Diabetes Endothelial Keratoplasty Study (DEKS): Impact of Diabetes on Corneal Transplant Success and Endothelial Cell Loss

Version Number: v.1.2

26 January 2023

Funded by: National Eye Institute

PROTOCOL VERSION HISTORY

VERSION NUMBER	AUTHOR(S)	APPROVER	EFFECTIVE DATE	REVISION DESCRIPTION
1.0	J. Lass M. Price L. Szczotka-Flynn C. Bauza	J. Lass	7/15/21	Original protocol
1.1	J. Lass M. Price L. Szczotka-Flynn C. Bauza	J. Lass	10/21/21	Edits to inclusion and exclusion criteria, enrollment of second eye, donor diabetes definitions, statistical analyses, and source documentation and congruence in adverse event definitions to align with the JCHR global adverse event application
1.2	J. Lass M. Price L. Szczotka-Flynn C. Bauza	J. Lass	2/3/23	Clarified donor diabetes determination used for randomization and donor tissue selection is based on eye bank assessment, not reclassification determination after post-mortem HbA1c value is returned

KEY ROLES

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AE	Adverse Event
AGEs	Advanced glycation endproducts
ARDL	Advanced Research and Diagnostics Laboratory
BMI	Body Mass Index
CC	Coordinating Center
CCTS	Collaborative Corneal Transplantation Studies
CDC	Centers for Disease Control and Prevention
CDS	Cornea Donor Study
CFR	Code of Federal Regulations
CIARC	Cornea Image Analysis Reading Center
CRF	Case Report Form
CRFA	Cornea Research Foundation of America
CPTS	Cornea Preservation Time Study
CWRU	Case Western Reserve University
DEKS	Diabetes Endothelial Keratoplasty Study
DMAC	Data Management and Analysis Center
DMEK	Descemet membrane endothelial keratoplasty
DRAI	Donor Risk Assessment Interview
DRCR.net	Diabetic Retinopathy Clinical Research Network
DSAEK	Descemet stripping automated endothelial keratoplasty
DSMC	Data Safety and Monitoring Committee
EBAA	Eye Bank Association of America
ECD	Endothelial cell density
ECL	Endothelial cell loss
EK	Endothelial keratoplasty
FDA	Food and Drug Administration
FECD	Fuchs endothelial corneal dystrophy
GCP	Good Clinical Practice
HbA1c	Hemoglobin A1c
Hg	Mercury
HIPAA	Health Insurance Portability and Accountability Act
ICH	International Council for Harmonisation
IOL	Intraocular lens
IOP	Intraocular pressure
IRB	Institutional Review Board

ABBREVIATION	DEFINITION
JCHR	Jaeb Center for Health Research
LASIK	Laser assisted in situ keratomileusis
MedDRA	Medical Dictionary for Regulatory Activities
MIGS	Minimally invasive glaucoma surgery
NIH	National Institutes of Health
OARRS	Online Adverse Reaction Reporting System
QA	Quality assurance
QC	Quality control
RBM	Risk-based monitoring
Rho-kinase	Rho-associated protein kinase inhibitor or ROCK inhibitor
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
UHEI	University Hospitals Eye Institute
US	United States
VEGF	Vascular endothelial growth factor
YAG	Yttrium aluminum garnet

Signature Page

Diabetes Endothelial Keratoplasty Study (DEKS): Impact of Diabetes on Corneal Transplant Success and Endothelial Cell Loss

Protocol Identifying Number: DEKS

Version Number: v.1.2

26 January 2023

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SITE PRINCIPAL INVESTIGATOR STATEMENT OF COMPLIANCE

Protocol Title: Diabetes Endothelial Keratoplasty Study: Impact of Diabetes on Corneal Transplant Success and Endothelial Cell Loss

Protocol Version/Date: 1.2/January 26, 2023

I have read the protocol specified above. In my formal capacity as a Site Principal Investigator, my duties include ensuring the safety of the study participants enrolled under my supervision and providing the DEKS Coordinating Center at Case Western Reserve University, which serves as the Coordinating Center for the protocol, and the Jaeb Center for Health Research which serves as the Data Management and Analysis Center, with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site.

This trial will be carried out in accordance with ICH E6 Good Clinical Practice (GCP) and as required by the following (use applicable regulations depending on study location and sponsor requirements; examples follow): 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).

As the Principal Investigator, I will assure that no deviation from, or changes to the protocol will take place knowingly without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), or other approved Ethics Committee, except where necessary to eliminate an immediate hazard(s) to the trial participants.

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Participants Protection Training and GCP Training. Further, I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Investigator's Signature _____ Date: _____ / _____ / _____
dd mmm yyyy

Investigator's Name: _____

Site Name/Number: _____

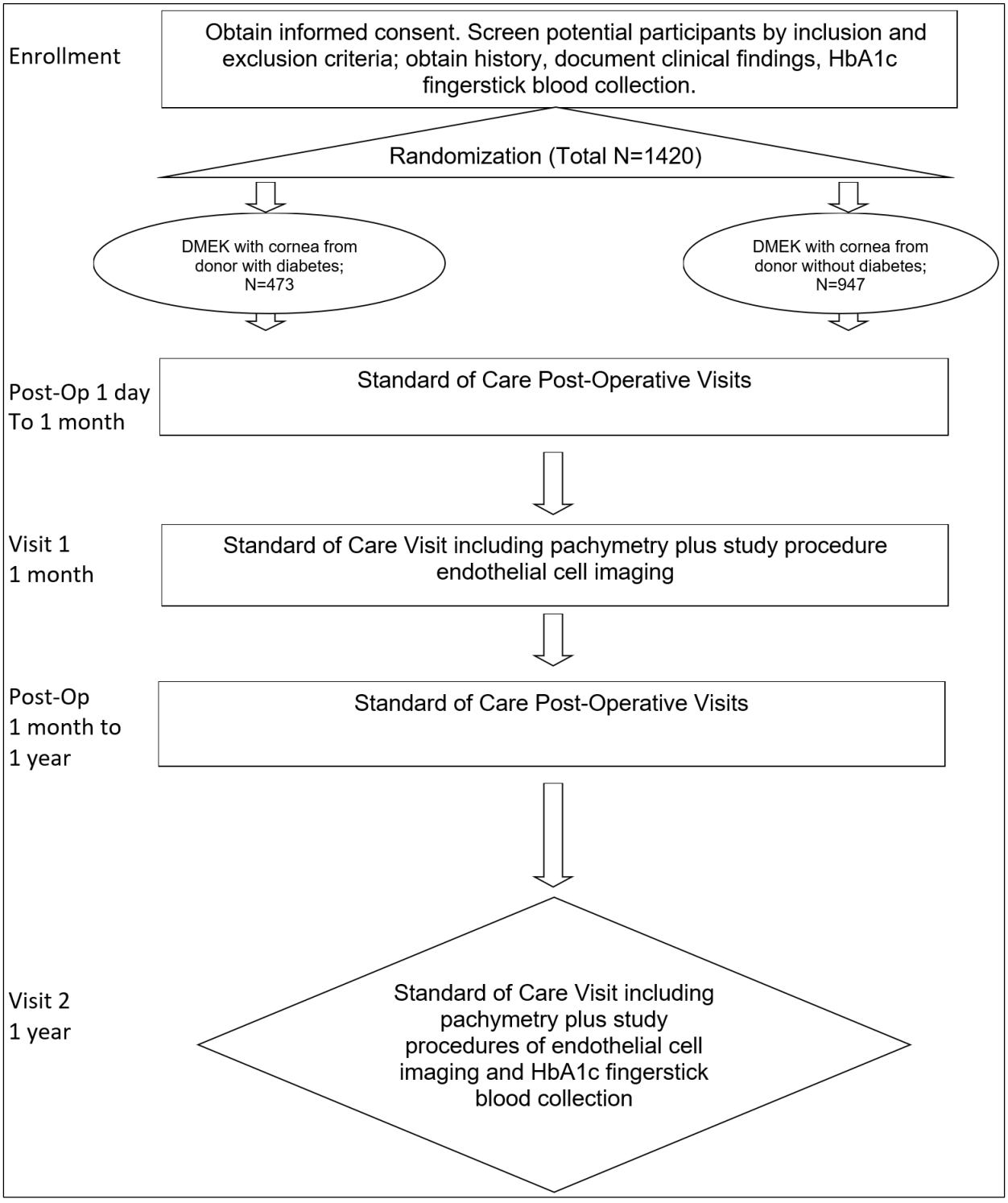
PROTOCOL SUMMARY

Title	Diabetes Endothelial Keratoplasty Study: Impact of Diabetes on Corneal Transplant Success and Endothelial Cell Loss (DEKS)
Précis	The association of diabetes in the cornea donor with transplant success and loss of endothelial cells one year following Descemet membrane endothelial keratoplasty (DMEK) will be evaluated in a double-masked multi-center trial in which study eyes will be assigned to receive either a cornea from a donor without diabetes or a cornea from a donor with diabetes.
Objectives	• To determine if the 1-year graft success rate following DMEK performed with corneas from donors without diabetes is superior to the graft success rate with cornea donors with diabetes. • To determine if the 1-year central endothelial cell loss (ECL) following DMEK performed with corneas from donors without diabetes is superior to the central ECL when corneas from donors with diabetes are used. • To explore the relationship of severity of diabetes in the donor, as measured by eye bank-determined diabetes risk categorization scores, post-mortem hemoglobin A1c (HbA1c), and skin advanced glycation endproducts (AGE) and oxidation markers, with 1-year graft outcomes (i.e., graft success and ECL) following DMEK in corneas from donors with diabetes.
Study Design	Prospective, randomized, double-masked (participant and clinical site) clinical trial
Number of Sites	~30 clinical sites and ~16 eye banks in the United States
Endpoint	**Primary Efficacy Outcome:** Graft failure within 1 year of surgery **Key Secondary Efficacy Outcomes:** Change in central endothelial cell density (ECD) at 1 year after surgery **Key Safety Outcomes:** Endophthalmitis, microbial keratitis (bacterial, fungal, parasitic) within 3 months of DMEK, and other unexpected ocular serious adverse events (SAEs). **Other Key Analyses:** Develop separate models for predicting 1) risk of graft failure and 2) ECL based on severity of diabetes in the donor as measured by eye bank-determined diabetes risk categorization scores, post-mortem HbA1c, and skin advanced glycation endproducts (AGE) and oxidation markers.
Population	**Participant Inclusion Criteria:** 1) Age range 30- < 91 years with minimum life expectancy of at least 1 year 2) Willingness to return to study site for follow up at 1 month and 1 year 3) Fluent in English or Spanish 4) Willingness to have fingerstick blood sample collected to determine HbA1c level at entry and at 1 year. The participant must agree to have their primary care provider contacted (or an appropriate referral provided) if they were not known to have diabetes and the HbA1c suggests they may have diabetes. Similarly, if already known to have diabetes and the HbA1c is high, the participant must agree to have their primary care provider contacted or an appropriate referral provided. **Study Eye Eligibility Criteria** **<u>Inclusion:</u>** 1. At least one eye clinically recommended for DMEK that is able to be scheduled for DMEK between 5 to 90 days after enrollment 2. If second eye is enrolled, must be scheduled for DMEK between 7 days and 6 months after DMEK on the first eye. 3. Presence of a condition related to endothelial dysfunction which will be treated by DMEK • Eligible indications for DMEK include: a. Presence of Fuchs endothelial corneal dystrophy (FECD) meeting at least one of the following:

