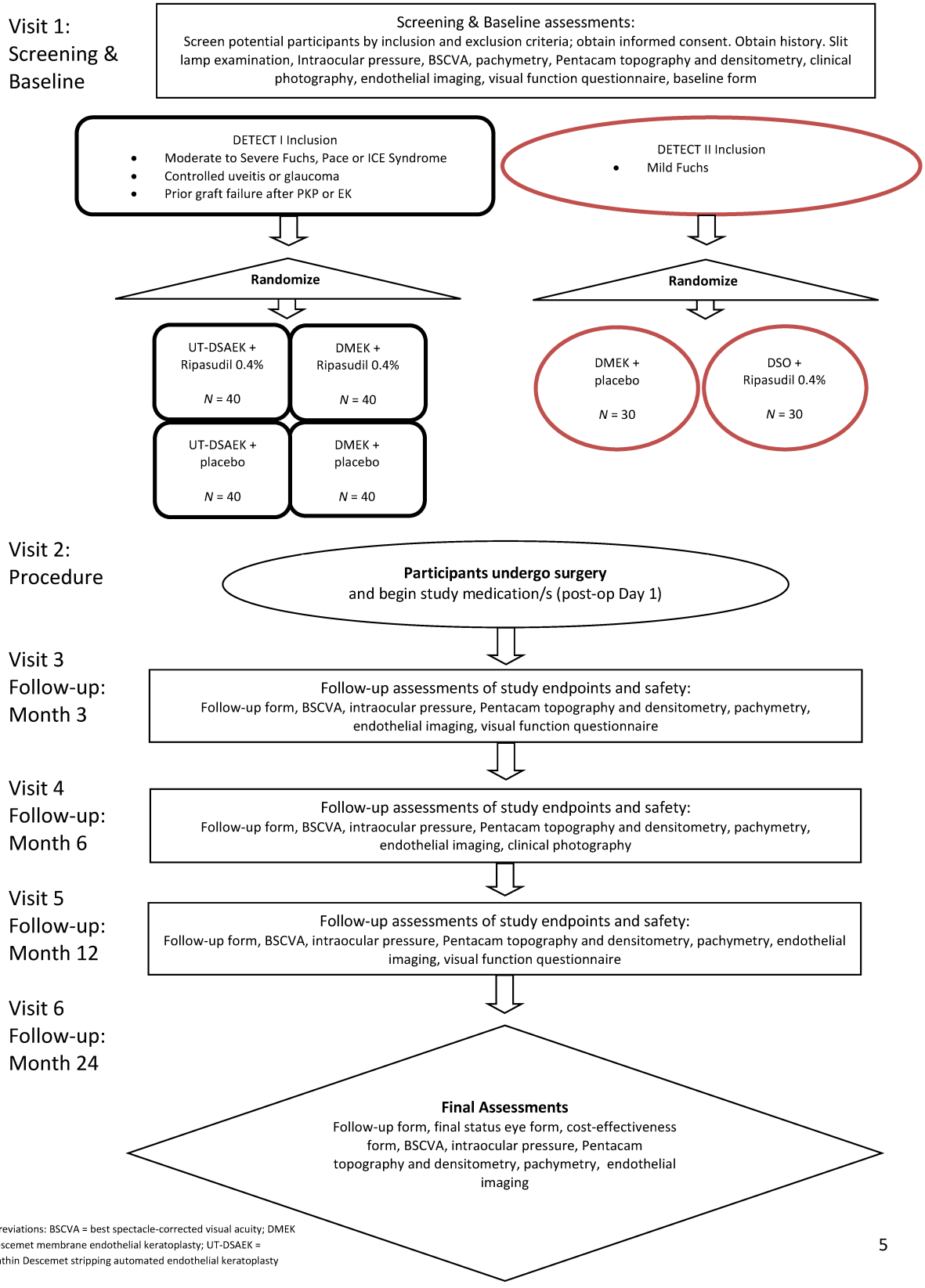
All patients will be randomized to undergo one of two types of endothelial keratoplasty. All patients will be randomized to receive either topical ripasudil 0.4% or topical placebo. Patients will begin topical medicines post-op day 1. They will take their assigned study medication 4x/day for 3 months.

DETECT II

Study participants will all undergo either DMEK + topical placebo or DSO + ripasudil. All patients will be randomized to undergo one of these two surgical interventions. All patients randomized to DSO will receive topical ripasudil 0.4% post-operatively and all patients randomized to DMEK will receive topical placebo. Patients will begin topical medicines post-op day 1. They will take their assigned study medication 4x/day for 3 months.

Study Duration: 5 years
Participant Duration: 24 months

1.2 SCHEMA



Abbreviations: BSCVA = best spectacle-corrected visual acuity; DMEK = Descemet membrane endothelial keratoplasty; UT-DSAEK = ultrathin Descemet stripping automated endothelial keratoplasty

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Screening Pre-enrollment	Enrollment/Baseline, Visit 1 Day 0	Procedure, Visit 2 Day 5 (+/- 14 days)	Follow-up, Visit 3 Month 3 (+/- 1 month)	Follow-up, Visit 4 Month 6 (+/- 1 month)	Follow-up, Visit 5 Month 12 (+/- 2 months)	Final Follow-up, Visit 6 Month 24 (+/- 4 months)
Review Inclusion/Exclusion criteria	X						
Informed consent		X					
Demographics		X					
Medical history		X					
Randomization			X*				
Administer study intervention			X				
Slit lamp examination		X		X	X	X	X
Intraocular pressure		X		X	X	X	X
Pachymetry		X		X	X	X	X
Pentacam topography and densitometry		X		X	X	X	X
Endothelial imaging	X	X		X	X	X	X
Clinical photography†		X			X		
BSCVA/ETDRS/MRx		X		X	X	X	X
Baseline form		X					
Follow-up form				X	X	X	X
Final form							X@
Visual function questionnaire		X		X		X	
Cost-effectiveness form							X
Interval History				X	X	X	X

*Randomization performed approximately one week prior to surgery
†Clinical photography also taken upon adverse event
@If participant does not complete the study, final form will be filled out at time of withdrawal or loss-to-follow-up
BSCVA = best spectacle corrected visual acuity, ETDRS = Early Treatment Diabetic Retinopathy Study, MRx = manifest refraction

2 INTRODUCTION

2.1 STUDY RATIONALE

The field of corneal transplantation is evolving rapidly with few rigorous studies to guide the implementation of novel surgical techniques or medical therapies.[1,2] Posterior lamellar keratoplasty, which replaces only the posterior cornea including the diseased endothelium and Descemet membrane, has led to faster recovery, fewer complications, and better visual acuity outcomes compared to traditional penetrating keratoplasty (PKP).[3] According to the Eye Bank Association of America, selective endothelial transplantation accounted for approximately 59% of all corneal transplants performed in the US in 2018.[4,5]

It is unknown which endothelial keratoplasty (EK) technique results in the best outcomes while maintaining an optimal safety profile. Descemet membrane endothelial keratoplasty (DMEK), which replaces only DM and endothelium, has the potential to further improve outcomes compared with Descemet stripping endothelial keratoplasty (DSAEK), which remains the most common keratoplasty approach for endothelial dysfunction conditions in the US.[6] Descemet Endothelial Thickness Comparison Trial - Therapeutic Exploratory Study (DETECT-TES) was an outcome-masked, two-surgeon therapeutic exploratory study (TES) that randomized patients with primary endothelial disease to Ultrathin (UT)-DSAEK (donor grafts 70-90 µm thick) versus DMEK and found that DMEK had 1.4 lines better visual acuity at 12 months (95% CI 2.2 to 0.1;*P*<0.001).[7] However, two other similar small studies were unable to detect a difference between DMEK and either UT-DSAEK or Nanothin-DSAEK (donor grafts less than or equal to 50 µm thick).[8,9]

Additionally, DMEK may have higher complication rates, such as primary graft failure.[10,11] DETECT-TES noted more rapid decline in central endothelial cell densities (ECDs) over time compared with UT-DSAEK although this was not statistically significant [12 months: UT-DSAEK, 2070 ± 292 cells/mm^2; DMEK, 1855 ± 448 cells/mm^2 (*P*=0.051)]. *The long-term implications of endothelial cell loss (ECL) on graft survival are important, especially if visual acuity in UT-DSAEK group is similar and this should be further investigated.*

Although corneal transplantation has improved over time, it still carries a risk of vision threatening complications such as endophthalmitis, graft rejection and endothelial failure, making medical therapy an attractive alternative. The topically administered rho kinase (ROCK) inhibitor, ripasudil, has been shown to protect against apoptosis and promote endothelial cell proliferation *in vitro* and in a human *ex vivo* model.[12-14] One RCT has demonstrated improved recovery of corneal clarity in Fuchs Endothelial Corneal Dystrophy (FECD) after Descemet stripping only (DSO) with adjuvant topical ripasudil.[15,16]A small series of Pseudophakic/Aphakic Corneal Edema (PACE) patients achieved complete corneal clearing after cultured donor endothelial cells supplemented with ripasudil were injected into the anterior chamber.[17] Therefore, adjuvant ripasudil may address the biggest challenge facing corneal transplant surgeons today, protection against ECL peri-operatively and long-term maintenance of endothelial cell health. *Here, we propose a multi-center, outcome-masked clinical trial in a 2 x 2 factorial design randomizing patients to 1) DMEK versus UT-DSAEK and 2) Adjuvant topical ripasudil 0.4% versus placebo.*

2.2 BACKGROUND

Although many of the treatments in ophthalmology are surgical in nature, there are few clinical trials to guide the implementation of surgical techniques or treatments.[18] Posterior lamellar keratoplasty, which replaces only diseased endothelium has led to faster recovery times, fewer complications, and better visual acuity outcomes compared to traditional penetrating keratoplasty (PKP).[3] Currently, Descemet's Stripping Automated Endothelial Keratoplasty (DSAEK) is the most common type of posterior lamellar keratoplasty because of its relative ease and good outcomes.[19] A newer technique, Descemet's Membrane Endothelial Keratoplasty (DMEK), where only Descemet's membrane and the endothelium is transplanted, has the potential to further improve visual acuity outcomes, produce fewer higher-order corneal aberrations and decrease rejection rates.[20-24] However, donor preparation, increased intra-operative times, and problems with donor attachment in DMEK are all important limitations.[25,26]

Ultrathin-DSAEK (UT-DSAEK), with donor grafts less than 100 µm thick, has been shown to have superior visual acuity outcomes compared with traditional DSAEK and may have similar results to DMEK without the technical difficulties.[6] Several large prospective series show similar visual outcome results and rates of immunologic rejection between UT-DSAEK and DMEK, however comparisons are difficult.[27,28] The two largest published series for each technique include a large cohort of 500 DMEK procedures performed by two surgeons[29] and a series of 285 UT-DSAEK procedures performed by a single surgeon.[27] DMEK resulted in improved 6-month visual acuity results compared with UT-DSAEK, with more study participants having 20/25 or better visual acuity (75% DMEK versus 61% UT-DSAEK) and more 20/20 or better visual acuity (41% DMEK versus 26% UT-DSAEK). In the DMEK series 2.2% of patients required repeat grafting and were excluded from visual acuity analysis. In these two series, the procedures appear to be quite comparable in terms of re-bubble and primary graft failure rates. The visual acuity results and complication rates of DMEK from specialized centers may not be generalizable to most corneal surgeons. Melles series reported outcomes of 431 DMEKs performed by 18 experienced corneal surgeons in 11 countries.[30] In this study only 43.8% of participants achieved 20/25 or better visual acuity by 6 months and only 18.8% achieved 20/20 visual acuity or better. What is most concerning, however, is that 79 participants (18%) required re- operation, sometimes up to their 5th DMEK.