	◆ Phakic FECD with or without cataract • Triple procedure including DMEK for FECD, cataract extraction and posterior chamber intraocular lens implantation (IOL) is allowed ◆ Pseudophakic FECD with posterior capsule supported, sulcus supported, or scleral-fixated posterior chamber IOL b. Pseudophakic corneal edema with posterior capsule supported, sulcus supported, or scleral-fixated posterior chamber IOL without FECD c. Failed Descemet stripping automated endothelial keratoplasty (DSAEK) or DMEK originally performed for the same indications above without current exclusionary criteria, as described below **Exclusion Criteria:** 1. Pregnant or planning to become pregnant prior to the DMEK study surgery, based on verbal report. 2. Lack cognitive capacity such that consent could not be provided. 3. Presence of a condition that has a high probability for failure (e.g., failed penetrating keratoplasty, uncontrolled uveitis) 4. Stromal vascularization that will impede assessment of recipient stroma clarity 5. Other primary endothelial dysfunction conditions including posterior polymorphous corneal dystrophy and congenital hereditary corneal dystrophy. 6. Indication for surgery that is not suitable for DMEK (e.g, keratoconus, stromal dystrophies and scars) 7. Aphakic corneal edema with or without FECD 8. Anterior chamber IOL in study eye prior to DMEK or planned placement of anterior chamber IOL during DMEK 9. Presence of vitreous in the anterior chamber 10. Planned IOL exchange of an anterior chamber IOL with a posterior chamber IOL in study eye at time of study DMEK 11. Pre-operative central sub-epithelial or stromal scarring that could impact post-operative recipient stromal clarity assessment 12. Presence of anterior synechiae 13. Peripheral anterior synechiae in the angle greater than a total of three clock hours 14. Uncontrolled glaucoma with or without prior filtering surgery, tube shunt placement, or MIGS. *Uncontrolled glaucoma is defined as intraocular pressure > 25mm Hg.* 15. Controlled glaucoma with prior tube shunt placement for glaucoma (*controlled glaucoma with MIGS or trabeculectomy is allowed*) 16. Fellow eye visual acuity < 20/200 due to an ocular condition other than a cornea disease that would be a candidate for DMEK 17. IOP <8 mmHg 18. Topical Rho kinase inhibitor, including Rhopressa, used within 1 month prior to study entry and anticipated during the course of the study 19. Fellow eye enrolled in the DEKS that has met study-criteria for graft failure.
Sample Size	Up to 1420 completed surgeries (~947 donors without diabetes and ~473 donors with diabetes) with a goal of 1278 completing the 1-year follow-up
Treatment Groups	Study eyes will be assigned to receive either a cornea from a donor without diabetes or a cornea from a donor with diabetes. Assignment selection will use a minimization procedure which will prioritize assignments in a 2:1 distribution of tissue from donors without known diabetes to tissue from donors with known diabetes as determined by the eye bank. Participants with 2 study eyes cannot receive a cornea from a donor with known diabetes in both eyes. The minimization procedure will attempt to achieve balance on several factors, including the surgeon and whether the recipient had diabetes versus not.
Participant Duration	12 months follow-up after DMEK

Protocol Overview/Synopsis	Each of the ~ 30 clinical sites is expected to recruit 40-50 cases over 30 months. Eligibility is assessed during a routine examination by an investigator; there are no examination procedures required to assess participant eligibility other than those that are part of standard patient care. Eye eligibility is determined at the time DMEK is scheduled, meaning that the eligibility of the second eye will be assessed at the time surgery on the second eye is being scheduled. Surgery on the second eye must be performed between 7 days and 6 months (182 days) after DMEK on the first eye. DMEK will be performed according to the investigator's usual routine. Aspects of the surgical technique and procedure will be tracked, but not standardized. Data to be collected will include incision size, insertion method, air or gas usage, other procedures, operative complications, and other key operative details. Donor diabetes classification will be made initially at the time of tissue assignment. Historical records will be the principal method to determine diabetes status. In parallel, postmortem donor HbA1c will be collected and analyzed at the Advanced Research and Diagnostics Laboratory (ARDL) at the University of Minnesota. There may be a small percentage of donors classified by the eye bank as non-diabetic who will be reclassified as diabetic due to an elevated HbA1c. Therefore, the final classification of donor diabetes status for analysis will include: 1) Diabetic Donor = known diabetes history or postmortem HbA1c $\geq$ 6.5% or 2) Non-Diabetic Donor= no known history and postmortem HbA1c < 6.5% (or HbA1c is missing or unanalyzable). Surgeons and participants will be masked to the donor parameters, except the FDA-approved hypothermic (2-8 degrees C) storage solution being employed. Postoperative management will be at the discretion of the surgeon based on his/her usual practices. Key aspects of pharmacologic management will be collected on the data forms. Study eyes of participants will be examined at a baseline/enrollment visit, at the time of the scheduled DMEK procedure, and at post-operative study visits at 1 month and 12 months after surgery in addition to post-op visits according to the investigators' usual routine. At the 1-month visit all significant events during this first postoperative period will be recorded (e.g. rebubbling, repositioning, elevation of IOP > 25 mmHg). At the 12-month visit, all significant events from one month to one year will be recorded (e.g. secondary procedures, graft rejection episodes, elevation of IOP > 25 mmHg). Procedures at each visit will follow the surgeon's standard of care including dilated fundus examinations to check and grade diabetic retinopathy (at baseline and 12 months), pachymetry (at 1 month and 12 months), and standardized measurements of recipient stroma clarity. Central endothelial cell imaging will be obtained at 1 month and 12 months. All participants, known diabetic and nondiabetic, will undergo a determination of diabetes status by having a fingerstick blood sample collection at baseline and at 12 months, with specimens sent to the ARDL of the University of Minnesota for HbA1c analysis. At baseline and the 12-month visit, a medical and ocular history will be obtained to track potential micro- and macrovascular complications related to diabetes.

SCHEMATIC OF STUDY DESIGN



Notes: HbA1c assessment at baseline may be obtained between enrollment and 1 month visits (inclusive); a minimization procedure is used for 'randomization'.

SCHEDULE OF STUDY VISITS AND PROCEDURES

Table 1. Schedule of Study Visits and Procedures

	RESEARCH (R)/ STANDARD CARE (SC)	ENROLLMENT	1M +/- 10 DAYS	12M -30, + 100 DAYS
Visit (V)		V	V	V
Comment		Screen/Enroll		
Eligibility Assessment	SC	X		
Medication History	SC	X	X[1]	X[2]
Slit lamp examination	SC	X	X	X
FECD grading	SC	X		
Assessment of other corneal dystrophies	SC	X		
Recipient corneal stroma clarity	SC		X[1]	X[2]
Evidence of graft rejection	SC		X[1]	X[2]
Presence of corneal vessels	SC	X		
Presence of corneal opacities	SC	X		
Donor positioning[1]	SC		X[1]	
Graft attachment	SC		X[1]	
Graft failure assessment[3]	SC		X[1]	X[2]
Intraocular pressure[1,2]	SC	X	X[1]	X[2]
Pachymetry	SC		X	X
Endothelial imaging	R		X	X
HbA1c sample collection (sent to central lab)	R	X[4]		X
Record additional procedures after initial DMEK	SC		X[1]	X[2]
Grading of diabetic retinopathy	SC	X		X
Review post-op complications and other untoward events	SC		X[1]	X[2]

[1]Record findings from surgery to 1-month visit
[2]Record findings from 1-month visit to 12-month visit
[3]Additionally submit graft failure form any time graft failure definition has been met
[4]Sample collection can span up to 1-month visit

Chapter 1: Background Information

1.1 Introduction and Rationale

Based on the increase in diabetes in the United States (US) over the past decade,[1,2] there has been a comparable increase in the percentage of corneas from donors with diabetes that meet the Eye Bank Association of America (EBAA) guidelines suitable for corneal transplantation. The EBAA, however, does not specifically report this at the national level. 18% of the donors in the Cornea Donor Study (CDS) obtained between 2000 and 2002 were diabetic.[3] Data from Midwire from 2016 and 2017 (total of 16,139 donor eyes) (Michael Titus, personal communication) showed that 27% of their transplanted tissue was from donors with diabetes, while Lions VisionGift (Ryan Williams, personal communication) was 34% of 4,260 donors during this same period, a 67% increase in the percentage of donors with diabetes used for transplantation over the past 15 years compared to the CDS data. And this in fact may be an underreporting of donors with diabetes, since many eye banks still have no specific field for the history for diabetes and data is retrieved from a write-in field, along with the problem of undiagnosed diabetes in the population which is reported as high as 21% (7.3 million) of the US population by the CDC in 2020.[4] The type of diabetes, duration, and treatment is also not captured. In the Cornea Preservation Time Study (CPTS) with data from 2012-14, a similar 18% of the donors as in the CDS were classified as diabetic.[5] Since then some eye banks in the CPTS with a more rigorous effort now track the presence of diabetes in the donor. Like the recent Eversight and Lions VisionGift data, they report on a monthly basis that their diabetic donors determined historically run as high as 40%. Thus, the epidemic continues unabated.

The literature has noted that the diabetic endothelium shows functional and morphometric differences as well as cell damage compared to non-diabetic endothelium that could compromise short term and long-term success of the keratoplasty.[6-26] Thus, some consider the use of donor corneas from individuals with diabetes to decrease graft success and endothelial cell density (ECD) following keratoplasty and the acceptance of these donors, when offered by the eye bank, has been at the discretion of the surgeon.

Additionally, the general consensus within internal medicine and endocrinology is that there may be increased rates and types of infections in patients with diabetes. However, the literature is not as firm. A recent case series reported risk for *Corynebacterium*-related ocular infections in patients with poor immunity including those with diabetes.[27] The association of diabetes with the rare and devastating rhino-orbito-cerebral involvement with mucormycosis is well known.[28] In case-control studies, patients with diabetes are 1.3-9.4 fold more likely than controls to develop microbial keratitis,[29,30] and type II diabetes mellitus has been significantly associated with chronic endophthalmitis following cataract surgery.[31] There is also good evidence that diabetes predisposes hosts to infection, especially (of relevance to the eye) in wounds, skin and mucous membranes.[32] Innate immunity may be altered in diabetes[32]; if the remaining immune cells in the donor tissue remain active once transplanted, their compromised immunity may predispose recipients to infection. Therefore, there is scientific rationale to suspect that diabetes in the donor and/or recipient may increase the risk of ocular infection after keratoplasty procedures although there is no evidence of such in the corneal transplant literature.

The CDS, the largest prospective study examining the effect of donor age on graft success and cell loss following penetrating keratoplasty for endothelial dysfunction conditions, did track the presence of diabetes in both the donor and recipient with limited historical information, and did not find an effect on either graft success or cell loss compared to donors without diabetes.[3] One major limitation of this study was that the presence of diabetes in donors was defined simply by presence or absence of diabetes. Vislisel et al for Descemet stripping endothelial keratoplasty (DSAEK) donors[33] and Price et al for Descemet membrane endothelial keratoplasty (DMEK) donors[34] found a similar lack of donor diabetes effect on graft failure, while both series had similar limitations on donor diabetes determination by historical grounds only. However, our group now has suggestive data on the negative impact of donor diabetes on endothelial keratoplasty (EK) outcomes from a secondary analysis of a large prospective multi-center clinical trial, the CPTS. In the CPTS, corneal tissue from donors with diabetes had a significantly greater risk of primary or early graft failure,[35] greater long term endothelial cell loss (ECL),[36] and increased risk of graft dislocation[37] following EK compared to corneas from donors without diabetes.

Since about 1/3 of the nationwide donor pool now includes tissue from donors with diabetes, and there are now conflicting results among studies that were not designed to assess the question of diabetic donor status directly, our group is interested in determining whether corneal tissue from donors without diabetes is indeed superior for EK outcomes, and if severity of donor diabetes correlates with graft success using novel biomarkers of glycemic burden. Finally, the most recent Online Adverse Reaction Reporting System (OARRS) data from the EBAA reported at the Medical Advisory Board meeting in November 2020 showed an unexplained increasing rate of primary and early failures associated with DMEK. The EBAA will be looking at increasing use of pre-loaded lenticules as one source of the issue, but admittedly there is an underreporting issue from surgeons and thus there is no comprehensive database of all the factors that could be contributing to this increasing rate of DMEK primary and early failures.

Building upon the strengths of the successfully completed CPTS structure,[5] the Diabetes Endothelial Keratoplasty Study (DEKS) will address these concerns through a prospective, masked, multi-center clinical trial in which the donor corneas are assigned by diabetes status *in the same distribution pattern (2:1 distribution of tissue from donors without diabetes to tissue from donors with diabetes) as is recognized nationally*. The DEKS will assess graft success and ECD through 1 year following DMEK to determine whether the surgical success rate with corneas from donors with well characterized diabetes (including post-mortem hemoglobin A1c (HbA1c)[38] and advanced glycation endproducts (AGE) testing)[39-42] is truly inferior to the rate with donors without diabetes. We hypothesize that the majority of donor corneas from individuals with diabetes will be suitable, but that a portion of donors with a higher diabetes severity scale,[43] and/or poorer control based on HbA1c will have a greater risk for graft failure and ECL. We will also examine from collected skin biopsies whether high levels of AGE biomarkers in donor skin tissue – which quantifies disease severity over many years[39-42] (and possibly coupled with elevated HbA1c levels) - will be associated with greater risk for graft failure and cell loss. This novel approach to characterization of donor tissue can provide a paradigm shift in the risk assessment of transplanted corneas from diabetic donors. The effect of recipient diabetes on keratoplasty success and cell loss will also be studied in a rigorous manner to determine the potential combined effect of donor and recipient diabetes status.

In summary, this study is designed to determine if non-diabetic donor corneas are superior to diabetic donor corneas in terms of both graft success and ECD outcomes, with an additional specific aim to determine whether donors with a higher diabetes severity scale,[43] and/or poorer control based on HbA1c are driving the effect. We will also determine whether a high AGE/A1c metric is also associated with the potential superiority finding and establish a novel composite score (severity score, HbA1c and AGE/A1c) based on these metrics that can be used to identify high versus low risk diabetic donors. This distinction may enable eye banks to potentially utilize the majority of donors with diabetes for EK surgery, while excluding the severely affected donors with diabetes.

With the growth of DMEK in the past 5 years,[44] including at our proposed clinical sites/surgeons, DMEK will be the only EK procedure to be utilized to test the study's diabetes question and its impact on the transplanted endothelium. Additionally, with the concerning rise in DMEK primary and early failures nationally reported in November 2020 from the EBAA OARRS data, the need for the DEKS is even greater. As the CPTS utilized DSAEK only as its procedure to test the impact of preservation time on graft success and cell loss which streamlined the analyses, having one procedure, DMEK, for the DEKS will similarly assist in the analyses of best practices surrounding DMEK.

The DEKS could have a major impact on the targeted use of corneas from diabetic donors whose tissues are more and more widely utilized for keratoplasty purpose in the US today. Use of biomarkers of disease severity new to the corneal transplant and eye banking field could lead to the development of novel technologies for rapid assessment of glycemic burden and tissue damage in diabetic donors. Finally, the DEKS may be able to sort out other donor, recipient, operative and postoperative factors that impact DMEK outcomes, including primary and early failures.

1.2 Potential Risks and Benefits

1.2.1 Known Potential Risks

1.2.1.1 Known Potential Risks of DMEK

The risks and discomforts for patients undergoing DMEK are the same regardless of study participation. Potential risks of DMEK include:

- mild pain for approximately one week after surgery
- temporary discomfort from the eye examination or eye drops, which may include stinging, itching, or redness
- serious infection or bleeding in 1 in 1,000 patients and serious problems related to anesthesia in 1 in 10,000
- in rare instances topical drops used during standard of care exams can cause an allergic reaction, seizures, and an irregular heartbeat
- development of a rapid rise in the eye pressure
- development of glaucoma

- additional surgery required due to healing problems or movement out of position of the donor cornea
- retinal swelling or detachment
- loss of vision
- rejection reactions occur approximately in about 2-3% of eyes within 5 years of surgery,[45] but are usually reversible if treated promptly with topical corticosteroids, but sometimes it leads to failure of the transplant
- endophthalmitis: a serious intraocular infection that requires prompt treatment and may cause permanent loss of vision or in severe circumstances loss of the eye
- corneal infection: a serious microbial infection of the cornea that requires immediate treatment and may result in permanent scarring and possible permanent loss of vision requiring a repeat of the corneal transplant
- rare chance of dissemination of a communicable disease from the donor tissue
- corneal scarring: permanent haze or cloudiness in the cornea that may result in permanent loss of vision and/or requiring a repeat of the corneal transplant
- corneal neovascularization: blood vessel growth into the cornea that could subject the transplant to a higher risk for rejection and/or permanent loss of vision, requiring a repeat of the corneal transplant
- corneal swelling: thickening of the cornea that may result in loss of vision which may or may not be reversible. If not reversible, another corneal transplant may be required to restore the vision
- wrinkling of the corneal layers: wrinkling of the donor cornea as it heals may result in blurred vision and require another corneal transplant

1.2.1.2 Known Potential Risks of Study Participation

The following are risks of procedures that are not necessarily part of routine care but are being performed for the purposes of this study:

- Fingerstick blood collection: Transient mild discomfort common. In about 1 in 10 times a small amount of bleeding under the skin will produce a bruise. A small scar may persist for several weeks. The risk of an infection is less than 1 in 1000.
- Subjects will be informed of their HbA1C results. Some who have not been previously diagnosed with diabetes might be uncomfortable to learn that their HbA1C level suggests that they may have diabetes. Similarly, those that already know they have diabetes might be uncomfortable to learn that their HbA1C level suggests their diabetes is not under good control.
- There are no known risks to the imaging procedures.

1.2.2 Known Potential Benefits

Study participants may not benefit directly from participation in this study. If the study identifies certain donor corneas as being associated with worse outcomes, eye banking practice could formally change to discourage use of potentially inferior tissue. In the future, if a study participant requires another DMEK in either the same eye or their other eye, the information obtained from this study might benefit them.

1.2.3 Risk Assessment

The risks for events noted in Section 1.2.1.1 are no greater when participating in the study compared with standard care. The likelihood of receiving a cornea from a donor with diabetes in this study will be the same or less compared to the manner in which corneal tissue is distributed nationally (see section 1:1). Other than the study procedures of allocation and distribution of donor tissue in a masked manner, finger stick blood collection and non-contact corneal photographs, the study procedures include collection of data from standard of care office visits, surgical techniques and post-operative care and evaluations.

1.3 General Considerations

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

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

Chapter 2: Study Enrollment and Participant Screening

2.1 Participant Recruitment and Enrollment

Note: throughout this document the term randomization may be used in reference to the study's assignment of donor tissue. However, the process is actually a minimization procedure as explained in Section 3.2.2 rather than traditional randomization; nonetheless for simplicity we retain the term randomization for descriptive purposes.

The goal is to randomize 1420 study eyes with at least 1278 study eyes followed with complete outcome assessments. Participants who have signed consent and started the screening process may be permitted to continue into the trial, if eligible, even if the randomization goal has been reached. Up to 2000 individuals may be enrolled (i.e., sign informed consent) to enable randomization of the 1420 study eyes.

Study participants will be enrolled at ~30 clinical centers in the US. All eligible participants will be included without regard to gender, race, or ethnicity. There is a restriction of a maximum of 20% of the number of eyes (284 eyes) to be randomized by a single surgeon toward the overall recruitment goal.

2.1.1 Informed Consent and Authorization Procedures

Potential eligibility may be assessed as part of a routine-care examination. Before completing any procedures or collecting any data that are not part of usual care, informed consent will be obtained. The informed consent process is described in Section 10.3.

The study will be discussed with the potential study participant by study staff. If the potential participant lacks the capacity to consent, then they should not be approached to consent for the study. The sites' policies and procedures shall be followed to determine capacity, and in general, if the person was able to consent for the transplant themselves, then they can consent for the study themselves. If they require a legally authorized representative to consent to the transplant on their behalf, then they should not be approached to participate in the study.

The potential study participant will be provided with the Informed Consent Form to read in a language understandable to the participant (in English or Spanish as applicable) and will be given the opportunity to ask questions. Potential study participants will be encouraged to discuss the study with family members and their personal physicians(s) before deciding whether to participate in the study. If the potential study participant is interested in the study, the paper Informed Consent Form will be signed by both the participant and an authorized study designee. The subject will be given a signed copy by the study team for their records.

As part of the informed consent process, each participant will be asked to sign an authorization for release of personal information, which may be within the consent form or a separate form. The investigator, or his or her designee, will review with the potential participant the study-specific information that will be collected and to whom that information will be disclosed. After speaking with the participant, questions will be answered about the details regarding authorization.

A participant is considered enrolled when the informed consent form has been fully executed and HIPAA authorization has been provided.

2.2 Participant Inclusion Criteria

Individuals must meet all of the following participant-level inclusion criteria and have at least one eye meeting the study eye inclusion criteria in order to be eligible to participate in the study.

2.2.1 Study Participant-level Criteria

1. Age range 30-<91 years with minimum life expectancy of at least 1 year.
2. Willingness to return to study site for follow up at 1 month and 1 year.
3. Fluent in English or Spanish.
4. Willingness to have fingerstick blood sample collected to determine HbA1c level at entry and 1 year.
    a. The participant must agree to have their primary care provider contacted (or an appropriate referral provided) if they were not known to have diabetes and the HbA1c suggests they may have diabetes. Similarly, if already known to have diabetes and the HbA1c is high, the participant must agree to have their primary care provider contacted or an appropriate referral provided.