Descemet Endothelial Thickness Comparison Trial – Therapeutic Exploratory Study (DETECT-TES) was a two-surgeon, outcome-masked clinical trial that randomized 50 eyes of 38 patients with primary endothelial disease to UT-DSAEK versus DMEK and found that study participants randomized to DMEK had 1.8 lines better visual acuity at 6 months (*P*<0.001), 1.4 lines better visual acuity at 12 months (P<0.001) and 1.3 lines better visual acuity at 24 months (P<0.001) with similar complication rates (**Figure 1**).[31] Although DETECT-TES was a well-designed and well-powered study for the primary outcome of visual acuity, there were several limitations including the fact that it reported outcomes of only two

surgeons and may not be generalizable to most corneal specialists. It was also not powered to detect differences in important secondary outcomes such as re-bubble rate, primary graft failure, change in endothelial cell densities (ECDs) or vision-related quality of life. These limitations, along with the fact that there have been one other small randomized clinical trial and one other prospective comparative case series which have not found a difference in visual acuity outcomes between DMEK and UT-DSAEK or Nanothin-DSAEK (<50µm), support the need for additional rigorous study comparing these techniques.[8,9] *A larger, multi-center, multi-surgeon randomized clinical trial would help to confirm the primary outcome of DETECT-TES and differentiate important secondary outcomes over 2 years. Furthermore, it will be designed to assess longer-term outcomes such as graft survival and rejection in the same cohort with subsequent grant funding.*

There was a suggestion that the mean change in ECD was worse over time among DMEK patients in DETECT, although this was not statistically significant. Mean ECD at 3 and 6 months were similar between groups; however, by 12 months the mean ECD was 1855 cells/mm^2 in DMEK and 2070 cells/mm^2 in UT-DSAEK at 12 months (*P*=0.06). One of the most important challenges after corneal transplant is ECL which may lead to graft failure, a significant cost to individual patients and society. The Cornea Donor Study (CDS) found ECL of over 70% in penetrating keratoplasty after 10 years in surviving clear grafts.[32] Price et al found similar rates of ECL after DSAEK compared with PKP.[33] This is important because the post-operative ECDs have been shown to correlate with risk of subsequent graft failure in penetrating keratoplasty (PKP) and DSAEK.[34] The etiology of ECL is multifactorial and includes surgical trauma, and immune-mediated mechanisms. The rate and pattern of ECL after EK is different than that of PKP with a larger initial drop but slower rate of subsequent ECL. More peripheral trauma during PKP may result in better central ECDs at first, but peripheral migration of residual endothelial cells may cause the continued decline in central ECDs seen over time.[35] Prior case reports suggest that DMEK may have less ECL than PKP and DSAEK.[34,36-38] Our findings suggest that patterns of ECL may differ between UT-DSAEK and DMEK and that ECL may worsen more rapidly in DMEK. The reason for this is unclear, but may be similar to PKP with peripheral graft trauma during DMEK that results in peripheral migration of central endothelial cells to the graft edge. The *advantages of DMEK over UT-DSAEK may be less clear if ECL is found to be higher as this would likely lead to more late graft failures.*

If DMEK was indeed shown to have improved visual acuity, with higher endothelial cell loss, adjuvant Rho-kinase inhibitors may provide the optimal solution. The ROCK signaling pathway is a therapeutic target for a number of systemic and ocular diseases. Rho is a small GTPase that activates ROCK, a serine/threonine kinase, which has two isoforms, ROCK I and ROCK II. ROCK signaling has been implicated in a number of cell functions including cell adhesion, proliferation, differentiation and apoptosis.[39] Netarsudil recently became the first topically administered ROCK-inhibitor to be approved by the FDA for the treatment of glaucoma.[40] This non-selective ROCK-inhibitor is thought to improve trabecular meshwork outflow by increasing actin-myosin contraction.[40] Unfortunately, thus far Netarsudil has not been shown to have any effect on endothelial cell replication or function. Interest in this question is high with three small ongoing clinical trials listed on clinicaltrials.gov (NCT03575130, NCT03813056, NCT04057053).

Ripasudil, a selective ROCK-inhibitor that is approved for the treatment of glaucoma in Japan, may have an important adjuvant role in corneal transplantation. It been shown to promote endothelial cell proliferation and inhibit apoptosis *in vitro* and in primate and rabbit models.[12-14] One *ex vivo* study found that treating human endothelial cells with ripasudil reduced apoptosis.[41] In one small series published in the New England Journal of Medicine, patients with endothelial dysfunction from bullous keratopathy were shown to regain corneal clarity with injection of cultured donor corneal cells supplemented with ripasudil. However, it is unclear whether this would have occurred without the adjuvant ROCK-inhibitor or how this compares with current keratoplasty techniques since there was no control group.[17] There have also been case reports and one randomized clinical trial suggesting a benefit of adjuvant ripasudil after Descemet stripping only (DSO) in FECD.[15,16] *Adjuvant ripasudil has the potential to protect against apoptosis, promote cell health and proliferation which may profoundly impact ECL and the need for subsequent corneal transplantation.*

The NEI and the US Food and Drug Administration recommend that vision-related quality of life measures be used to assess interventions in ophthalmology. This has become particularly important in an era of limited health care resources. DETECT-TES found both a clinically significant and statistically significant improvement in vision-related

quality of life as measured by the NEI-VFQ at 12 months in those undergoing either DMEK or UT-DSAEK (*P*<0.001). Given that a 4-point change in overall VFQ score is considered to be a small clinically significant change, an increase of approximately 13 points from baseline on average is remarkable.[42] Interestingly, however, it was unable, to find a difference in vision-related quality of life between treatment arms. This may be related to a lack of power to find a difference given the small numbers in the study, however, *if there is no improvement in vision-related quality of life among DMEK patients, the advantages of DMEK over UT-DSAEK are less compelling.*[43]

The number of DMEK procedures performed each year in the US has increased dramatically in recent years. According to the Eye Bank Association of America, DMEK accounted for less than 15% of endothelial keratoplasties in the US in 2015, whereas DSAEK accounted for approximately 50% of all corneal transplants. In 2017 the number of DMEK surgeries increased to 26% of all endothelial keratoplasties and in 2018 DMEK surgeries increased by another 41%.[44] The increase is likely due to a combination of factors including the results of DETECT-TES, improvements in eyebank prepared tissue (pre-stripped, pre-punched, pre-loaded), and standardization of surgical techniques.[11,45] DMEK may provide better visual acuity outcomes in carefully selected patients and at specialized centers; however, it may also carry more risks and a higher cost to society. Understanding the long-term implications of the potential ECL identified in DMEK on graft survival will be crucial to protecting the donor pool. The role of adjuvant topical selective ROCK-inhibitors in supporting endothelial cell health remains unclear. *Our proposed multi-center outcome-masked randomized placebo-controlled trial will profoundly impact clinical practice with regard to these surgical and adjuvant medical therapies. It also has the potential to yield longer-term outcome data, although that is beyond the scope of this 5-year funding.*

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Study participants will not have any increased risk or cost for participating in this study. Participants in the study have the same risks as those undergoing corneal transplantation in other settings. Many of the risks in this study are inherent risks of corneal transplantation; thus, there is no known increased risk associated with participation in this study.

As with any surgery, there are risks associated with these surgeries, and both surgeries have similar risks. There is a risk of infection or bleeding which could result in vision loss. There may be damage to the tissue graft during tissue preparation, and the tissue graft can fail to attach to the cornea; thus, there may be a need for repeat air injection. Below, the other risks associated with each type of surgery are listed:

- UT-DSAEK: There may be slightly decreased vision after surgery compared to DMEK, although this is unknown. The healing process may also be slower. Some think there is an increased risk of rejection compared to DMEK, although this is also unknown. There is a small risk of intraocular pressure rise with painful glaucoma attack in the immediate postoperative period that can cause permanent glaucomatous optic nerve damage resulting in visual field deficits.
- DMEK: There may be increased risk of tissue loss, repeat air injection, detachment of the tissue graft to the cornea compared with UT-DSAEK. There may be higher costs associated with this surgery, although these additional costs will be covered by insurance. There is a small risk of intraocular pressure rise with painful glaucoma attack in the immediate postoperative period that can cause permanent glaucomatous optic nerve damage resulting in visual field deficits.
- DSO: The healing process may be slower than after DMEK or UT-DSAEK. There is a risk that a second surgical for endothelial keratoplasty would be required. Topical ripasudil may cause a local allergic reaction, eye redness or irritation, or upset stomach.

Blindness due to infection may occur in extremely rare cases. There may also be differences in rates of difficulty with donor preparation, increased intra-operative times, graft rejection and graft survival, and donor attachment in DMEK versus UT-DSAEK. Even if there is a total detachment during DMEK, we will continue to move forward with secondary treatment. These risks will be addressed by the enrollment site by making sure all protocols are being followed thoroughly and by following up with participants after surgery.

There are no known risks directly related to the Endoserter, jones tube, or cannulas. There may be some discomfort during follow-up testing (BSCVA, IOP, slit lamp, Pachymetry, Pentacam, endothelial imaging and OCT testing and photo imaging inside the eye), but this will be kept to a minimum. The participant will be asked to tell the doctor if any of this testing feels painful.

Anesthesiology will be determined as per the participants needs. They will generally be awake with medications given to help them relax and feel comfortable. This is called monitored anesthesia care. Other participants may require general anesthesia care. While this is generally safe, there are small increased risks to the heart and lungs which will be discussed with the anesthesiologist. Both types of anesthesia are routinely used for DMEK and DSAEK surgeries, although generally monitored anesthesia care is the typical route.

The most commonly reported adverse effects of topical ripasudil 0.4% include irritation, blepharitis, honeycomb edema, conjunctival congestion, conjunctival hyperemia, and conjunctival inflammation. Other risks include conjunctival hyperemia, guttatta-like bodies with reversible morphologic changes.

2.3.2 KNOWN POTENTIAL BENEFITS

Corneal transplantation is among the most commonly performed transplant surgeries in the world, and endothelial keratoplasty is the most common type of corneal transplantation. There is reasonable evidence that our interventions (DMEK and ripasudil) will improve outcomes for those undergoing endothelial keratoplasty. If our hypotheses are correct, there could potentially be a profound societal benefit for future patients.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Knowledge gained from this study may help improve outcomes for patients undergoing endothelial keratoplasty, and may prolong the lifespan of transplanted tissue. Given the minimal risks to participants in this study, we feel the benefits of the important knowledge we expect to gain from this study outweigh the risks.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Specific Aim 1: To determine whether DMEK has superior post-operative best-spectacle corrected visual acuity (BSCVA) compared to UT-DSAEK and DSO	BSCVA at 12 months	The most clinically important outcome for patients
Specific Aim 2: To determine if rho-kinase inhibitors affect endothelial cell loss in patients undergoing endothelial keratoplasty	Endothelial cell density at 12 months	Endothelial cell loss corresponds with risk of subsequent graft failure
Secondary		
Specific Aim 1: To determine whether DMEK has superior post-operative BSCVA compared to UT-DSAEK and DSO	BSCVA at 3, 6, and 24 months	The most clinically important outcome for patients
Specific Aim 2: To assess the effect of surgery type on endothelial cell loss	Endothelial cell loss at 3, 6, 12, 24 months	Endothelial cell loss corresponds with risk of subsequent graft failure