2.2.2 Study Eye Criteria

1. At least one eye clinically recommended for DMEK that is able to be scheduled for DMEK between 5 and 90 days after enrollment.

2. If second eye is enrolled, must be scheduled for DMEK between 7 days and 6 months after DMEK on the first eye.

3. Presence of a condition related to endothelial dysfunction which will be treated by DMEK.

    Eligible indications for DMEK include:

    a. Presence of FECD meeting at least one of the following:
        i. Phakic FECD with or without cataract
            1. Triple procedure including DMEK for FECD, cataract extraction and posterior chamber intraocular lens implantation (IOL) is allowed
        ii. Pseudophakic FECD with posterior capsule supported, sulcus supported, or scleral-fixated posterior chamber IOL
    b. Pseudophakic corneal edema with posterior capsule supported, sulcus supported, or scleral-fixated posterior chamber IOL without FECD
    c. Failed DSAEK or DMEK originally performed for the same indications above without current exclusion criteria below, as described below.

NOTES:

- *Stromal vascularization is acceptable, as long as not visually significant (by investigator's judgement)*

- *Penetrating keratoplasty and DSAEK will not be included procedures; only DMEK*

2.3 Participant Exclusion Criteria

Individuals meeting any of the following exclusion criteria at baseline will be excluded from study participation.

1. Pregnant or planning to become pregnant prior to the DMEK study surgery, based on verbal report.
2. Lack cognitive capacity such that consent could not be provided. Such individuals are excluded because they are at greater risk for post-operative complications if they do not follow proper instructions (use of drops, ocular protection, physical positioning) and therefore can confound study outcomes.
3. Presence of a condition that has a high probability for failure (e.g., failed penetrating keratoplasty, uncontrolled uveitis)
4. Stromal vascularization that will impede assessment of recipient stroma clarity
5. Other primary endothelial dysfunction conditions including posterior polymorphous corneal dystrophy and congenital hereditary corneal dystrophy
6. Indication for surgery that is not suitable for DMEK (e.g, keratoconus, stromal dystrophies and scars)
7. Aphakic corneal edema with or without FECD
8. Anterior chamber IOL in study eye prior to DMEK or planned placement of anterior chamber IOL during DMEK
9. Presence of vitreous in the anterior chamber
10. Planned IOL exchange of an anterior chamber IOL with a posterior chamber IOL in study eye at time of study DMEK
11. Pre-operative central sub-epithelial or stromal scarring that could impact post-operative recipient stromal clarity assessment
12. Presence of anterior synechiae
13. Peripheral anterior synechiae in the angle greater than a total of three clock hours
14. Uncontrolled glaucoma with or without prior filtering surgery, tube shunt placement, or MIGS. *Uncontrolled glaucoma is defined as intraocular pressure > 25mm Hg.*
15. Controlled glaucoma with prior tube shunt placement for glaucoma (*controlled glaucoma with MIGS or trabeculectomy is allowed*)
16. Fellow eye visual acuity < 20/200 due to an ocular condition other than cornea disease that would be a candidate for DMEK
17. IOP <8 mmHg
18. Topical Rho kinase inhibitor, including Rhopressa, used within 1 month prior to study entry and anticipated during the course of the study
19. Fellow eye enrolled in the DEKS that has met study criteria for graft failure.

Study eye eligibility is determined at the time DMEK surgery is scheduled. A participant can contribute two study eyes if both eyes are eligible. The eligibility of the second eye, if applicable, will be assessed at the time surgery on the second eye is scheduled; second eye surgery as part of the study can be performed between 7 days and 6 months (182 days) after DMEK on the first eye. If participant agrees to second eye study participation, he/she will not receive a cornea from a donor with known diabetes in both study eyes. Specifically, if the first eye received a cornea from a donor with known diabetes, after thorough medical record review as part of standard eye banking practices (see Section 3.2.2), the second eye will receive a cornea from a donor without known diabetes. (Study surgery could thus potentially be cancelled if a donor cornea without known diabetes is not available). If the first eye received a cornea from a donor without known diabetes, the second eye will proceed through the minimization algorithm for assignment of donor tissue. Note, because of the reclassification process based on HbA1c testing at the central laboratory, this means that donors not known to have diabetes after thorough eye bank staff medical record review, may still have diabetes that was not known at the time of the keratoplasty procedure. This testing is not standard practice and could delay proceeding with the keratoplasty if the cornea could not be placed until the result (HbA1c value < or >= 6.5%) was returned. Therefore, there is a possibility that a participant who agreed to both eyes participating in the study can in both eyes receive a cornea from a donor classified as having diabetes based upon post-mortem study-specific HbA1c laboratory values (>=6.5%) that are not acquired in standard practice.

2.4 Screening/Pre-operative Procedures

After informed consent has been signed, a potential participant will be evaluated for study eligibility through the elicitation of a medical history and a routine examination including a dilated eye examination. There are no examination procedures required to assess patient eligibility other than those that are part of standard patient care. Historical data (which may predate the date of informed consent) may be obtained from the medical record after informed consent has been signed to acquire baseline data described below.

2.4.1 Data Collection and Testing

The following procedures/data will be performed/documented for each study eye, unless otherwise specified:

- Inclusion and exclusion criteria assessed
- Demographics (date of birth, sex, race and ethnicity)
- Contact information (site enters into limited access study database)
- Medical history (including history and complications of diabetes, last HbA1c, and any ocular treatments for diabetes)
- Current diabetic and selected systemic and topical ocular medications
- Slit lamp examination (including FECD grading, assessment of other corneal dystrophies, presence of corneal vessels, presence of corneal opacities, anterior synechiae and lens status)
- Intraocular pressure

- Dilated fundus exam for grading of diabetic retinopathy

Once participant eligibility has been confirmed and informed consent has been obtained, a fingerstick blood sample will be collected for HbA1c determination prior to or within about 1 month of surgery. A fingerstick blood sample for HbA1c determination will be collected at the 12-month follow-up visit as well to monitor diabetes control and also to detect new diabetes in the original non-diabetic group.

Screening procedures will last approximately 1 hour.

- Those participants that were unaware they had diabetes will be reclassified as diabetic for study purposes based on this measurement. See Clinical Site Manual of Procedures for details regarding dissemination of participant HbA1c results.

2.5 Screen Failures

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

Chapter 3: Donor Eligibility and Cornea Assignment, Eye Banks

3.1 Eye Bank Procedures

Eye bank procedures will mimic standard procedures as closely as possible to minimize disruption to their normal routine, with the exception of procedures related to donor assignment including classification of donor diabetes status and specular microscopy (including calibration, technician certification, and procedure for obtaining specular images). Eye banks will be able to use any FDA-approved hypothermic (2-8ºC) storage solution for intermediate term storage.

3.2 Donor Information, Procurement of Tissue and Other Samples

1. Donor information will be obtained by the eye bank's usual routine.
   The eye banks must have authorization for research use in order to obtain a blood sample and skin tissue biopsy for the study. The eye banks may need to add a statement to their usual authorization forms referencing the DEKS to assure that they have proper authorization to take blood and skin biopsy samples for the purpose of the study. The Coordinating Center (CC) will work with each eye bank and provide templates for authorization for research.
2. Eye banks will follow their usual routine for procurement of tissue.
   In addition to the usual cornea tissue procurement, the eye banks will also need to draw/obtain a whole blood sample from the potential donor for cadaveric HbA1c testing. If there is proper authorization for skin biopsies referred to above, the eye bank should also obtain a skin tissue sample for AGE testing at the time of recovery. See specific DEKS Eye Bank Procedures Manual, Advanced Research and Diagnostics Laboratory (ARDL)) Eye Bank Central Laboratory Manual of Procedures, and the AGE Testing Program and Procedure Manual for details.

3.2.1 Donor Cornea Tissue Suitability

1. All eye banks will follow their procedural routine for procurement of cornea tissue and determination of suitability for DMEK in accordance with the Medical Standards and Procedure Manual of the EBAA. This includes standard serologic testing, specular microscopy, and slit lamp examination.
2. The following eligibility criteria will apply to all donor cornea tissue assigned to study eyes.
   - Must meet current EBAA standards for human corneal transplantation
   - Age of donor at time of death 50–75 years
   - If the donor body was refrigerated or eyes on ice within 12 hours of death, the body or eye may stay refrigerated up to 24 hrs; if no refrigeration then the death to preservation time should be <12 hrs
   - Preservation time ≤ 11 days
   - Eye bank determined minimum ECD (at screening) of ≥ 2300 cells/mm^2
   - Pleomorphism/Polymegethism: At eye bank and medical director discretion

- Guttae: no true guttae present
- No evidence of central endothelial cell damage/trauma or dystrophy, such as FECD, by slit lamp examination

3.2.2 Determining Diabetic Status

Eye Banks should review various data collection documents and consult with treating physicians to help collect information to confirm diabetes status. If there are conflicting data found within the various data collection documents, continue to request additional primary care records, consults, and other sources of electronic health records as available. Examples include, as available:

- DRAI – Donor Risk Assessment Interview[46] – the current uniform DRAI for tissue banks has the following question: Did he/she EVER have diabetes? If yes a) for how many years? b) Was it treated? If yes How? The eye banks should add this question to the "eye only" DRAI to obtain diabetes information in addition to medical information. Eye Banks should also obtain the name of the treating physician in order to perform a follow up consult.
- Hospital medical record
- Physician consults
- Primary Care/Internist Medical Records

Defining Diabetic Status

For the purpose of tissue assignment for the minimization procedure, the eye bank will define the donor as having diabetes using ICD-10 codes and medical records with respect to diagnoses and treatments. Refer to the Eye Bank Manual of Procedures for detailed definitions.

Only donors definitely diabetic or definitely not diabetic by above approach will be eligible for the study. Those indeterminant may be used for keratoplasty, but not eligible for the study.

Diabetes Risk Categorization

Once diabetes status determination is complete, if found to have diabetes, additional information will be obtained to determine diabetes risk categorization on a 1 to 5 scale.

Specifically, the following aspects will be determined to calculate the diabetes risk categorization score:

- Any history of diabetes mellitus [1 point]
- Body mass index > 30kg/m^2 [1 point]
- Hypertension [1 point]

Additionally, 2 points are added to the score for any of the following, regardless of how many are chosen:

- Diabetes mellitus history of at least 10 years
- Insulin dependent outpatient

- Possible comorbidities
    - peripheral nerve damage/neuropathy
    - renal failure and/or dialysis, chronic kidney disease
    - stroke
    - myocardial infarction
    - leg ulcers or amputations
    - peripheral vascular disease
    - diabetic retinopathy with proliferative disease including vitreous hemorrhage
    - diabetic retinopathy with history of retinal laser
    - diabetic retinopathy with history of vitrectomy
    - diabetic retinopathy with history of intravitreal anti-VEGF therapy

Once diabetes status and severity are determined a computer program will be able to run a minimization procedure to select a donor cornea for a given participant DMEK surgery. For the first eye of each participant, if only one donor cornea is available, then this cornea is selected. Otherwise, if all available donor corneas are the same donor diabetes classification, then a cornea is randomly selected from all available. Otherwise, if at least one donor cornea is from each donor diabetes classification, a minimization procedure will select based on achieving balance on several factors, including the surgeon, and the donor diabetes risk categorization score.