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Specific Aim 1: To assess the safety profile of each surgery type	Complications over the course of the entire study period, including re-bubble rate, graft rejection, and primary graft failure	Complications rates are important to consider when deciding on a surgery type
Specific Aim 2: To determine if rho-kinase inhibitors affect endothelial cell loss in patients undergoing endothelial keratoplasty	Endothelial cell density at 3, 6, and 24 months	Endothelial cell loss corresponds with risk of subsequent graft failure
To determine the effect of study interventions on quality of life	National Eye Institute Visual Function Questionnaire (VFQ) post-operatively at 3 months and 12 months as compared to baseline	Quality of life is an important patient outcome and will help determine if any differences in BSCVA or endothelial cell density are clinically relevant. The previous DETECT-TES found DMEK to have significantly better visual acuity outcomes but no difference in VFQ
To determine the effect of study interventions on adverse events	Adverse events over entire study period	To ensure safety of the interventions
To assess the relative role of light scatter on visual acuity	Pentacam densitometry at baseline and post-operatively at 3, 6, 12, 24 months	Correlates with clinical outcomes, and may provide a sensitive surrogate endpoint for future clinical trials
To assess the relative role of higher-order aberrations on visual acuity	Pentacam HOA at baseline and post-operatively at 3, 6, 12, 24 months	Correlates with clinical outcomes, and may provide a sensitive surrogate endpoint for future clinical trials
To assess the effect of ripasudil on graft thickness	An RCT comparison of graft thickness as measured by pachymetry on donor tissue post-operatively at 6 and 12 months controlling for baseline	Objective measure of the effect of ripasudil on graft function
To assess the relative role of graft thickness on visual acuity	Graft thickness as measured by pachymetry on donor tissue pre-operatively and post-operatively at 3, 6, 12, 24 months	Important to understand how graft thickness affects visual acuity to guide future surgeries
To assess the cost-effectiveness of each intervention	Cost-effectiveness analysis at 24 months	Important factor to consider when recommending surgery
To assess the effect of surgery type on refractive outcomes	Manifest refraction at baseline and post-operatively at 3, 6, 12, 24 months	Important to assess the relationship between surgery and refraction

4 STUDY DESIGN

4.1 OVERALL DESIGN

DETECT I
Descemet Endothelial Thickness Comparison Trial (DETECT) I is a multi-center, outcome-masked, placebo-controlled clinical trial randomizing 160 patients in a 2x2 factorial design. The purpose of this study is to determine differences in visual outcomes between two types of corneal transplant surgeries, ultrathin Descemet stripping automated endothelial keratoplasty (UT-DSAEK) and Descemet membrane endothelial keratoplasty, and to determine the effect of rho-kinase inhibitors on endothelial cell loss in patients with moderate Fuchs endothelial dystrophy, bullous keratopathy, prior graft failure, controlled uveitis or controlled glaucoma. Patients presenting to Oregon Health & Science University, Stanford University, University of Pennsylvania, University of California Davis, or to Dartmouth-Hitchcock Medical Center with isolated endothelial dysfunction who are good candidates for both types of endothelial keratoplasty performed in this study will be eligible for inclusion. Participants will be randomized to one of four treatment groups in this 2x2 factorial design study:

1) UT-DSAEK plus topical ripasudil 0.4%
2) UT-DSAEK plus topical placebo
3) DMEK plus topical ripasudil 0.4%
4) DMEK plus topical placebo

We hypothesize that DMEK will result in improved visual acuity at all time points compared to UT-DSAEK. We anticipate that DMEK will have higher ECL than UT-DSAEK. We hypothesize that endothelial cell loss at 12 months will be lessened in those receiving rho-kinase inhibitors, after controlling for pre-operative endothelial cell density.

DETECT II
Descemet Endothelial Thickness Comparison Trial (DETECT) II is a multi-center, outcome -masked trial randomizing 60 patients with Mild Fuchs endothelial dystrophy to DMEK versus DSO with adjunctive Ripasudil. The purpose of this study is to determine visual outcomes between these two surgical interventions.
Participants will be randomized 1:1 to one of two treatment groups:

5) DMEK plus topical placebo
6) DSO plus topical ripasudil 0.4%

We anticipate that DMEK will result in improved visual acuity at all time points compared to DSO. We hypothesize that DMEK will have higher ECD at 12 months that DSO.

All study participants will be masked to their intervention. The refractionist performing the BSCVA will also be masked. Due to the nature of the intervention, the surgeon and technician performing study visit endothelial cell density and other imaging will not be masked as to surgery type (but will be masked as to study medication); however, the image graders at the Proctor Reading Center and CIARC will be masked. All study medications and placebo will be labelled identically to ensure adequate masking of study physicians and patients. An interim analysis will be performed once primary outcome data is available for one half of the patients (see **Section 9.4.6** of this protocol for details). The enrollment period is 24 months.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

Randomized control trials are known to be the least biased form of evidence. The factorial design of this trial is efficient, allowing us to perform two randomized trials simultaneously. It is very unlikely that there will be an interaction between surgery and ripasudil. All patients will receive standard of care treatment.

4.3 JUSTIFICATION FOR DOSE

Route of administration and dosage of topical ripasudil 0.4% are consistent with previous studies of the drug. Topical placebo will be administered in the same way as topical ripasudil to mask the patient, physician, and study staff. The topical placebo solution will have the same clear appearance as ripasudil to maintain masking.

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the final 24-month visit, as shown in the Schedule of Activities (SoA), **Section 1.3**.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate, an individual must meet all of the following criteria:

DETECT I

- Dysfunctional endothelium from Fuchs Endothelial Corneal Dystrophy (FECD) with guttata extending beyond 4.5 mm of the central cornea or severe edema without visualization of guttata
- Dysfunctional endothelium from Pseudophakic Corneal Edema (PCE) or Iridocorneal Endothelial Syndrome (ICE) or other primary endothelial dysfuction such as Posterior Polymorphous Corneal Dystrophy (PPMD)
- Dysfunctional endothelium from prior graft failure after PKP or EK
- Controlled uveitis (defined as quiet for > 3 months off topical steroids with or without systemic immunosuppression) or no uveitis
- Controlled glaucoma with topical medications and/or prior trabeculectomy or tube shunt without ongoing hypotony (IOP < 5 mmHg) or no glaucoma
- Good candidate for corneal transplantation for either DMEK or UT-DSAEK
- Willingness and ability to undergo corneal transplantation
- Willingness to consistently use study medications (i.e. ROCK-inhibitors)
- Willingness to participate in follow-up visits
- Age greater than 18 years

DETECT II

- Dysfunctional endothelium from FECD with few guttata extending beyond 4.5 mm
- Peripheral endothelial cell count >1500 cells/mm2
- Good surgical candidate for either procedure as determined by the surgeon
- Willingness and ability to undergo corneal transplantation
- Willingness to consistently use study medications (i.e. ROCK-inhibitors)
- Willingness to participate in follow-up visits
- Age greater than 18 years

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

DETECT I

- Aphakia, or anterior chamber IOL or scleral fixated IOL in study eye prior to or anticipated during EK
- Pre-operative central sub-epithelial or stromal scarring that the investigator believes is visually significant and could impact post-operative stromal clarity assessment

- Peripheral anterior synechiae (iris to angle) in the angle greater than a total of three clock hours
- Visually significant optic nerve (ok to have small visual field defects) or macular severe pathology
- Inability to comply with post-operative instructions (i.e. unable to position)
- Pregnancy
- Cataract surgery within the last 3 months
- Fellow eye visual acuity <20/200

DETECT II

- Other primary endothelial dysfunction such as PPMD
- Any prior intraocular surgery other than cataract surgery
- Cataract surgery within the last 3 months
- >3 clock hours of ANY anterior or posterior synechiae
- >1 quadrant of stromal corneal vascularization
- Visually significant optic nerve or macular pathology
- Fellow eye visual acuity <20/200
- Pregnancy
- Inability to comply with post-operative instructions (i.e. unable to position)
- Hypotony (Intraocular pressure <10mmHg)
- Peripheral anterior synechiae (iris to angle) in the angle greater than a total of three clock hours
- Aphakia, or anterior chamber IOL or scleral fixated IOL in study eye prior to or anticipated during EK
- Pre-operative central sub-epithelial or stromal scarring that the investigator believes is visually significant and could impact post-operative stromal clarity assessment

5.2.1 PREGNANCY EXCLUSION:

Women of childbearing age will have to take a urine test prior to enrolling in the study. Women of childbearing age will need to use birth control while taking Ripasudil. Women will check with their doctor about what kind of birth control methods to use and how long to use them. For this study women of childbearing age would only need to use birth control for the three months of taking Ripasudil.

Pregnancy is a contraindication to Ripasudil. If participant gets pregnant during treatment with ripasudil they will need to stop taking it. Participant will continue in study but will not be on ripasudil. We will follow the patient until the end of the study.

5.3 LIFESTYLE CONSIDERATIONS

None.

5.4 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

In this study, all patients who are randomized will be included in the primary analysis, regardless of whether or not they actually receive the assigned intervention. Randomization will occur just days before the participant's surgery to ensure that all randomized patients receive their assigned surgical intervention. Due to the nature of the intervention, patients may have weeks to months between enrollment in the study and randomization/surgery, leaving time for patients to drop out of the study before randomization. All required information will be collected from screen failures.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

Patients with diseased or damaged endothelium from Fuchs Endothelial Dystrophy or Pseudophakic Bullous Keratopathy will be recruited from the cornea subspecialty clinics of participating institutions for inclusion in the study. For eligible patients the study will be explained in English or the patient's native language using a translator for non-English speakers, in addition to the risks and benefits of participating in the study. The patients will be assured that they can receive all appropriate treatment whether or not they participate in the study. The side effects and drug interaction of all study drugs as well as endothelial keratoplasty are also explained in the consent forms. The study personnel (the study ophthalmologist with the help of the study coordinator) will make sure the patient understands risks, benefits and responsibilities of participating in this trial. Then the study personnel will obtain the patient signature on the consent form.

After the initial screening visit, this trial includes 6 study visits over 24 months. These study visits are:

1. Baseline (pre-operative)
2. Surgery
3. 3 Month Follow-Up (visit window: 2-4 months post-surgery)
4. 6 Month Follow-Up (visit window: 5-7 months post-surgery)
5. 12 Month Follow-Up (visit window: 10-14 months post-surgery)
6. 24 Month Follow-Up (visit window: 20-28 months post-surgery)

For both trials, patients will be randomized by the eye bank one week prior to surgery. At each visit, the study coordinator will work with the patient to schedule their next study visit. The study coordinator will give the patient written documentation of their upcoming visits, and will follow-up with a phone call as their appointments approach. Using this retention strategy, the DETECT-TES had 100% follow-up at 12 months. We are confident that these studies will have high retention as well.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Ultrathin Descemet stripping automated endothelial keratoplasty (UT-DSAEK)
Tissue grafts will be cut to the right thickness using a microkeratome prepared at the eye bank per standard eye bank protocol (about 60-90 microns). The grafts will be punched in the operating room to 7.0- 7.5 mm. A 4 mm corneal incision will be used, with Endoserter as the means of inserting the graft, an FDA approved device for this purpose. Donor tissue or media will be sent for fungal culture by the enrollment site.

Descemet membrane endothelial keratoplasty (DMEK)
Endothelial grafts will be pre-peeled at the eye bank, pre-punched to 7.0 - 7.5 mm and pre-loaded at the eye bank. The endothelium will be stained with trypan blue. The recipient descemets membrane will be stripped to 7.0 - 7.5 mm. A 2.4 mm corneal incision will be used and the graft will be inserted with a modified Jones tube injector, micro Jones tube injector, LEITR glass cannula, micro Stephens glass cannula, or Geuder cannula. The tap technique will be used to position the graft. Donor tissue or media will be sent for fungal culture by the enrollment site.

Descemet Stripping Only (DSO)
The pupil center will be marked in mesopic conditions to guide centration. A 4mm diameter imprint centered on the central mark will be used to guide the descemetorhexis. A reverse sinskey hook will be used to gently initiate a tear in Descemet's membrane and Gorovoy DSO forceps (or similar instrument) will be used to complete the descemetorrhexis

without disturbing the underlying stroma. The central endothelium will then be gently peeled and removed with forceps.

Ripasudil 0.4%

Ripasudil 0.4% is a selective rho-kinase (ROCK) inhibitor that has been shown to promote endothelial cell proliferation. Patients randomized to receive topical ripasudil will begin medication on post-op day 1. They will take this medication 4 times per day for 3 months.

Placebo

For participants randomized to placebo, they will receive topical placebo in place of topical ripasudil. The placebo will be sodium chloride 0.9%. Those randomized to placebo will receive the topical placebo on the same medication schedule described above for ripasudil.

6.1.2 DOSING AND ADMINISTRATION

The schedule of medications for the studies are below:

DETECT I Dosing schedule for medications

Medication	Day 1-8	Day 9 – 30	Month 2-3	Month 4	Month 5	Month 6- 12
Ripasudil 0.4% or placebo	4x/day	4x/day	4x/day			
Prednisolone	4x/day	4x/day	4x/day	3x/day	2x/day	1x/day
Ofloxacin	4x/day					

DETECT II Dosing schedule for medications

	Medication	Day 1 – 8	Day 9- 30	Month 2	Month 3	Month 4	Month 5	Month 6-24
DSO-R	Ripasudil 0.4%	4x/day	4x/day	4x/day	4x/day			
	Dexamethasone 0.1%	4x/day	4x/day					
	(Dexamethasone) placebo			4x/day	4x/day	3x/day	2x/day	1x/day
	Ofloxacin	4x/day						
DMEK	(Ripasudil) placebo	4x/day	4x/day	4x/day	4x/day			
	Dexamethasone 0.1%	4x/day	4x/day	4x/day	4x/day	3x/day	2x/day	1x/day
	Ofloxacin	4x/day						

Participants randomized to receive topical placebo instead of topical ROCK-inhibitors will receive the placebo on the same medication schedule as ripasudil outlined above. If at any time the masked treating physician deems it appropriate to change the patient's treatment, he/she may do so.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

We will obtain ripasudil 0.4% from Mimaki Family Pharmacy, Osaka, Japan, and Japan Health (bio-japan), Japan under IND #154317. Partner pharmacies will store and compound study medications: Rancho Park Compounding Pharmacy (for Stanford University, UC Davis, and OHSU); Penn Investigational Drug Services (for University of Pennsylvania); and Dartmouth Health Investigational Drug Services (for Dartmouth Hitchcock Medical Center). Medications and masked placebos will be purchased by the study and distributed to each enrolled patient individually by the partner pharmacies.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Transfer of topical study medication or placebo to an identical bottle by the compounding pharmacy will ensure patient and physician remain masked to treatment arm.

6.2.3 PRODUCT STORAGE AND STABILITY

All study medications should be stored at 15-25°C and should be protected from sunlight. Expiration dates will be clearly labeled. The shelf life for ripasudil will be 97 days. For study drug accountability see Appendix Study Drug Accountability Log. For study drug preparation, study drug storage, and study drug distribution see Appendix RP SOP 9.00 V 1.0, RP SOP 3.15 V 1.1, RP SOP 3.10 V 1.2, RP SOP 3.20 V 1.5.

6.2.4 PREPARATION

All preparation will be performed by the partner pharmacies and dispensed directly to the patient.

6.2.5 STUDY MEDICATION TRACKING

Once it has been determined that a patient is eligible for the study, their name and mailing address will be entered in REDCap, along with a Baseline Pharmacy Information Form which provides the partner pharmacy with information about allergies and current medications. All study drug tracking will occur within the REDCap database. Randomization into the study will trigger an email notification to the UCSF Study Coordinator, who will communicate with the respective partner pharmacy. The partner pharmacy will be informed of 1) which study arm the patient has been randomized to, and 2) the surgery date. The UCSF Study Coordinator will enter the date and time communication with pharmacy personnel into the REDCap database. All study medication will be dispensed directly to the patient from the corresponding partner pharmacy.

Several days prior to surgery, the pharmacy will prepare the study medication and log into REDCap to enter information about study drug shipment: date and time shipped; lot number; and FedEx tracking info. The site study coordinator or treating physician will contact the patient to ensure receipt of the study drug (Y/N; date received) and review instructions. This will also be logged into the REDcap database. At each study visit, the patient will be asked to bring all bottles of study related medication to review at follow up visits. At the 3- month study visit the study drug bottles will be collected by the study coordinator and any remaining medication will be pulled from the dropper with a syringe to measure the remaining volume. The study coordinator will log the volume in REDCap. Medication adherence questions will be included in the follow-up form at the 3-month study visit.

6.2.6 STUDY MEDICATION PREPARATION

Please see Rancho Park Standard Operating Procedures for full details. Ripasudil will be received and stored according to the conditions and temperature requirements set by the manufacturer. Ripasudil, a commercially available sterile

preparation, will be repackaged (6.5mL Ripasudil into a 10mL sterile dropper) using simple one step transfer of the product with no additional manipulation. Beyond Use Date (BUD) stability testing has been approved for 120 days.

The repackaged sterile investigational product will be packaged in an identical container as placebo which will be a commercially available artificial tear. Pharmacies will not repackage the artificial tears but will re-label the outside of the bottle following requirements of California Code, Business and Professions Code – BPC 4076 to match the repackaged Ripasudil so that they have identical appearances. The pharmacist will perform final quality control of the product before it is released to the shipping department.

6.2.7 STUDY MEDICATION DISTRIBUTION

Once the study drug has been prepared, it will be released to the shipping department where it will be shipped directly to the patient. Validated shipping methods per storage conditions and temperature requirements will be ensured during shipment. Medications and masked placebos will be provided to each patient individually from the partner pharmacy.

In addition to ripasudil/placebo, DETECT II patients will receive a second study medication. Initially all DETECT II patients will receive dexamethasone. The enrolling physician will prescribe 10mL of dexamethasone eye drops to begin post-operatively. After 30 days, partner pharmacies will provide the second study medication. Those randomized to DSO will switch to a placebo and those randomized to DMEK will continue on dexamethasone. Medications and masked placebos will be purchased by the study and distributed to each enrolled patient individually by the partner pharmacies.

6.2.8 CONCOMITANT MEDICATIONS

There are no known drug interactions with Ripasudil. There are no known side effects when taking Ripasudil with two or more medications concurrently.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

For both trials, patients will be randomized by the eye bank 1-3 weeks prior to surgery. In DETECT I, each study eye will be randomly assigned separately to the two treatment groups (DMEK/UT-DSAEK, ripasudil/placebo). Block randomization will be performed using a computer program (Statistical package R; Version 3.6; R Foundation for Statistical Computing, Vienna Austria) by the data coordinating center. Once an eye is enrolled in the study, the study coordinator will assign the study participant's eye an ID (alpha-numeric code).

Prior to surgery, the eye bank will assign the study participant corneal tissue. Once the corneal tissue has been assigned, the eye bank will look at the treatment assignment with regard to surgical treatment and prepare the tissue accordingly. For DETECT II, the tissue will be randomized and assigned to the patient if the patient is randomized to DMEK. The eye bank will also inform the surgeon of the study participant's randomization assignment with regard to type of surgery.

The eye bank will randomize tissue using the Microsoft Excel RANDBETWEEN formula. A new file will be created for each study eye and uploaded to REDCap. The eye bank will highlight the randomized tissue in the Excel file (the randomization runs every time the file is opened), so the original randomization is documented. If tissue does not pass post-processing evaluation, becomes ineligible for some other reason, or it does not meet study parameters, e.g. >90 microns, the randomization process will be repeated, the same Excel file can be used.

Once the study eye has been assigned a study participant ID and randomized to treatment group they will be included in the intention to treat analysis. (Note: See **MOP – Section 4.3** for full donor tissue procurement and assignment information).

All study participants will be masked to their intervention. The refractionist performing the BSCVA will also be masked. Due to the nature of the intervention, the surgeon and technician performing study visit endothelial cell counts and

other imaging will not be masked as to surgery type (but will be masked as to study medication); however, image graders at the Proctor Reading Center and CIARC will be masked. Eye bank personnel will not be given any identifying information.

6.4 STUDY INTERVENTION COMPLIANCE

We will have several measures in place to assess compliance with medications. We will have outpatient medication logs to track compliance and missed doses, to be filled out by the patient. At follow-up visits, patients will be asked to bring their used medication bottles.

6.5 CONCOMITANT THERAPY

The masked treating physician will be allowed add or change any therapy deemed necessary, including surgery. These changes will be recorded and reported.

6.5.1 RESCUE MEDICINE

The masked treating physician will be allowed to add or change any therapy deemed necessary, including surgery. These changes will be recorded and reported.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation from assigned study intervention does not mean discontinuation from the study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

The data to be collected at the time of study intervention discontinuation will include the following:

- Reason for study intervention discontinuation
- New prescribed treatment

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request. The reason for participant discontinuation or withdrawal from the study will be recorded on the final status eye form, which will be filled out at time of participant withdrawal (instead of at study completion) for patients withdrawing from the study. Subjects who sign the informed consent form and are randomized but do not receive the study intervention may not be replaced and will be included in the intent to treat analysis. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced and will be included in the primary analysis.

7.3 LOST TO FOLLOW-UP

All efforts will be made to prevent loss to follow-up during the trial. We will assess whether missing outcomes are differential by arm. If we find differential loss to follow-up, or if primary outcomes are missing in >15% of eyes, we will conduct a sensitivity analysis that uses inverse probability of censoring weights to correct for potential bias due to potentially informative censoring, following best practice guidelines for the prevention and treatment of missing outcomes in clinical trials.[46-48]

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

The following masked efficacy assessments will be performed:

- **Visual acuity assessment.** BSCVA/ETDRS/MRx will be used to assess visual acuity. Visual acuity will be assessed only by a trained, masked optometrist to ensure proper procedures are followed across sites. See **MOP – Section 4.4** for the visual acuity protocol.
- **Slit lamp examination.** Performed by the treating physician.
- **Imaging assessments**. Non-contact imaging will be performed with Pentacam topography and densitometry, clinical photography, and endothelial imaging. Historical tests/images within one month prior to enrollment will be acceptable if treating physician deems results representative of the current health of the eye. See **MOP – Section 4.5 and CIARC Appendix** for detailed descriptions of imaging procedures. Performed by trained study staff.
- **Visual function questionnaire (VFQ).** The National Eye Institute Visual Function Questionnaire (NEI-VFQ), a validated questionnaire to evaluate the effect of vision on the patient's quality of life, will be administered. Performed by trained study staff.

Please see the SoA in **Section 1.3** of this protocol for a detailed schedule of all study activities.

Visual acuity assessment, slit lamp examination, and Pentacam imaging will be performed during the screening process. These are standard of care procedures that the patient would receive even if they were not being screened for the study. The masked treating physician may use the results of these assessments to change the participant's treatment in any way they deem necessary.

8.2 SAFETY AND OTHER ASSESSMENTS

The following assessments will be administered to monitor patient safety:

- **Interval history from the patient** to ask about side effects, etc.
- **Visual acuity assessment.**
- **Intraocular pressure.**
- **Slit lamp exam to identify complications.** Ongoing AEs and SAEs will be followed until resolved.
- **Assessment of study intervention adherenc**e. See Study Intervention Compliance, **Section 6.4**.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) 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 participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

The following guidelines will be used to describe AE severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious."

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

8.3.3.3 EXPECTEDNESS

The treating physician will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the adverse event form. Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. AEs to anticipate are ocular in nature.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The masked treating physician will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit,

the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization. Each AE is to be classified by the Investigator as SERIOUS (SAE) or NONSERIOUS (NSAE). If a SAE occurs during the study, the investigator at the enrollment site must fill out the serious adverse event form and email it to the Medical Monitor at UCSF, within 24 hours of the occurrence of the SAE and or when it was discovered. The AEs are recorded as they are presented from patients and or are discovered.

8.3.5 ADVERSE EVENT REPORTING

All AEs will be recorded in both the follow-up form and in a specific adverse event form.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

All AEs should be followed until they are resolved or until a stable clinical endpoint is reached. Each AE is to be classified by the Investigator as SERIOUS (SAE) or NONSERIOUS (NSAE). If an SAE occurs, the investigator must complete the serious adverse event form in REDCap for review by the Medical Monitor at UCSF, Dr. Jeremy Keenan, within 24 hours of the occurrence of the SAE. The investigator must provide written follow-up reports until the SAE or clinically significant AE has resolved or until a stable clinical endpoint is reached. Notification of an SAE or clinically significant AE must also be submitted to the Lead PI/Clinical Coordinating Center for submission to WCG IRB and Data and Safety Monitoring Committee (DSMC) in accordance with its requirements. Quarterly Serious Adverse Events summary reports will be sent to the Data and Safety Monitoring Committee (DSMC). All AEs must be reported from the time that the subject provides informed consent through the last study visit.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into Research Electronic Data Capture (REDCap), a 21 CFR Part 11-compliant data capture system provided by the Data Coordinating Center at UCSF.