For participants with two study eyes, if the first eye received a cornea from a known diabetic donor, then the second eye will receive a cornea from a donor the eye bank determined as non-diabetic, if available. If a cornea from a donor the eye bank determined as non-diabetic is not available, then surgery must be postponed or performed outside of the study. If the first eye received a cornea from a donor the eye bank determined as non-diabetic, then the minimization procedure will run and the second eye could receive a cornea from a known diabetic or nondiabetic donor.

The most important aspect of the protocol for the eye banks is maintaining masking of the surgeon and surgeon staff from any direct or indirect knowledge of the diabetic status of the donor tissue assigned to study participants.

The surgeon will be masked to all other donor parameters except storage solution. NO eye bank's standard reports and labels (including donor tissue report, donor cornea container label, recipient information form, package insert form and adverse reaction report) **can be sent to the surgeon**. Study-specific documentation will be used in lieu of these reports. See the Eye Bank Manual of Procedures for a summary of the EBAA waivers and other references, and examples of the DEKS Donor Tissue Label, DEKS Donor Tissue Report, DEKS Adverse Reaction Report, and DEKS Shipping Label.

3.2.3 Eye Bank Procedures for Study Images

Detailed procedures for study images will be provided in the DEKS-Cornea Image Analysis Center (CIARC) Eye Bank Imaging Manual. In screening images for eye bank prepared DMEK tissue, a minimum of central ECD of ≥ 2300 cells/mm^2 determined by the eye bank at screening will be required along with fulfilling all medical director determined criteria for quality post prep, including assessment of cell damage within the anticipated donor diameter.

3.2.4 DMEK Lenticule Preparation

Detailed procedures are found in the DEKS Eye Bank Procedures Manual. Each DEKS eye bank will perform their DMEK lenticule preparation following their usual technique while documenting technique, and observations as to any problems during the stripping. Most importantly, each eye bank will ensure that the technician performing the prep will be masked as to the diabetes status of the donor.

For both the eye bank- and surgeon- prepared DMEK preps, the extent of endothelial cell damage post prep utilizing trypan blue will be assessed by the masked technician (or surgeon). The masked technician (or surgeon) will also perform an assessment of scroll tightness modified from earlier description whenever possible[48]. Whether eye-bank or surgeon-prepared, the preferred time from DMEK donor preparation to surgery will be at the discretion of the surgeon, but tracked. Also, if corneal tissue is pre-loaded by the eye bank, the eye bank will follow its normal loading procedure according to their standard operating procedures. The loading system will be recorded (i.e., injector, storage container) and the preferred time from loading to surgery will be at the discretion of the surgeon and tracked.

3.3 Laboratory Testing

1. HbA1c
    - De-identified whole blood samples will be sent to the central laboratory (ADRL at the University of Minnesota) for sample analysis.[53]
    - De-identified skin biopsy samples for AGE testing will be sent to Dr. Vincent Monnier's laboratory at CWRU for sample analysis.

Chapter 4: Transplantation and Follow-up Procedures at Clinical Sites

4.1 DMEK Procedure

DMEK will be performed according to the investigator's usual routine. Aspects of the surgical technique and procedure will be tracked, but not standardized. Data to be collected will include but are not limited to incision size, insertion method, air or gas usage, other procedures (e.g. cataract surgery), and operative complications (e.g., difficult donor preparation, difficult placement).

The surgeon will be masked to donor parameters (e.g. donor age, donor ECD), except the FDA-approved hypothermic (2-8ºC) storage solution being employed.

4.2 Post-Operative Management

Postoperative management will be at the discretion of the surgeon based on his/her usual practices. Key aspects of pharmacologic management (e.g. topical corticosteroid usage, glaucoma medications) will be collected on the data forms.

4.3 Study Visits

Protocol-specified post-operative follow-up visits (and visit windows) for the first eye, established to conform to the usual practice and timed from surgery date, will be scheduled as outlined in Section 4.3.1 below.

4.3.1 Study Visits and Windows

Protocol-specified post-operative follow-up visits (and visit windows) for the first eye, established to conform to the usual practice and timed from surgery date, will be scheduled as outlined as below.

Table 2. Study Visits and Windows

VISIT (TARGET DAY)	ALLOWABLE WINDOW (AROUND TARGET DAY/WEEK)
1 Month (Day 30)	+/- 10 days *note an out of window but not missed visit will be allowed up to 100 days and baseline HbA1c assessment is allowable within this time period as well
12 Months (Day 364)	-30 / +100 days

Additional contacts or visits may occur as needed. Additional visits are expected to be performed per standard of care at the discretion of the investigator. A data form will be completed for each protocol visit (1 month and 12 months) capturing data from that visit plus selected data from the medical record from prior standard of care visits. If graft failure is determined and/or a regraft is required on a non-protocol visit, a graft failure form should be completed if and when this occurs.

4.3.2 Procedures at Study Follow-up Visits

The following data will be collected and procedures will be performed at each protocol-specified post-operative follow-up visit, unless otherwise specified:

- Medication history
- Slit lamp examination:
  - Recipient corneal stroma clarity
  - Evidence of graft rejection
  - Donor positioning (*1 month*)
  - Graft attachment (*1 month*)
  - Other abnormalities of interest
- Graft failure assessment
- Intraocular pressure
- Pachymetry
- Endothelial imaging
- HbA1c sample collection (*may be performed up to 1 month visit after surgery for baseline measurement and then at 1 year*)
- Record additional procedures after initial DMEK surgery
- Grading of diabetic retinopathy (*1 year*)
- Post-operative complications and other untoward events

4.4 Early Termination Visit

Participants will be asked to come for an end of study visit in the event of withdrawal or early termination for another reason. If graft failure occurs in the absence of re-graft, follow-up is not terminated prematurely. However, if the graft is replaced, follow-up will be discontinued.

Chapter 5: Clinical Procedures

5.1 Corneal Assessment and Slit Lamp Examination

The slit lamp examination should be performed per the investigator's usual routine. Specific details of the data collected from the slit lamp examination are found in the site procedures manual. Any corneal images obtained for standard of care and therefore part of the medical record may be considered study data.

5.1.1 Recipient Corneal Stroma Clarity

The recipient corneal stroma clarity will be assessed by slit lamp examination using the following validated 3-level classification[5]

- clear central recipient stroma
- equivocally cloudy central recipient stroma
- clouded central recipient stroma.

Specific details regarding the grading of recipient corneal stroma clarity for DMEK are found in the Site Procedures Manual. Investigators will be provided a high resolution color standard scale and will be trained and certified on this classification scheme prior to enrolling participants.

5.1.2 Graft Rejection Assessment

Graft rejection will be assessed during the slit lamp examination using a modification of the Collaborative Corneal Transplantation Studies (CCTS) classification and as used in the CPTS.[5,49] Graft rejection will be classified as definite, probable/ possible, or not present. Details of the assessment of graft rejection are found in the Site Procedures Manual.

The management of suspected graft rejection episodes will be according to the investigator's clinical discretion.

5.1.3 Graft Attachment Assessment

Graft attachment and position will be assessed by the investigator's routine. If the donor button is detached, the percentage of detachment will be documented. The investigator's management of the detachment and/or repositioning will be standard of care and tracked for study purposes.

5.2 Graft Failure Assessment

Graft failure will be assessed and defined as the occurrence of one of the following:

- Cornea which requires regrafting for any reason
- Cornea which remains cloudy without clearing, according to the following:

  (1) cloudy cornea which does not clear in the first postoperative week and stays cloudy through 8 weeks post-operatively

  OR

  (2) cloudy cornea which was initially clear in the first postoperative week but becomes and remains cloudy for 3 months without clearing.

- A study participant whose cornea becomes cloudy (clouded recipient central stroma, based on the DEKS grading scale) will be treated by the investigator's usual routine.

For eyes meeting the definition of graft failure above, the principal cause of graft failure will be classified as one of the following:

- Early failure (cloudy cornea in the first postoperative week which does not clear or requires a regraft within 8 weeks), associated with surgical complications
- Primary donor failure (cloudy cornea in the first postoperative week which does not clear or requires a regraft within 8 weeks), in the absence of surgical complications
- Graft rejection (defined as a clouded recipient central stroma following an allograft reaction);
- Non-rejection graft failure (defined as a graft that initially had a clear central recipient stroma and becomes cloudy due to causes other than an immune event. These include: surface failure, infection, glaucoma/hypotony, endothelial decompensation, interface irregularity or opacity, pre-existing stromal scarring, blunt or penetrating trauma, and other causes);
- Refractive/visual graft failure (defined as a graft that requires regrafting due to inadequate vision while the recipient central stroma remains clear).

5.3 Intraocular Pressure

Intraocular pressure will be measured using the investigator's usual routine.

5.4 Pachymetry

Corneal thickness will be measured using the investigator's usual routine. If there is a choice, slit scanning topography (i.e. Pentacam) corneal thickness maps are preferred. If no measurement can be obtained (e.g. if the cornea is too thick), this will be noted on the data form.

5.5 Specular Microscopy

At the 1 month and 12 month visits, specular microscopy of the central endothelium will be obtained on all participants that have not experienced graft failure.[5,50,51] Detailed procedures for obtaining best image quality and image transmission will be provided in the DEKS-CIARC Clinical Site Imaging Manual.

5.6 Grading of Retinopathy

Fundus exams will be performed on all enrolled participants at the initial visit and the 12 month visit to grade the presence or absence of diabetic retinopathy; if present, the degree will be grading according to the DRCR.net grading scales.[52] Specifics are found in the Clinical Site Procedures Manual.

5.7 Additional Procedures after initial DMEK Surgery

Data on additional procedures performed on the study eye after the initial DMEK surgery will be collected, including, but not limited to:

- air bubbling/repositioning in the first month
- cataract surgery and placement of intraocular lens (anterior chamber, posterior chamber)
- YAG capsulotomy
- refractive procedure (e.g. limbal relaxing incision, LASIK)
- glaucoma surgery (e.g. trabeculectomy, laser trabeculoplasty, tube shunt, mini shunt, other)

5.8 Laboratory Testing

2. HbA1c
    - De-identified capillary whole blood samples obtained around the Baseline and 12-month visits will be sent to the central laboratory (ADRL at the University of Minnesota) for sample analysis.[53]

Chapter 6: Unanticipated Problems and Adverse Event Reporting

6.1 Unanticipated Problems

Site investigators will promptly report to the CC all unanticipated problems meeting the criteria below and the JCHR IRB must be notified within seven calendar days of recognition. For this protocol, an unanticipated problem is an 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 IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places participants or others at a greater risk of harm than was previously known or recognized (including physical, psychological, economic, or social harm)

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

6.2 Adverse Events

6.2.1 Definitions

Adverse Event (AE): Any untoward medical occurrence in a study participant, irrespective of the relationship between the adverse event and the device(s) under investigation.