The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

AEs and SAEs will be discussed on an individual level with the masked treating physician. At the end of the study, all study participants will be given a report of the results in their native language.

8.3.8 EVENTS OF SPECIAL INTEREST

Not applicable.

8.3.9 REPORTING OF PREGNANCY

Women of childbearing age (ie women who have been told by their doctors that they need to take birth control to avoid pregnancy) will be required to take a urine test prior to enrolling in the study. Women of childbearing age will need to use birth control while taking Ripasudil. Women will check with their doctor about what kind of birth control methods to use and how long to use them. For this study women of childbearing age would only need to use birth control for the three months of taking Ripasudil.

There is no available data on the risk of taking Ripasudil during pregnancy; however, the systemic absorption from a topical eye drop is low. If a participant gets pregnant while taking the study drug, they will stop taking the study drug. Participants will continue in study and we will follow the patient until the end of the study- regardless of their ability to continue the study drug.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

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

8.4.2 UNANTICIPATED PROBLEM REPORTING

The site investigator will concurrently report unanticipated problems (UPs) to the Medical Monitor and the Lead PI/Clinical Coordinating Center for submission to WCG IRB. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- Any deaths related to the study intervention must be reported by the site investigator to the Medical Monitor and the Lead PI/Clinical Coordinating Center for submission to WCG IRB within 1 business day of the investigator becoming aware of the death.
- UPs will be reported to the Medical Monitor and the Lead PI/Clinical Coordinating Center for submission to WCG IRB within 5 days of the investigator becoming aware of the event.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 30 days of WCG IRB's receipt of the report of the problem from the lead investigator.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Participants will be notified of UPs in aggregate. When UPs affect an individual, they will be notified individually by the masked treating physician.

8.4.4 NONCOMPLIANCE

The site investigator will concurrently report instances of Non-Compliance to the Study Chair/Clinical Coordinating Center for submission to WCG IRB.

It is the responsibility of investigators and research staff to follow the written protocol approved by the IRB. When investigators and/or research staff do not follow the written protocol it will require reporting to the Study Chair/Clinical Coordinating Center and the Lead IRB if it meets one or more of the categories below:

1) Report within 5 days: Violations (failure to follow the protocol) that harmed, or **could have** harmed, participants/subjects or others or that indicate increased risk of harm;

2) Report within 5 days: Deviations committed to eliminate an immediate hazard for a participant/subject; or

3) Report at Continuing Review or Next Report: Other researcher failure (due to the action or inaction of the investigator or research staff) to follow the protocol that do not impact subject safety, participant's rights, or the completeness, accuracy and reliability of study data

Promptly reportable events include:

Report within one business day:

- **Unexpected Death:** Unexpected death of a locally enrolled participant/subject who has not withdrawn from the research when the death is unanticipated and possibly related to the research.
- **Breach of Confidentiality:** Any instance where records of research participants were improperly disclosed or shared.

Report within 5 business days:

- **Increase in Risk:** Information that indicates a new or increased risk, or a safety issue. For example: New information (e.g., an interim analysis, safety monitoring report, publication in the literature, sponsor summary report, or investigator finding) indicates an increase in the frequency or magnitude of a previously known risk, or uncovers a new risk. An adverse event that indicates a potential increase in risk or reduction in benefit (such as those that may prompt a change to the protocol or consent form).
- Withdrawal, restriction, or modification of a marketed approval of a drug, device, or biologic used in a research protocol.
- Protocol violation that harmed or could have harmed subjects or others or that indicates participants/subjects or others might be at increased risk of harm.
- Complaint of a participant/subject that indicates participants/subjects or others might be at increased risk of harm or at risk of a new harm.
- Any changes significantly affecting the conduct of the research.
- Harm: Any harm experienced by a participant/subject or other individual that, in the opinion of the investigator, is unexpected and at least probably related to the research procedures.
    - A harm is "unexpected" when its specificity or severity is inconsistent with risk information previously reviewed and approved by the IRB in terms of nature, severity, frequency, and characteristics of the study population.
    - A harm is "probably related" to the research procedures if, in the opinion of the investigator, the research procedures more likely than not caused the harm.
- Non-compliance: Allegation of investigator or study team noncompliance or finding of investigator or study team noncompliance.
- Audit: Audit, inspection, or inquiry by a federal agency (e.g. FDA Form 483).
- Report: Data safety monitoring reports from councils, committees, or boards charged with data and safety oversight activities; or other reports such as FDA non-approval letters.

- Researcher error: Failure to follow the protocol due to the action or inaction of the investigator or research staff.
- Confidentiality: Unauthorized disclosure of confidential information.
- Protocol Deviation: Change to the protocol taken without prior IRB review to eliminate an apparent immediate hazard to a subject.
- Incarceration: Incarceration of a subject in a study not approved by the IRB to involve prisoners.
- Complaint: Unresolved subject complaint.
- Suspension: Suspension or premature termination by the sponsor, investigator, institution or other IRB.
- Disqualification / Termination: Change in qualification of any member of the study team based on state medical board, hospital medical staff action, or other disqualification by professional board or employer.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Specific Aim 1: To determine whether DMEK has superior post-operative best spectacle corrected visual acuity (BSCVA) compared with UT-DSAEK or DSO with a similar safety profile.

a. *We hypothesize that the DMEK group will have improved visual acuity compared with UT-DSAEK at all time points.*
b. *We hypothesize that the DMEK group will have improved visual acuity compared with DSO at all time points.*

Specific Aim 2: To determine the benefit of adjuvant rho-kinase inhibitors on endothelial cell loss in patients who received UT-DSAEK and DMEK.

a. *We hypothesize that endothelial cell loss will be higher after DMEK than UT-DSAEK.*
b. *We hypothesize that endothelial cell loss will be lessened among those receiving ripasudil, after controlling for the pre-operative ECD in DMEK and UT-DSAEK.*

Primary Endpoints:

- Best spectacle-corrected visual acuity (BSCVA) at 12 months
- Endothelial cell loss at 12 months

Secondary Endpoints:

- Specific Aim 1: BSCVA at 3, 6, and 24 months
- Specific Aim 1: Complications over the course of the entire study period, including re-bubble rate, graft rejection, primary graft failure, and need for further surgery.
- Specific Aim 2: Endothelial cell density at 3, 6, and 24 months
- National Eye Institute Visual Function Questionnaire (VFQ) post-operatively at 3 months and 12 months as compared to baseline
- Adverse events over entire study period
- Corneal haze and higher-order aberrations as measured by Pentacam at baseline and post-operatively at 3, 6, 12, 24 months
- Graft thickness as measured by optical coherence tomography and pachymetry on donor tissue pre-operatively and post-operatively at 6 and 12 months
- Cost-effectiveness analysis at 24 months
- Manifest refraction at baseline and post-operatively at 3, 6, 12, 24 months

9.2 SAMPLE SIZE DETERMINATION

DETECT I

We will power the study for each comparison. For both primary analyses, we used a standard sample size formula based on an ANCOVA that conditions on a baseline outcome measure.[49] Given that endothelial cell density and visual acuity are different, non-correlated outcomes (*R*=0.08 in the DETECT pilot study) with different randomization, each will have its own alpha of 0.05. Specifically, we will be comparing:

I. With 80 patients per group (160 total), we estimate that we will have at least 90% power to detect a difference in logMAR of 0.11 at 12 months, assuming an outcome SD of 0.2, a 2-sided alpha of 0.05, and 10% loss-to-follow-up. Under the same assumptions but 80% power, the minimum detectable difference in logMAR is 0.09. We assumed a higher SD in the calculation compared with the SD estimated in the DETECT pilot (adj. SD=0.147) to allow for more complex eyes enrolled in the trial.

II. With 80 patients per group (160 total), we estimate that we will have at least 90% power to detect an 7% difference in ECL between enrollment and 12 months, assuming an outcome SD of 0.134 estimated from the DETECT pilot study, a 2-sided alpha of 0.05, and 10% loss-to-follow-up. If we assume a larger outcome SD for more complex eyes (SD=0.18), we will have at least 90% power to detect a 10% difference in ECL.

DETECT II

With 30 patients per group (60 total), we estimate that we will have at least 90% power to detect a difference in logMAR of 0.13, assuming an outcome SD of 0.147, a 2-sided alpha of 0.05, and 10% loss-to-follow-up. The SD was estimated from the DETECT pilot study and was adjusted for correlation between baseline and 12-month measurements (outcome SD = 0.163, correlation = 0.428, adjusted SD = 0.147). Under the same assumptions but 80% power, the minimum detectable difference is 0.11 logMAR.

9.3 POPULATIONS FOR ANALYSES

Analyses will be intention-to-treat (ITT); patients will be analyzed according to their randomized group, regardless of adherence to treatment.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Model fits will be examined using residual versus fitted value plots, and we will examine jackknife influence plots to assess potential high leverage observations. If such observations have an undue influence on the final result (in either direction), this will be reported. Inadequate model fit will be addressed using transformations, as well as higher order terms in the regression if needed. The number of such exploratory models will be reported. Such fits will be reported. Robust (Huber) regression may be reported as a methodological sensitivity analysis.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT

DETECT I

1. UT-DSAEK versus DMEK: The primary analysis will be 12-month BSCVA. We will use a mixed effects linear regression model to evaluate BSCVA measured at 12 months with fixed effects for surgical treatment arm (expressed as a binary indicator variable for UT-DSAEK versus DMEK), drug treatment arm (expressed as a binary indicator variable for Ripasudil versus Placebo) and baseline BSCVA. We will include random effects for clinic site and patient.

2. Adjuvant Ripasudil versus Placebo: The primary analysis will be 12-month ECL. We will use a mixed effects linear regression model to assess 12-month ECD with fixed effects for treatment arm (expressed as a binary indicator variable for Adjuvant Ripasudil versus placebo), surgery (expressed as a binary variable for UT-DSAEK versus DMEK), baseline ECD (as measured by the eye bank). We will include random effects for clinic site and patient.

We have no reason to expect that ripasudil would work better with one surgery versus another. A secondary analysis of the primary outcomes will also include a fixed effect interaction term for Drug x Surgery. This will enable us to estimate the effect of UT-DSAEK versus DMEK on BSCVA with- and without the adjuvant ripasudil, and to estimate the effect of Ripasudil on ECL separately by type of surgery. If there is a statistically significant interaction between treatments with respect to either outcome we will report stratified results, though we concede that the trial will have low power to detect an interaction unless the interaction effect is twice as large as the detectable effect sizes reported above in **Section 9.2**.

DETECT II

1. DMEK versus DSO: The primary analysis will be 12-month BSCVA. We will use a mixed effects linear regression model to evaluate BSCVA measured at 12 months with fixed effects for treatment arm (expressed as a binary indicator variable for DMEK versus DSO) and baseline BSCVA. We will include random effects for clinic site and patient.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINTS

We will use the definition of graft failure and displacement previously outlined by the Corneal Preservation Time Study (CPTS).[50] Graft failure will be defined as the occurrence of one of the following: 1) cornea which requires re-grafting for any reason; 2) cornea which remains cloudy [per the CPTS grading scale of recipient stroma clarity without clearing either if a) cloudy on the first postoperative day which does not clear within 8 weeks, or b) initially clear postoperatively but becomes and remains cloudy for 3 months.

CPTS Graft Failure Classification

1) Early: cloudy or equivocal recipient stroma on the first postoperative day that does not clear or requires a re-graft within 8 weeks and is associated with intra- and/or perioperative complications

2) Primary Donor: cloudy or equivocal recipient cornea on the first postoperative day that does not clear or requires a re-graft within 8 weeks in the absence of surgical complications*

3) Graft rejection: clouded recipient central stroma following an allograft reaction that was initially clear

4) Non-rejection: graft that had clear central recipient stroma on the first post-operative visit but becomes cloudy due to causes other than an immune event (e.g. surface failure, infection, glaucoma/hypotony, endothelial decompensation, interface irregularity/opacity, stromal scarring, blunt or penetrating trauma, or other causes

5) Refractive/visual: graft that requires re-grafting due to inadequate vision while the recipient central stroma is clear

* Differs from EBAA Medical Standards which defines primary donor failure as a re-graft that is performed > than 8 weeks after keratoplasty.[51]

CPTS Graft displacement Classification

1) Donor positioning: recorded as centered, decentered, total dislocation in anterior chamber, total dislocation in posterior chamber
2) Partially detached: yes, no
3) If partially detached: central, peripheral, both & % of detachment: 0 to less than 25%, 25 to less than 50%, 50 to less than 75%, 75 to 100%

Other Secondary Outcomes.
Within each secondary outcome group, we will correct for multiple comparisons across the two factorial treatments using a Holm-Bonferroni approach to control the family-wise error rate.

In DETECT I, we will examine ECL at 12 months as a key secondary outcome for the UT-DSAEK versus DMEK comparison and will examine BSCVA at 12 months as a key secondary outcome for the ripasudil versus placebo comparison.

Vision-Related Quality of Life. VFQ will be compared between groups using the National Eye Institute Vision-Function Questionnaire 25 (NEI-VFQ-25) at 3 and 12 months controlling for 1-day VFQ. This will be conducted using linear regression with baseline and assignment variables. We estimate the study will be sufficiently large to detect a difference in NEI-VFQ of 4 points, assuming a 2-sided alpha of 5%, 90% power, 110 patients per group, outcome standard deviation of 10.1 and correlation between baseline and follow-up of 0.51 (standard deviation and correlation estimated from NEI-VFQ measurements in the DETECT pilot).[52]

Endothelial cell morphology. We will use methods similar to the primary analysis for ECL to study the impact of ripasudil on the endothelial cell morphology by comparing the coefficient of variation (CV), variability in hexagonal cell shape (HEX) at the 3-month time point while still on ripasudil as well as at 6, 12 and 24 months after cessation of the study drug.

Pentacam Scheimpflug Tomography. Is a rotating scheimpflug camera, which provides 3 dimensional images of the cornea. In addition to topographic maps with keratometric readings of the anterior and posterior cornea, Pentacam reports on the total corneal power, corneal thickness maps, higher order aberrations and densitometry.

- *Higher order aberrations:* comparing the quantitative measure of irregular astigmatism, expressed in microns as the root mean square (RMS) of the Zernike polynomials across the pupil (approximately central 4 mm of the pupil) controlling for baseline measurements
- *Densitometry:* comparing a measure of corneal reflectance (i.e. scarring) in gray scale units controlling for baseline measurements
- Statistical analysis will be similar to that describe above, linear mixed effects regression using treatment assignment and baseline values as covariates, using the same template as we did for BSCVA.
- For a final supplementary analysis, we will also repeat the BSCVA analyses, but now including Pentacam densitometry at baseline as a predictor. We will report both the regression coefficient for baseline densitometry as a predictor, as well as the coefficient for treatment.

Cost-effectiveness analysis. A supplementary analysis will use individual-level cost outcomes as well as individual-level health outcomes. We propose to report standard cost-effectiveness acceptability curves based on bootstrap resampling at the individual level from both control and intervention subjects (for a statistical, clinical-trial based, cost-effectiveness analysis from a health care system perspective).[53] The outcome variable will be cost per line of vision gained.

Treatment allocation survey. We will perform a formal assessment of study participant masking as outlined in SAP 6.4.1

Adverse Events. All adverse events, including the number of re-bubble procedures, graft failure and graft rejection will be tabulated and reported. Statistical comparisons will be conducted using Fisher's exact test, but with the caution that failure to find evidence of a difference cannot be used to infer a lack of risk difference for rare outcomes such as primary graft failure since the study is not powered to examine these.

9.4.4 SAFETY ANALYSES

Total adverse events and total serious adverse events will be reported to the Data Safety Monitoring Committee (DSMC), tabulated by surgery and drug. Serious adverse event reporting to the DSMC and the medical monitor will be conducted within 24 hours. Individual adverse events will be tabulated by treatment allocation and included in DSMC reports, on a schedule to be determined by the empaneled DSMC prior to enrollment.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

Baseline statistics will be similar to those in DETECT-TES, which you can see below.[31] We will be looking at comparable baseline demographic characteristics, including age and sex; clinical characteristics, including diagnosis, BSCVA, central corneal thickness, manifest refraction, concurrent cataract surgery; and donor characteristics including age, sex, death to preservation time, death to surgery time, endothelial cell count pre- and post-processing, and central graft thickness in UT-DSAEK grafts.

Table 1. Baseline Characteristics

	UT-DSAEK (N=25)	DMEK (N=25)	P*
Patient characteristics			
Age (yrs), mean (range)	68 (51–95)	68 (61–81)	0.96
Female sex, N (%)	16 (64%)	13 (52%)	0.39
Diagnosis, N (%)			
Fuchs	24 (96%)	24 (96%)	1.00
PBK	1 (4%)	1 (4%)	
ETDRS BSCVA, mean ± SD			
logMAR	0.27±0.21	0.34±0.29	0.34
Approximate Snellen	20/40	20/40	
CCT (µm), mean ± SD	610±44†	608±52	0.93
Spherical equivalent (diopters), mean ± SD	−0.4±1.9	−0.8±2.2	0.53
Surgery, N (%)			
Pseudophakic EK	8 (32%)	7 (28%)	0.76
Triple EK	17 (68%)	18 (72%)	
Donor characteristics			
Age (yrs), mean (range)	64 (54–75)	62 (50–76)	0.38
Female sex, N (%)	8 (32%)	5 (20%)	0.33
Death to preservation (hrs), mean ± SD	9±4	9±3	0.80
Death to surgery (days), hrs ± SD	6±1	6±1	0.61
ECC preprocessing (cells/mm^2), mean ± SD	2792±210	2797±222	0.94
ECC postprocessing (cells/mm^2), mean ± SD	2796±238	2771±150	0.66
Central graft thickness, mean ± SD (range)	73±12 (37–88)	–	–

BSCVA = best spectacle-corrected visual acuity; CCT = central corneal thickness; DMEK = Descemet membrane endothelial keratoplasty; ECC = endothelial cell count; EK = endothelial keratoplasty; ETDRS = Early Treatment Diabetic Retinopathy Study; logMAR = logarithm of the minimum angle of resolution; PBK = pseudophakic bullous keratopathy; SD = standard deviation; UT-DSAEK = ultrathin Descemet stripping automated endothelial keratoplasty.
*t tests for continuous variables and chi-square tests for categoric variables.
†One missing value.

9.4.6 PLANNED INTERIM ANALYSES

A Data and Safety Monitoring Committee (DSMC) has been empaneled by the NEI. This committee consists of 5 individuals, and includes (a) cornea specialists, (b) an independent biostatistician, and (c) a bioethicist. The committee will meet in person at least once per year and will convene biannual teleconferences for progress reports. *Ad hoc*

meetings as needed may also be convened. All study protocols will be subject to review and approval by Institutional Review Boards.

The Data and Safety Monitoring Committee will meet to review the interim efficacy data for each trial when primary outcome data is available on one third of the study subjects. The DSMC will make one of the following recommendations:

- Continue the trial without modifications
- Continue the trial with study modifications
- Terminate enrollment or treatment in the trial because of safety concerns
- Terminate enrollment or treatment in the trial because of efficacy
- Terminate enrollment or treatment in the trial because of futility

Interim reports for the DSMC will be prepared by the Data Coordinating Center. These reports will include (a) recruitment overall, and by study site, (b) compliance, and (c) retention. The reports will also list study outcomes, and all adverse outcomes, including medication side effects, primary graft failure, graft rejection, and mortality. The DSMC will determine the database closure dates for each report in advance; archival copies of the (a) main database, and (b) study analysis files as they exist at the time of each report will be maintained. All reports will be sent using secure email to the members of the DSMC two weeks prior to each meeting.

The DSMC will make decisions with the benefit of pre-specified decision guidelines. These guidelines will be agreed upon at the initial meeting, and are expected to include (a) safety, (b) efficacy, (c) clinical importance, (d) effect of baseline covariates, or (e) validity.

We propose the same interim analysis approach for both DETECT I and DETECT II trials.

Benefits. An unmasked interim analysis will be conducted to determine whether or not sufficient evidence has accumulated to justify stopping one or both factorial treatments. The guidelines for efficacy will use group sequential boundaries for judging the statistical significance of the primary outcome measure. The trial will use the Lan and DeMets flexible alpha spending approach with a power function, with $\alpha^*(t^*) = \alpha\,(t^*)^{\Theta}$, where $\alpha = 3.561$ (chosen so that the two-sided *P*-value to stop the trial for efficacy is 0.001).[54] We propose a single interim analysis at the midpoint of the trial, i.e. when full outcome data are available for one half of the subjects. If one surgery method is clearly superior with respect to BSCVA, with no evidence for interaction with the ripasudil, then future patients will receive the better surgery method and still be randomized to ripasudil vs placebo. If ripasudil is clearly superior to placebo with respect to ECD, with no evidence for interaction by type of surgery, then future patients will receive ripasudil and would still be randomized to surgery type. At the interim analysis, tests for interaction will have very low power and so any decision to stop a single treatment early for benefit would be done in consultation with the DSMC.

Harm. Stopping one or both treatments for harm will be done at the judgment of the DSMC. Several endpoints will be examined, including (a) the best spectacle-corrected visual acuity at 12 months (b) adverse events, and especially (c) serious adverse events, including primary graft failure or mortality. While the analysis would consider maldistribution of predictive factors such as (a) age (b) visual acuity at presentation, and (c) diagnosis of PACE, it is recognized that ethical considerations require careful considerations of statistical tests as well as qualitative judgments in the light of experience.

Futility. Early discontinuation due to the unlikeliness of significant findings for both primary analyses conditional on interim results may be considered pending discussion with the DSMC.

9.4.7 SUB-GROUP ANALYSES

There is no reason to suspect that age, sex, race/ethnicity will influence our primary or secondary outcomes, particularly because we have not found these factors in prior endothelial keratoplasty studies including DETECT-TES.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Participant data will be listed by measure and time point.

9.4.9 EXPLORATORY ANALYSES

Tertiary outcomes

The effect of endothelial keratoplasty on refractive outcomes will be evaluated using the baseline refraction

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.5.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the study interventions, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention.

Prior to initiating the informed consent process, enrolling clinicians will assess patient decisional and cognitive ability through review of the following questions:

Does the patient understand the study?

Were the patient's questions answered?

Is the patient willing to participate?

Does the patient have the ability to make this decision?

If the answer to any question is "no," the patient will not be enrolled in the study. If the clinician can answer "yes" to all of the above questions, he/she will proceed with the informed consent process.

10.1.5.1 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, sponsor, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, WCG IRB, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, WCG IRB, and/or Food and Drug Administration (FDA).

10.1.3 CONFIDENTIALITY AND PRIVACY

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

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

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

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

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Data Coordinating Center. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by the Data Coordinating Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the Data Coordinating Center at UCSF.

Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local

level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at the Data Coordinating Center at UCSF. After the study is completed, the de-identified, archived data will be transmitted to and stored on the Research Electronic Data Capture system (REDCap).