Serious Adverse Event (SAE): Any untoward medical occurrence that:

- Results in death.
- Is life-threatening; (a non-life-threatening event which, had it been more severe, might have become life-threatening, is not necessarily considered a serious adverse event).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions (sight threatening).
- Is a congenital anomaly or birth defect.
- Is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the participant or may require medical/surgical intervention to prevent one of the outcomes listed above).

6.2.2 Reportable Adverse Events

Related and unrelated ocular adverse events will be reported only in the study eye as there is no plausible reason to believe that the DMEK procedure could affect a non-study eye. Since the DEKS does not involve investigational drugs or devices and participants in this study would have undergone DMEK regardless of study participation, related adverse events are those limited to events that are related or possibly related to randomization of donor tissue or study procedures of blood sample collection and specular microscopy. Eye banks will follow their standard procedures for reporting of AEs related to the donor cornea as required to the FDA and the EBAA.

6.2.2.1 Events Requiring Expedited Reporting

The following events will require expedited reporting to the CC (see 6.3).

- Endophthalmitis
- Microbial keratitis (bacterial, fungal, parasitic) within 3 months of DMEK

6.2.2.2 Other Adverse Events

All deaths will be reported, with cause of death if known; other systemic events will not be reported unless determined to be related to study participation.

The following events will be reported on a separate online AE form:

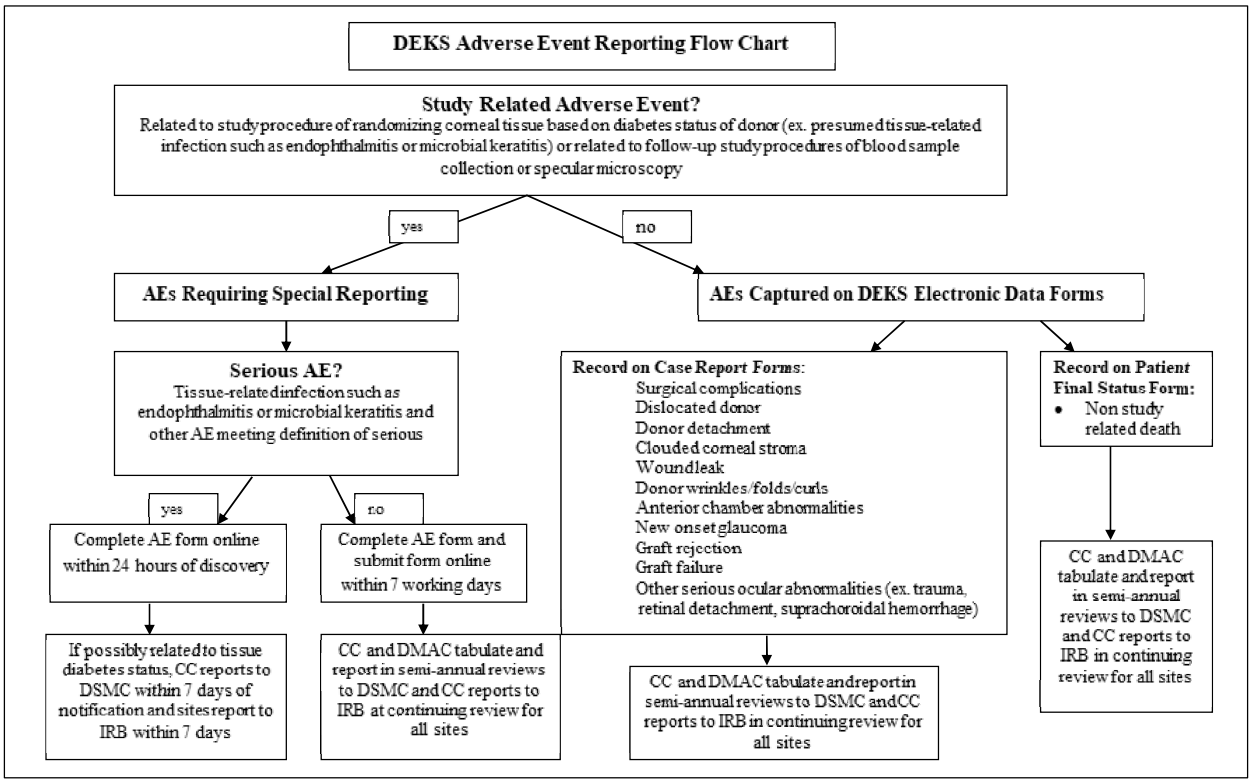
- non-serious events related to study procedure of blood sample collection that requires some form of medical intervention *(note: expected events such as pain, bruising, irritation at site of fingerstick do not require reporting)*
- non-serious events related to study procedure of specular microscopy that requires medical intervention

The following ocular adverse events of interest, even if serious, will be reported on electronic study visit case report forms and not on separate AE Forms:

- Surgical complications
- Dislocated donor
- Donor detachment
- Clouded recipient corneal stroma
- Wound leak
- Donor wrinkles/folds/curls
- Anterior chamber abnormalities
- New onset glaucoma
- Graft rejection
- Other serious ocular abnormalities (ex. trauma, retinal detachment, suprachoroidal hemorrhage)

6.2.2.3 Clinical Site AE Reporting Flow Chart

The following flow chart outlines the required AE reporting procedures described above:



6.2.3 Relationship of Adverse Event to Study Procedure

The study investigator will assess the relationship of any adverse event requiring reporting on an AE form to be related or unrelated by determining if there is a reasonable possibility that the adverse event may have been caused by a study procedure (in the DEKS study procedures include randomization of donor tissue by donor diabetes status, blood sample collection and specular microscopy).

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

Yes

There is a plausible temporal relationship between the onset of the adverse event and the study procedure, and the adverse event cannot be readily explained by the participant's clinical state, intercurrent illness, or concomitant therapies; and/or the adverse event follows a known pattern of response to the study procedure; and/or the adverse event abates or resolves upon discontinuation of the study procedure and, if applicable, reappears upon re-challenge.

No

Evidence exists that the adverse event has an etiology other than the study procedure (e.g., preexisting medical condition, underlying disease, concurrent illness, or concomitant medication); and/or the adverse event has no plausible temporal relationship to study device, drug or procedure.

6.2.4 Severity (Intensity) of Adverse Events

The severity (intensity) of an adverse event requiring reporting on an AE form will be rated on a three-point scale: (1) mild, (2) moderate, or (3) severe. A severity assessment is a clinical determination of the intensity of an event. Thus, a severe adverse event is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

- MILD: Usually transient, requires no special treatment, and does not interfere with the participant's daily activities.
- MODERATE: Usually causes a low level of inconvenience, discomfort or concern to the participant and may interfere with daily activities, but is usually ameliorated by simple therapeutic measures and participant is able to continue in study.
- SEVERE: Interrupts a participant's usual daily activities, causes severe discomfort, may cause discontinuation of study device, and generally requires systemic drug therapy or other treatment.

6.2.5 Expectedness

For a serious adverse event that is considered possibly related to a study procedure as noted in 6.2.1, the Medical Monitor will classify the event as unexpected if the nature, severity, or frequency of the event is not consistent with the risk information.

6.2.6 Coding of Adverse Events

Each AE form is reviewed by the Medical Monitor to assess safety and to verify the coding and the reporting that is required.

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

6.2.7 Outcome of Adverse Events

The outcome of each reportable adverse event (requiring completion of an AE form) will be classified by the investigator as follows:

- RECOVERED/RESOLVED (COMPLETE RECOVERY) – The participant recovered from the AE/SAE without sequelae. Record the AE/SAE stop date.
- RECOVERED/RESOLVED WITH SEQUELAE – AE/SAE where the subject recuperated but retained pathological conditions resulting from the prior disease or injury. Record the AE/SAE stop date.
- FATAL – A fatal outcome is defined as the SAE that resulted in death. Only the event that was the cause of death should be reported as fatal. AEs/SAEs that were ongoing at the time of death; however, were not the cause of death, will be recorded as "resolved" at the time of death.

- ONGOING NOT RECOVERED/NOT RESOLVED – An ongoing AE/SAE is defined as an ongoing event with an undetermined outcome.
  - An ongoing outcome will require follow-up by the site in order to determine the final outcome of the AE/SAE.
  - The outcome of an ongoing event at the time of death that was not the cause of death, will be updated and recorded as "resolved" with the date of death recorded as the stop date.
- ONGOING (MEDICALLY STABLE) – AE/SAE is ongoing, but medically stable. For example, a chronic condition where no further change is expected.

If any reportable adverse events are ongoing when a participant completes the study (or withdraws), data collection will continue until the specific adverse event is resolved. Note: participants should continue to receive appropriate medical care for all adverse events after their participation in the study ends, even if they are not reportable adverse events.

6.3 Timing of Event Reporting

The events noted in 6.2.2.1 and any SAEs possibly related to study participation must be reported to the CC within 24 hours of the site becoming aware of the event. This can occur via phone or email, or by completion of the online serious adverse event form. If the form is not initially completed, it should be completed as soon as possible after there is sufficient information to evaluate the event. The site will be responsible for reporting the event to the JCHR IRB within 7 days.

All other reportable AEs (as defined in 6.2.2.2) should be submitted by completion on the online form within 7 days of the site becoming aware of the event.

The CC will notify all participating investigators of any adverse event that is serious, related, and unexpected. Notification will be made within 10 working days after the CC becomes aware of the event.

Each principal investigator is responsible for reporting serious study-related adverse events to the JCHR IRB as the overseeing IRB. Sites must report all serious, related adverse events within seven calendar days to the IRB.

6.4 Safety Oversight

The study Medical Monitor will review all adverse events that are reported during the study. Additionally, the Medical Monitor will review compiled safety data at periodic intervals (generally timed to the review of compiled safety data by the DSMC).

The DSMC will review compiled safety data at periodic intervals. The DSMC can recommend modifications to the study protocol or suspension or outright stoppage of the study if deemed necessary based on the totality of safety data available. Details regarding DSMC review will be documented in a separate DSMC document.

Chapter 7: Miscellaneous Considerations

7.1 Pregnancy Reporting

If pregnancy occurs, the participant will remain in the study since the study intervention will have been completed and remaining in the study consists of only low risk follow-up procedures. The occurrence of pregnancy will be reported to the CC.

7.2 Participant Compensation

Participant compensation will be specified in the informed consent form.

7.3 Participant Withdrawal

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

7.4 Confidentiality

For security and confidentiality purposes, both the donor and the human subject participants will be assigned identifiers that will be used instead of their names for study purposes. However, protected health information, such as name and social security number, will need to be shared with the specific eye bank processing the participant's donor tissue (eye banks require additional unique patient identifiers) as part of the EBAA standard requirements of transplant processing and notification; this information will be shared directly between the clinical site and their respective eye bank as per standard practices and therefore will not be stored in the study database. Alternatively, participant name and contact information will be shared by the sites through the study database and will be accessible (limited access) to members in the CWRU Coordinating Center for purposes of contacting participants that may be lost to follow-up. De-identified or coded participant information may also be provided to research sites involved in the study. The samples sent to the laboratories will be coded for HbA1c and AGE testing, and the labs will not receive any identifying information other than date of collection. Similarly, the images sent to CIARC will be coded and are not identifiable other than date of collection.

Chapter 8: Statistical Considerations

8.1 Statistical and Analytical Plans

The approach to sample size and statistical analyses are summarized below.

8.2 Statistical Hypotheses

The primary outcome for this study is graft failure by 12 months. The null/alternative hypotheses are:

a. *Null Hypothesis*: There is no difference in the probability of graft failure by 12 months between the diabetic group and the non-diabetic group.

b. *Alternative Hypothesis*: The probability of graft failure by 12 months is different for the diabetic and non-diabetic groups.

8.3 Sample Size

The sample size is calculated based on a **superiority** (2-sided test) design with the goal to determine that the graft failure rate of the recipients of tissue from donors with diabetes is different than the graft failure rate of recipients of tissue from donors without diabetes. Numbers in table are total sample size for both donor groups combined (lost to follow up not accounted for), assuming different distributions of tissue from persons without:with diabetes.

Proportion diff	1:1						2:1						3:1					
	Failure Rate						Failure Rate						Failure Rate					
	12%	10%	8%	6%	5%	4%	12%	10%	8%	6%	5%	4%	12%	10%	8%	6%	5%	4%
10%	590	532	472	408	376	342	651	585	516	444	405	369	764	684	604	516	468	424
8%	880	788	690	588	534	480	975	870	759	642	582	519	1144	1020	888	748	676	600
6%	1486	1318	1140	954	856	756	1650	1461	1260	1047	936	825	1940	1716	1476	1224	1092	956
5%	2082	1836	1576	1304	1164	1018	2316	2037	1746	1437	1278	1113	2728	2396	2048	1680	1492	1296
4%	3160	2770	2362	1930	1706	1478	3522	3084	2622	2133	1881	1623	4156	3632	3084	2504	2204	1892
3%	5450	4750	4012	3236	2836	2424	6090	5298	4467	3591	3138	2673	7192	6252	5264	4224	3684	3132

For the final sample size, the following has been assumed:

- Type I error of 5%
- 90% power
- Non-diabetic 1-year failure rate (P_{ND}) of 4%
- 5% difference between P_{ND} and P_D
  - CPTS results
    - Failure rate for donors with diabetes: 9%
    - Failure rate for donors without diabetes: 4%
- 2:1 distribution of tissue from donors without diabetes to tissue from donors with diabetes
  - Recent distribution estimates from eye banks showing regional differences
    - Midwire 2015 data (N=11,834 donors): 36% had diabetes
    - Midwire 2016-2017 data (N=16,139 donors): 27% had diabetes

- Eversight 2018 data (N=10,672 donors): 24% had diabetes
- Lions Visiongift 2015-2018 data (N=4,260 donors): 34% had diabetes

This requires a total of 1278 study eyes before adjusting for lost to follow-up. Increasing the calculated sample size by 10% to account for loss to follow-up gives a total of **1420 study eyes (~947 donors without diabetes and ~473 donors with diabetes)**.

- A participant can enroll both eyes into the study, if both are eligible. It is anticipated that about 30% of participants will have two study eyes, based on the CPTS data[54], data from single site study of Price et al for DMEK[55], and a participating surgeon survey, indicating more common bilateral cases associated with DMEK, the endothelial keratoplasty procedure in the DEKS, compared to DSAEK which was the procedure in the CPTS. This would mean it is expected that approximately **1092 participants would need to be enrolled in order to enroll 1420 study eyes**.

Although both eyes of a study participant can be enrolled, and/or both eyes of the donor may be provided for the study, the sample size calculation does not adjust for these correlations, for the reasons noted below.

- If participants with two study eyes receive a cornea from a donor with known diabetes for the first eye, the second eye will receive a cornea from a donor without known diabetes. If the first study eye received a cornea from a donor without known diabetes, then there is a 1/3 chance that the second study eye will receive a cornea from a donor with known diabetes. Note, the primary analyses will adjust for these correlations. Those classified as having *known diabetes* are participants with a diabetes diagnosis prior to tissue donation or reclassified as diabetic based on post-mortem HbA1c values.

Regarding donors, CPTS found there were no cases of graft failures occurring from the cornea mates of the same donor because of the low graft failure rate and low number of cornea mates in the study;[35] a similar trend is expected in the DEKS and sample size will not be adjusted for this. If there are cases of graft failures occurring from cornea mates both assigned in the study, sensitivity analysis will account for the correlation in the models.

8.4 Outcome Measures

8.4.1 Primary Efficacy Endpoint

- Graft failure within 1 year of surgery as defined in the SAP

Graft failure will be assessed and defined as the occurrence of one of the following:

- Cornea which requires regrafting for any reason
- Recipient cornea remains cloudy after surgery without clearing for 8 weeks or longer

For eyes meeting the definition of graft failure above, the principal cause of graft failure will be classified and described in the SAP. Further, the date of graft failure will also be described in the SAP.

8.4.2 Secondary Endpoint

Endothelial cell density at 1 year is considered a secondary endpoint.

8.5 Analysis Dataset and Sensitivity Analyses

The primary analysis will follow the intent-to-treat principle, with the following exceptions: it will exclude eyes that did not have DMEK surgery, received a non-study donor cornea tissue, had an anterior chamber IOL implanted during surgery, or experienced a suprachoroidal hemorrhage. It is highly unlikely that any of these events could be related to diabetes status of the donor.

Safety outcomes will be reported for all enrolled study eyes that have DMEK surgery, irrespective of whether the participant completed the study.

Sensitivity analyses for the primary and secondary outcomes, such as adjusting for significant operative complications and/or recipient diabetes, will be described in the SAP.

8.6 Analysis of the Primary Efficacy Endpoint

Summary statistics (Kaplan-Meier estimates of 1-year graft failure with 95% confidence intervals) will be reported by donor diabetes status groups (with versus without diabetes). Further, point estimates and 95% confidence intervals for donor diabetes status group differences will be presented.

One-year graft failure between donor diabetes status groups will be compared using a proportional hazards model while adjusting for corneal diagnosis and accounting for participants who have two study eyes included in the study using a frailty model. The proportional hazards assumption will be tested. If this assumption is violated, then hazard ratios will be presented separately over time. There will be no imputation of missing data in the primary analysis.

8.7 Analysis of the Secondary Endpoint

For ECD, only participants with a gradable 1-year image who have not experienced graft failure 1-year after DMEK will be included in the analysis. There will be no imputation of missing data. Summary statistics (mean ± SD or median (IQR)) appropriate to the distribution will be tabulated by donor diabetes status group and at baseline, 1-year and for the changes from baseline to 1-year.

Repeated measures regression models with unstructured covariance including data from baseline, 1 month, and 1 year will be used to compare donor diabetes status effects while adjusting for baseline ECD, corneal diagnosis, and recipient study eyes (random effect. A point estimate, 95% confidence interval, and two-sided p-value will be reported for the donor diabetes status effect based on the linear regression model. Residual values will be examined for an approximate normal distribution. If residual values from the regression model have a skewed distribution then an appropriate transformation, truncation, or a nonparametric analysis based on ranks will be performed. Descriptive analyses (e.g., boxplots) stratified by donor diabetes status group will be given at baseline, 1 month, and 1 year.

8.8 Safety Analyses

The safety analysis will include events occurring on or after the day of surgery until and including the 12-month visit, or Day 464, whichever occurs first.

All reportable adverse events, operative complications and procedures, post-operative complications and procedures (e.g., dislocation of donor, graft detachment, air injection), and abnormalities noted on ocular exam will be tabulated by donor diabetes status group. These will include but not be limited to:

- Endophthalmitis
- Microbial keratitis
- IOP>25 mmHg
- Corneal Thickness >750 microns
- Definite signs of graft rejection
- Recipient stromal clarity = cloudy

8.9 Protocol Adherence and Retention

The following tabulations and analyses will be performed by donor diabetes status group:

- Protocol and procedural deviations
- Flow chart accounting for all enrolled participants
- Participants who dropped and reasons

8.10 Baseline Descriptive Statistics

Baseline demographic and clinical characteristics will be tabulated overall, and by donor diabetes status group. Additionally, baseline characteristics will be tabulated for cases with graft failure, cases with incomplete follow-up without graft failure, and cases with complete follow-up without graft failure.

Donor characteristics will also be tabulated.

8.11 Planned Interim Analyses

No formal interim efficacy analyses are planned for this study. Compiled safety data reports will be reviewed by the DSMC semi-annually.

8.12 Sub-Group Analyses

Subgroup analyses/assessments of effect modification (interaction) will be conducted separately for the graft failure within the first year after surgery and ECD at one-year. These analyses will be considered exploratory. Additionally, interpretation of the analyses will depend on whether the overall analysis demonstrates a significant treatment group difference; in the absence of such an overall difference, subgroup analyses will be interpreted with caution. The general approach for these exploratory analyses will be to add an interaction term for the subgroup factor, which will be noted in the SAP, by diabetes group into the models used for the primary analyses. For

continuous variables, results will be displayed in subgroups based on cutpoints although the analysis will utilize the variable as continuous. If there is insufficient sample size in a given subgroup, the cutpoints for continuous measures may be adjusted per the observed distribution of values. Cutpoint selection for display purposes will be made masked to the outcome data.

8.13 Multiple Comparison/Multiplicity

There will be no adjustment for primary and secondary analyses.

For comparison of all exploratory analyses, Benjamini-Hochberg false discovery rate adjusted p-values will be calculated within several subcategories.

8.14 Analysis of the Tertiary Exploratory Endpoint

Exploratory analyses will evaluate the effect of donor diabetes status on 1-year graft failure rate with donor diabetes severity defined in the alternative ways as measured by eye bank-determined diabetes risk categorization scores, post-mortem HbA1c, and skin AGEs and oxidation markers. Additionally, the effect of donor diabetes status on 1-year ECD with alternative definitions for donor diabetes severity will be examined. The alternative definitions will be detailed in the SAP.

Potential confounders or interaction terms determined in the analyses of graft failure and ECD will be explored in adjusted models mirroring the primary analyses.

8.15 Additional Tabulations and Analyses

8.15.1 Additional Tabulations

The following tabulations will be performed by donor diabetes status group:

- Additional post-operative study eye procedures
- Immunizations or vaccinations vs signs of graft rejection
- Ocular and systemic diabetic complications

8.15.2 Additional Analyses

The association of donor, recipient, and operative factors potentially related to graft failure within 1-year following DMEK will be evaluated and detailed in the SAP.

Chapter 9: Data Collection and Monitoring

9.1 Case Report Forms and Other Data Collection

Since data are captured retrospectively, direct data entry onto electronic case report forms (CRFs) is not feasible. Thus, paper worksheets will be provided to capture relevant post-operative data for transcription later, which is the preferred method of data capture. When utilized, these will be considered source documentation and must be maintained in the study records. If data is transcribed from the medical record onto electronic forms, the medical record is considered the source document. The source documents must be readily verifiable against the values entered into eCRF, and if paper worksheets are utilized they may be compared to concurrent medical records as well.