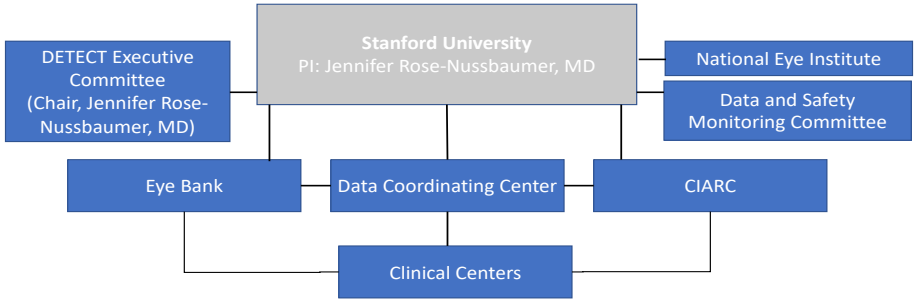
When the study is completed, access to study data will be provided through REDCap.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Study Chair, Director of the Clinical Coordinating Center	**Director of the Data Coordinating Center**	**Medical Monitor**
Jennifer Rose-Nussbaumer, MD Associate Professor	Tom Lietman, MD Director and Professor	Jeremy Keenan, MD MPH, Professor
Stanford University	Francis I. Proctor Foundation, University of California, San Francisco	Francis I. Proctor Foundation, University of California, San Francisco
2370 Watson Court Palo Alto CA 94303	490 Illinois St San Francisco, CA 94158	490 Illinois St San Francisco, CA 94158
650-722-7422	415-502-2662	415-476-6323
rosej@stanford.edu	Tom.Lietman@ucsf.edu	Jeremy.Keenan@ucsf.edu

Please see ***MOP Section 2*** for detailed descriptions of study investigators and staff.

10.1.5.1 STUDY ORGANIZATION



Please see **Overall Study Organization, Administration and Procedures** for additional detail.

EXECUTIVE COMMITTEE

An Executive Committee will oversee the study. The Committee will have representation from each of the Clinical Centers and Resource Centers (Eye Bank, Data Coordinating Center, and CIARC): Jennifer Rose-Nussbaumer, MD (Stanford), Winston Chamberlain, MD (OHSU), Charles Lin, MD (Stanford), Bennie Jeng, MD (University of Pennsylvania), Christopher Stoeger, MBA (Lions VisionGift), Ben Arnold, PhD (Proctor/UCSF), Tom Lietman, MD (Proctor/UCSF), Jonathan Lass, MD (CWRU/CIARC), Beth Ann Benetz (CWRU/CIARC), William Gensheimer (DHMC), and Jennifer Li, MD (UC Davis). This committee will act as the administrative and executive arm of the clinical trial and will meet monthly to provide oversight for the study and make decisions on day-to-day operational issues such as:

- Monitor study progress and data collection process
- Discuss any quality control issues that have arisen in the Clinical Coordinating Center (CCC) and Data Coordinating Center (DCC)
- Evaluate and adopt changes in study procedures as necessary
- Communicate with and implement recommendations from the Data and Safety Monitoring Committee
- Make executive decisions on the allocation of resources
- Establish policies on publications and authorship
- Approve and oversee ancillary studies

A kick-off meeting will be held prior to the start of the study to ensure standardization and harmonization across Clinical Centers and between Clinical Centers and Resource Centers. The committee will meet in person 1-2 times per year at annual ophthalmology meetings such as AAO and ASCRS.

10.1.5.2 CLINICAL AND RESOURCE CENTERS

Stanford University, UCSF, OHSU, University of Pennsylvania, UC Davis, Dartmouth-Hitchcock Medical Center (DHMC), and Case Western Reserve University (CWRU) will jointly execute this trial. All sites except for UCSF and CWRU will be responsible for recruitment and enrollment, intervention implementation, and follow-up visits. The Study Chair/Clinical Coordinating Center is based at Stanford University and will take the lead on the writing of study-related materials, writing manuscripts, and preparing presentations. The Data Coordinating Center at UCSF will take the lead on all data collection and analyses. The Cornea Image Analysis Reading Center (CIARC) at CWRU will determine ECD, morphometric parameters (coefficient of variation, % hexagonal cells) and ECL. Lions VisionGift in Portland, OR and Sierra Donor Services in Sacramento, CA will serve as the eye banks for this study.

Stanford University, Palo Alto, USA. The Byers Eye Institute at Stanford University is dedicated to combating blindness and preserving sight by delivering an effective, integrated collection of comprehensive vision care specialties. The Byers Eye Institute will serve as the Clinical Coordinating Center and oversee the resource centers at UCSF and CIARC at CWRU.

Jennifer Rose-Nussbaumer, MD is the study PI and will serve as the director of the CCC at Stanford in addition to enrolling patients. After completing her MD as UCSF, she trained in ophthalmology at Oregon Health & Sciences University. She completed a fellowship in Cornea at the UCSF and stayed on faculty until she transitioned to Stanford in 2021. Her clinical practice is focused on corneal transplantation including lamellar keratoplasty. As the Coordinating Center, Stanford will train all study personnel, maintain the most current protocol, consent documents and HIPAA authorization for reference. Stanford will ensure protection of all study-related data by using de-identified information over a secure server.

Charles C. Lin, MD is a board-certified ophthalmologist and cornea specialist. He received his AB in Environmental Science and Public Policy from Harvard University, graduating summa cum laude. He attended medical school at the University of California, San Francisco, where he received his M.D. with honors. Following an internship in Internal Medicine at Cedars-Sinai Hospital, he completed his ophthalmology residency at the University of California, San Francisco. He received subspecialty Cornea, External Disease, and Refractive Surgery training at the F.I. Proctor

Foundation and University of California, San Francisco. He spearheads the cornea transplant program at Stanford University and has launched cutting edge surgical procedures including ultra-thin DSAEK, DMEK and DALK at Stanford. Dr. Lin is the principal investigator for the DETECT-TES study at Stanford University.

University of California, San Francisco, USA. The Proctor Foundation, an organized research unit at the University of California, San Francisco, is led by its Director, Tom Lietman, Ruth Lee and Phillips Thygeson Distinguished Professor. The Foundation has a 72-year history of research in ocular infectious and inflammatory disease and runs one of the leading corneal fellowship training programs in the United States. The Foundation has 9 core research faculty, with a current portfolio of more than $50 million of grants from the NIH and the Bill and Melinda Gates Foundation (BMGF). The Foundation has an equal number of UCSF affiliate faculty, and multiple Associate Proctor Researchers around the world. The Coordinating Center at Proctor runs 10-15 NIH and BMGF clinical trials at any given time. Dr. Lietman, will be the Director of the Data Coordinating Center (DCC) for this study and serve on the DETECT Executive Committee as well. UCSF will be responsible for overseeing data collection and analysis, including image acquisition and transmission to the CIARC. The DCC will prepare weekly reports at each clinical center outlining enrollment progress at each site, study patients who are in window for study visits, complete study visits, and missing data. The DCC will also prepare open and closed reports for the Data Safety and Monitoring Committee.

Corneal Imaging Analysis Reading Center (CIARC), Case Western Reserve University, Cleveland, USA.

CIARC is the leading centralized reading center for endothelial image analysis in the United States, in part based on its experience over the past nearly 20 years with the NEI-supported Specular Microscopy Ancillary Study (SMAS) and the Cornea Preservation Time Study (CPTS), leading to numerous publications and providing key insights into the donor, recipient, operative, and postoperative factors that influence ECL following PKP (SMAS) and DSAEK (CPTS). The CIARC will perform dual grading and adjudication, when required, of each image using established methods for determination of endothelial cell density and morphometric parameters. The CIARC will perform eye bank (2) and clinical site (5) training, external calibration for specular microscopes as well as eye bank and clinical site technician certification (minimum of 2 technicians per site). The CIARC will analyze 3 eye bank screening images of the central donor endothelium and 3 images of the grafted central endothelium postoperatively at 6, 12 and 24 months for the 220 study eyes for a total of 2,640 images.

Jonathan Lass, MD is the Charles I Thomas Professor and Vice Chair for Academic Affairs in the CWRU Department of Ophthalmology and Visual Sciences and has been the Medical Director of the Cornea Image Analysis Reading Center (CIARC) since 1988. He served as Medical Director for the CIARC for the NEI-funded Specular Microscopy Ancillary Study (SMAS) and the Study Chair as well as Medical Director for the CIARC in the NEI-funded Cornea Preservation Time Study (CPTS). For the DETECT, as Medical Director of the CIARC, he will have the following responsibilities similar to his activities for the CPTS: 1) participate in the scientific plan for the DETECT; 2) oversee administrative functions outlined in the CIARC Operations Manual; 3) will interface with clinical sites and eye banks when direct physician contact is mandated; 4) review Scientific Director (CIARC) prepared reports to the Clinical Coordinating Center, Data Coordinating Center, DSMC, and the NEI; 5) meet on a weekly basis with the Scientific Director (CIARC) to review conduct of the study; 6) participate on regular Steering Committee calls of the DETECT administrative team; and 7) participate in data analysis, manuscript preparation, and presentations in conjunction with the to the Clinical Coordinating Center, Data Coordinating Center, DSMC, and the NEI.

Beth Ann Benetz, CRA, FOPS is Professor of Ophthalmology at the Case Western Reserve University School of Medicine and Scientific Director of the CIARC. For DETECT, as Scientific Director, she will have the following responsibilities similar to her activities for the CPTS: 1) participate in the scientific plan for the DETECT; 2) oversee the administrative functions and daily operations of the CIARC Resource Center as outlined in the CIARC Operations Manual; 3) ensure that DETECT eye bank and clinical site microscope images are properly calibrated for analysis, the eye bank and clinical site personnel are appropriately trained and certified to capture and transmit endothelial images for analysis and the Image Analysts are properly trained and certified to perform cell density and morphometric analyses; 4) review and, if necessary, act upon with additional individualized training, the quality reports monitoring the Readers' performance; 5) resolve outlier

review queries; meet on a weekly basis with the Medical Director (CIARC) to review conduct of the study; 6) participate on regular Steering Committee calls of the DETECT Steering Committee; and 7) participate in data analysis, manuscript preparation, and presentations in conjunction with the to the Clinical Coordinating Center, Data Coordinating Center, DSMC, and the NEI.

Oregon Health & Science University, Portland, USA. The Casey Eye Institute has state-of-the-art facilities and access to specialists in all areas of eye care. The Casey Eye Institute will serve as one of the sites for recruitment, treatment/intervention, and follow-up visits. Winston Chamberlain, MD, PhD works as a lead surgeon at Casey Eye Institute. Dr. Chamberlain received his BS in biology from California Institute of Technology. He holds a PhD in immunology and an MD from the University of Colorado Health Sciences Center. He is board certified in ophthalmology, and a member of the American Academy of Ophthalmology. His research interests include the outcomes of corneal transplant surgery with the femtosecond laser and DSAEK techniques, and inflammatory responses in the cornea. Dr. Chamberlain is the principal investigator for the DETECT-TES study at OHSU.

University of Pennsylvania, Philadelphia, USA. The Department of Ophthalmology is comprised of 17 sub-specialties, with over 59 faculty, 15 residents, 8 fellows, and 250 employees. The full-time faculty performs more than 130,000 outpatient visits and 2,400 surgeries each year. In addition to providing outstanding clinical care, the Department is a leader in ophthalmic research, education, and community outreach. Over the past five years (FY16-present), the Department ranked third in the nation in total funding from the National Eye Institute ($90.1M for 244 awards). The residency program ranks in the top ten nationwide in terms of research output, and the Alumni Publication Percentile (collective h-index of the residency program) rose to the top 2%. The Department provides high-quality care to underserved populations both internationally and locally.

Bennie H. Jeng is Professor and Chair of the Department of Ophthalmology at the University of Pennsylvania, School of Medicine. He earned his bachelor's degree *summa cum laude* from Washington University and his M.D. from the University of Pennsylvania School of Medicine. He then completed his ophthalmology residency and chief residency at the Cole Eye Institute of the Cleveland Clinic, which was followed by a fellowship in cornea and external diseases at the Francis I. Proctor Foundation/University of California San Francisco (UCSF) in 2003. He then returned back to the Cole Eye Institute to serve on faculty, during which time he was the recipient of a K-grant from the NIH and also earned a Master's degree in Clinical Investigation. He subsequently returned to Proctor/UCSF as an Associate Professor and then Full Professor, where he served as co-director of the UCSF cornea service, Director of the Proctor/UCSF Cornea Fellowship program, and as Chief of Ophthalmology at the San Francisco General Hospital. He was an R01-funded researcher at UCSF, and he served as the chair of the Department of Ophthalmology and Visual Sciences at the University of Maryland for nine years. He is currently an investigator on several clinical trials, including 2 that are NIH-funded.

University of California, Davis, Sacramento, USA. The University of California, Davis Eye Center is a leader in collaborative vision research and state-of-the-art, world-class eye care. The UC Davis Eye Center will serve as one of the sites for recruitment, treatment/intervention, and follow-up visits. Dr. Jennifer Li is a Professor and Director of Cornea and External Disease at the Department of Ophthalmology and Visual Sciences at the University of California, Davis. Dr. Li received her Bachelor of Science *cum laude* from Yale University. She subsequently obtained her medical degree and completed her residency in ophthalmology at the Baylor College of Medicine in Houston, Texas. This was followed by a fellowship in Cornea, External Disease and Refractive Surgery at UC Davis. She is board certified in ophthalmology and an active member of the American Academy of Ophthalmology, the Cornea Society, and the Eye Bank Association of America. Her academic and research interests focus on eye banking and corneal transplantation techniques and outcomes.

Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire, USA. As a major academic medical center in Northern New England, the Dartmouth-Hitchcock Medical Center (DHMC) Section of Ophthalmology is dedicated to providing comprehensive medical and surgical care of the eye in a robust multi-specialty practice. DHMC will serve as one of the sites for recruitment, treatment/intervention, and follow-up visits. William G. Gensheimer, MD is a board-certified

ophthalmologist and corneal specialist at the Section of Ophthalmology at Dartmouth-Hitchcock Medical Center and the Chief of Ophthalmology at the Veteran Affairs Medical Center in White River Junction, VT. Dr. Gensheimer received his Bachelor of Arts from Cornell University and his MD from the University of Rochester School of Medicine and Dentistry with distinction in research and community service. He completed his Ophthalmology residency at the Flaum Eye Institute at the University of Rochester and a fellowship in Cornea, External Disease, and Refractive Surgery at the University of Colorado. He then served in the U.S. Air Force as Chief of Cornea Service at the Warfighter Eye Center in Maryland until joining DHMC. He currently has multiple funded research projects in the areas of corneal transplant surgery, teleophthalmology, and ocular trauma.

Lions VisionGift, Portland, Oregon, USA
Lions VisionGift (LVG) is a nonprofit 501(c)(3) organization dedicated to the mission: to honor donors by advancing sight for all humankind. Since its inception in 1975, over 60,000 people have received the gift of sight from tissue recovered, screened, and processed by LVG. Additionally, thousands of gifts have been distributed for ocular research and surgical training. LVG has locations in both Portland, Oregon and Boston, Massachusetts. Locations are FDA registered and Eye Bank Association of America (EBAA) Accredited. LVG has a long history of supporting research studies including those aimed at providing novel tissue products for selective transplantation as well as participation in large multicenter clinical trials. To support these studies, both locations have state-of-the-art clean room facilities for corneal tissue processing for DMEK and DSAEK. LVG's laboratories have the required imaging equipment to ensure all quality and study parameters are met.

Jameson Clover, CEBT is the Vice President of Surgical Services at LVG. She has extensive experience from DETECT_TES for ensuring compliance with strict randomization and tissue parameters. She has many years of eye banking experience with service on numerous national EBAA committees as well as co-authoring a number of eye banking and corneal transplant related journal articles.

Chris Stoeger, MBA, CEBT, CTBS is the CEO of Lions VisionGift. Mr. Stoeger has over a quarter century of eye banking expertise at the local and national level. He has co-authored over 35 peer reviewed publications focused on corneal transplant safety, tissue evaluation, and novel tissue processing techniques. He has served on the Eye Bank Advisory Committee of the NIH-funded Cornea Preservation Time Study.

Sierra Donor Services, Sacramento, California, USA
Sierra Donor Services Eye Bank is a nonprofit donor network coordinating eye recovery, processing, and distribution in the states of Tennessee, California, and Nevada. SDSEB is part of the *DCI Donor Services, Inc.* family of organ and tissue procurement organizations that includes, *Tennessee Donor Services*, *Sierra Donor Services*, *New Mexico Donor Services*, and *DCI Donor Services Tissue Bank*.

10.1.6 SAFETY OVERSIGHT

A Data and Safety Monitoring Committee (DSMC) has been empaneled by the NEI. This committee consists of 5 individuals, and includes (a) cornea specialists, (b) an independent biostatistician, (c) a bioethicist, and (d) representation from all participating sites. The committee will meet in person at least once per year, and will convene biannual teleconferences for progress reports. *Ad hoc* meetings as needed may also be convened. All study protocols will be subject to review and approval by WCG IRB as the single IRB of record. Please see **Section 9.4.6** of this protocol for a detailed description of our planned interim analysis.

10.1.7 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

Clinical monitoring will be conducted by the Clinical Coordinating Center (CCC) at Stanford. A two-person team will make site visits to all enrollment sites three times per year to monitor study activities. At each visit, the CCC will check the quality of photographs, sit in refractions to ensure the proper refraction protocol is followed, and visit the clinic. During each visit the CCC will conduct a complete chart review of all patient charts to ensure data is being recorded in a complete fashion. The CCC will conduct regular weekly off-site reviews of data uploaded to REDCap by enrollment sites and data entered at UCSF to ensure 100% data verification.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

Each clinical site will perform internal quality management of study conduct, data collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.5.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into Research Electronic Data Capture (REDCap), a 21 CFR Part 11-compliant data capture system provided by the Data Coordinating Center at UCSF. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

REDCap is a secure and HIPAA-compliant electronic data capture tool. REDCap supports the use of data entry forms attached to a survey to enable research teams to securely collect data on survey respondents on forms that are tied to individual survey respondents. All incoming data gets intentionally filtered, sanitized, and escaped. This includes all data submitted in an HTTP Post request and all query string data found in every URL while accessing REDCap, among other modes through which user-defined data gets submitted in the application.

10.1.5.1 STUDY RECORDS RETENTION

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or MOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation, or within 7 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to National Eye Institute Program Official and the Lead PI/Clinical Coordinating Center at Stanford. Protocol deviations must be sent to WCG IRB within 5 business days of the investigator becoming aware of the event. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.1.11 PUBLICATION AND DATA SHARING POLICY

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results from this trial will be submitted and published on ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Consistent with the collaborative nature of the proposed research, the PI anticipates sharing all data generated by the study with collaborators. Analytic datasets that will be developed through the project will comply with the NIH Data Sharing Policy. The analytical datasets from this project will include patient-level data generated from the study visits.

This study will adhere to the National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. External investigators can contact the study PIs to initiate a request for study data to support new study proposals or manuscripts. Approval of such requests and initiation of collaborations will consider the following criteria:

1. The proposed project must be of high scientific merit.
2. The proposed project must be consistent with the overall goals and objectives of the parent study.
3. The proposed ancillary project must meet certain participant burden criteria (for any new primary data collection involving subjects), including:
    a. Acceptable to the subjects (e.g., risks, time, discomfort, privacy); and,
    b. Not hinder or disrupt clinical care provided by study sites
4. The proposed project's investigators must plan for adequate resources to effectively complete the project, including:
    a. Sufficient budget to cover costs of personnel and supplies; and
    b. Staff possessing the requisite expertise to meet the objectives of the project.
5. The proposed project should document any involvement of parent study investigators as part of the research team.

Approved requests for data will follow data sharing agreements that Stanford has with NIH. Data will be de-identified prior to release for sharing. However, there remains the possibility of deductive disclosure of subjects with unusual

characteristics and disclosure of Stanford proprietary information. Thus, researchers who seek access to individual level data will be required to sign a data sharing agreement prior to release for sharing. The agreement provides for: (1) a commitment to using the data for research purposes only and not to identify any individual participants or to disclose proprietary information; (2) a commitment to securing the data using appropriate computer technology; (3) a commitment to destroying or returning the data after analyses are completed; and (4) a commitment to meet any requirements that might be stipulated by WCG IRB.

10.1.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Eye Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10.2 ADDITIONAL CONSIDERATIONS

None

10.3 ABBREVIATIONS

AE	Adverse Event
BMGF	The Bill and Melinda Gates Foundation
BSCVA	Best Spectacle-Corrected Visual Acuity
CFR	Code of Federal Regulations
CCC	Clinical Coordinating Center
CDS	Corneal Donor Study
CIARC	Corneal Imaging Analysis Reading Center
CMP	Clinical Monitoring Plan
CONSORT	Consolidated Standards of Reporting Trials
CPTS	Corneal Preservation Time Study
CWRU	Case Western Reserve University
DAC	Data Analysis Committee
DCC	Data Coordinating Center
DETECT	Descemet Endothelial Thickness Comparison Trial
DETECT-TCS	Descemet Endothelial Thickness Comparison Trial – Therapeutic Confirmatory Study
DETECT-TES	Descemet Endothelial Thickness Comparison Trial – Therapeutic Exploratory Study
DHMC	Dartmouth-Hitchcock Medical Center
DMEK	Descemet Membrane Endothelial Keratoplasty
DOR	Division of Research
DSAEK	Descemet Stripping Automated Endothelial Keratoplasty
DSMC	Data and Safety Monitoring Committee
DSO	Descemet Stripping Only
ECD	Endothelial Cell Density
ECL	Endothelial Cell Loss
EC	Ethics Committee
eCRF	Electronic Case Report Forms
ETDRS	Early Treatment Diabetic Retinopathy Study
FDA	Food and Drug Administration

FECD	Fuchs Endothelial Corneal Dystrophy
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HOA	Higher Order Abberations
ICE	Iridocorneal Endothelial Syndrome
ICH	International Conference on Harmonisation
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IOP	Intraocular Pressure
IRB	Institutional Review Board
ITT	Intention-To-Treat
LOCF	Last Observation Carried Forward
logMAR	Logarithm of the Minimum Angle
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Operations and Procedures
MRx	Manifest Refraction
MUTT	Mycotic Ulcer Treatment Trial
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NEI	National Eye Institute
NEI-VFQ	National Eye Institute Visual Function Questionnaire
NIH	National Institutes of Health
NSAE	Non-Serious Adverse Event
OHRP	Office for Human Research Protections
OHSU	Oregon Health & Science University
PACE	Pseudophakic/Aphakic Corneal Edema
PCE	Pseudophakic Corneal Edema
PRC	Proctor Reading Center
PI	Principal Investigator
PKP	Penetrating Keratoplasty
QA	Quality Assurance
QC	Quality Control
REDCap	Research Electronic Data Capture
RCT	Randomized Control Trial
RMS	Root Mean Square
ROCK	Rho-Kinase Inhibitor
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCUT	Steroids for Corneal Ulcer Treatment Trial
SMAS	Specular Microscopy Ancillary Study
SoA	Schedule of Activities
SOP	Standard Operating Procedure
UCSF	University of California, San Francisco
UP	Unanticipated Problem
US	United States
UT	Ultrathin
UT-DSAEK	Ultrathin Descemet Stripping Automated Endothelial Keratoplasty
VFQ	Visual Function Questionnaire

10.4 PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Version	Date	Description of Change	Brief Rationale
2.0	February 2022	Addition of Dartmouth-Hitchcock Medical Center and UC Davis as enrollment centers	
		Change of IRB from UCSF to WCG	
		Inclusion of Lions VisionGift and Sierra Donor Services as Eye Banks	
		Clarification of Eye Bank procedures	
3.0	August 2022	Removal Kaiser Permanente, change of institution for Dr. Bennie Jeng from University of Maryland to University of Pennsylvania	
		AS-OCT only done at baseline, 6 and 12 month visits	
		Revise Schedule of Activities for Screening/Pre-Enrollment Visit	
		All patients are randomized one week prior to surgery	
		Add dosing schedule for DETECT II medication	
4.0	October 2022	Changed endothelial graft size to include a range from 7.0 – 7.5mm	
		Changed dexamethasone to prednisolone for DETECT II	
		Addition of Penn Investigational Drug Services and Dartmouth-Hitchcock Health Investigational Drug Services as partner pharmacies	

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