9.2 Study Records Retention

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

Study documents should be retained for a minimum of 3 years after completion of final grant reporting. 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 CWRU CC, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

9.3 Quality Assurance and Monitoring

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

A risk-based monitoring (RBM) plan will be developed and revised as needed during the course of the study, consistent with the FDA "Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring" (August 2013).[56]

For the DEKS, data of most importance for monitoring at the site are informed consent, proper characterization of donor and recipient diabetes status, and outcome assessments (graft failure). Therefore, the RBM plan will focus on these areas. All monitoring will be performed remotely. Elements of the RBM plan may include:

- Qualification assessment, training, and certification for sites and site personnel
- Oversight of Institutional Review Board (IRB) coverage and informed consent procedures
- Central (remote) data monitoring: validation of data entry, data edits/audit trail, protocol review of entered data and edits, statistical monitoring, study closeout
- Remotely monitored source data verification, site visit reports

- Tissue accountability
- Communications with site staff
- Patient retention and visit completion
- Quality control reports
- Management of noncompliance
- Documenting monitoring activities
- Adverse event reporting and monitoring

CC representatives or their designees may remotely "visit" the study facilities (eye banks as well as clinical sites) at any time in order to maintain current and personal knowledge of the study through review of records, comparison with source documents, observation and discussion of the conduct and progress of the study. The investigational site will provide access to all source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

9.4 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or procedure requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff.

Further details about the handling of protocol deviations will be included in the Site Manual of Procedures.

Chapter 10: Ethics/Protection of Human Participants

10.1 Ethical Standard

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

10.2 Institutional Review Boards

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

10.3 Informed Consent Process

10.3.1 Consent Procedures and Documentation

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

The participants should have the opportunity to discuss the study with their 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. They may need to complete execution of the informed consent form remotely using the process outlined in the clinical site Manual of Procedures.

The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.3.2 Participant and Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. 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.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, or regulatory agencies 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 and will be uploaded to a limited access database accessible to the CC at CWRU. 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 CWRU requirements.

Limited participant identifiable information (name and unique patient identifier) will be provided by the clinical sites to the eye banks per usual practices to satisfy EBAA regulations. Additionally, study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the JCHR. 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, eye banks and by JCHR research staff will be secured and password protected with access provided to the CC at CWRU, the Study Chairs and the CIARC as required. At the end of the study, all study databases will be fully de-identified and archived at the JCHR or the CWRU CIARC.

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

10.3.3 Future Use of Images and Data

Data collected for this study will be analyzed and stored at the JCHR. After the study is completed, the de-identified, archived data will be made publicly available. Further, some data and images may be requested and shared with other researchers under a Data Use Agreement as a limited data set, since other than date of collection, there are no other identifiable elements. No additional identifying information shall be provided in a manner that would make the human subjects readily identifiable.

Chapter 11: References

1. Stokes A, Preston SH. Deaths Attributable to Diabetes in the United States: Comparison of Data Sources and Estimation Approaches. *PLoS One*. 2017;12(1):e0170219.
2. American Diabetes Association. Statistics about Diabetes. American Diabetes Association. https://www.diabetes.org/resources/statistics/statistics-about-diabetes. Published 2021. Accessed April 20, 2021, 2021.
3. Lass JH, Riddlesworth TD, Gal RL, et al. The effect of donor diabetes history on graft failure and endothelial cell density 10 years after penetrating keratoplasty. *Ophthalmology*. 2015;122(3):448-456.
4. CDC National Diabetes Statistical Report. https://www.cdc.gov/diabetes/data/statistics-report/index.html. Accessed April 20, 2021.
5. Lass JH, Szczotka-Flynn LB, Ayala AR, et al. Cornea preservation time study: methods and potential impact on the cornea donor pool in the United States. *Cornea*. 2015;34(6):601-608.
6. Herse P, Adams L. Effect of hyperglycemia duration on rabbit corneal thickness and endothelial ATPase activity. *Acta Ophthalmol Scand*. 1995;73(2):158-161.
7. Kim J, Kim CS, Sohn E, Jeong IH, Kim H, Kim JS. Involvement of advanced glycation end products, oxidative stress and nuclear factor-kappaB in the development of diabetic keratopathy. *Graefes Arch Clin Exp Ophthalmol*. 2011;249(4):529-536.
8. Marano CW, Matschinsky FM. Biochemical manifestations of diabetes mellitus in microscopic layers of the cornea and retina. *Diabetes Metab Rev*. 1989;5(1):1-15.
9. Meyer LA, Ubels JL, Edelhauser HF. Corneal endothelial morphology in the rat. Effects of aging, diabetes, and topical aldose reductase inhibitor treatment. *Invest Ophthalmol Vis Sci*. 1988;29(6):940-948.
10. Inoue K, Kato S, Inoue Y, Amano S, Oshika T. The corneal endothelium and thickness in type II diabetes mellitus. *Jpn J Ophthalmol*. 2002;46(1):65-69.
11. Shimazaki J, Tsubota K, Yoshida A, Tornheim K, Laing RA. Changes of corneal redox state in diabetic animal models. *Cornea*. 1995;14(2):196-201.
12. Cisarik-Fredenburg P. Discoveries in research on diabetic keratopathy. *Optometry*. 2001;72(11):691-704.
13. Keoleian GM, Pach JM, Hodge DO, Trocme SD, Bourne WM. Structural and functional studies of the corneal endothelium in diabetes mellitus. *Am J Ophthalmol*. 1992;113(1):64-70.
14. Larsson LI, Bourne WM, Pach JM, Brubaker RF. Structure and function of the corneal endothelium in diabetes mellitus type I and type II. *Arch Ophthalmol*. 1996;114(1):9-14.
15. Lass JH, Spurney RV, Dutt RM, et al. A morphologic and fluorophotometric analysis of the corneal endothelium in type I diabetes mellitus and cystic fibrosis. *Am J Ophthalmol*. 1985;100(6):783-788.
16. Lee JS, Oum BS, Choi HY, Lee JE, Cho BM. Differences in corneal thickness and corneal endothelium related to duration in diabetes. *Eye (Lond)*. 2006;20(3):315-318.

17. McNamara NA, Brand RJ, Polse KA, Bourne WM. Corneal function during normal and high serum glucose levels in diabetes. *Invest Ophthalmol Vis Sci*. 1998;39(1):3-17.

18. Modis L, Jr., Szalai E, Kertesz K, Kemeny-Beke A, Kettesy B, Berta A. Evaluation of the corneal endothelium in patients with diabetes mellitus type I and II. *Histol Histopathol*.25(12):1531-1537.

19. Ravalico G, Tognetto D, Palomba M, Calderini S, Vattovani O. Corneal endothelial function in diabetes: a fluorophotometric study. *Ophthalmologica*. 1994;208(4):179-184.

20. Saini JS, Mittal S. In vivo assessment of corneal endothelial function in diabetes mellitus. *Arch Ophthalmol*. 1996;114(6):649-653.

21. Saini JS, Mittal S, Anand M. Cornea stress test--evaluation of corneal endothelial function in vivo by contact lens induced stress. *Indian J Ophthalmol*. 1997;45(1):19-24.

22. Sanchez-Thorin JC. The cornea in diabetes mellitus. *Int Ophthalmol Clin*. 1998;38(2):19-36.

23. Schultz RO, Matsuda M, Yee RW, Edelhauser HF, Schultz KJ. Corneal endothelial changes in type I and type II diabetes mellitus. *Am J Ophthalmol*. 1984;98(4):401-410.

24. Shenoy R, Khandekar R, Bialasiewicz A, Al Muniri A. Corneal endothelium in patients with diabetes mellitus: a historical cohort study. *Eur J Ophthalmol*. 2009;19(3):369-375.

25. Weston BC, Bourne WM, Polse KA, Hodge DO. Corneal hydration control in diabetes mellitus. *Invest Ophthalmol Vis Sci*. 1995;36(3):586-595.

26. Ziadi M, Moiroux P, d'Athis P, Bron A, Brun JM, Creuzot-Garcher C. Assessment of induced corneal hypoxia in diabetic patients. *Cornea*. 2002;21(5):453-457.

27. Aoki T, Kitazawa K, Deguchi H, Sotozono C. Current Evidence for Corynebacterium on the Ocular Surface. *Microorganisms*. 2021;9(2).

28. Rammaert B, Lanternier F, Poiree S, Kania R, Lortholary O. Diabetes and mucormycosis: a complex interplay. *Diabetes Metab*. 2012;38(3):193-204.

29. Chang YS, Tai MC, Ho CH, et al. Risk of Corneal Ulcer in Patients with Diabetes Mellitus: A Retrospective Large-Scale Cohort Study. *Sci Rep*. 2020;10(1):7388.

30. Arunga S, Kintoki GM, Gichuhi S, et al. Risk Factors of Microbial Keratitis in Uganda: A Case Control Study. *Ophthalmic Epidemiol*. 2020;27(2):98-104.

31. Nowak MS, Grzybowski A, Michalska-Malecka K, et al. Incidence and Characteristics of Endophthalmitis after Cataract Surgery in Poland, during 2010-2015. *Int J Environ Res Public Health*. 2019;16(12).

32. Knapp S. Diabetes and infection: is there a link?--A mini-review. *Gerontology*. 2013;59(2):99-104.

33. Vislisel JM, Liaboe CA, Wagoner MD, et al. Graft survival of diabetic versus nondiabetic donor tissue after initial keratoplasty. *Cornea*. 2015;34(4):370-374.

34. Price MO, Lisek M, Feng MT, Price FW, Jr. Effect of Donor and Recipient Diabetes Status on Descemet Membrane Endothelial Keratoplasty Adherence and Survival. *Cornea*. 2017;36(10):1184-1188.

35. Terry MA, Aldave AJ, Szczotka LB, et al. Donor, recipient, and operative factors associated with graft success in the Cornea Preservation Time Study. Ophthalmology. 2018;125:1700-1709.

36. Lass JH, Benetz BA, Patel SV, et al. Donor, Recipient, and Operative Factors Associated With Endothelial Cell Loss in the Cornea Preservation Time Study. JAMA *Ophthalmol*. 2019;137:185-193.

37. Aldave AJ, Terry MA, Szczotka-Flynn LB, et al. Effect of Graft Attachment Status and Intraocular Pressure on Descemet Stripping Automated Endothelial Keratoplasty Outcomes in the Cornea Preservation Time Study. *Am J Ophthalmol*. 2019;203:78-88.

38. Soper MC, Marcovina SM, Hoover CK, et al. Validity of Postmortem Glycated Hemoglobin to Determine Status of Diabetes Mellitus in Corneal Donors. *Cornea*. 2017;36(8):942-947.

39. Monnier VM, Genuth S, Sell DR. The pecking order of skin Advanced Glycation Endproducts (AGEs) as long-term markers of glycemic damage and risk factors for micro- and subclinical macrovascular disease progression in Type 1 diabetes. *Glycoconj* J. 2016;33(4):569-579.

40. Monnier VM, Sell DR, Strauch C, et al. The association between skin collagen glucosepane and past progression of microvascular and neuropathic complications in type 1 diabetes. *J Diabetes Complications*. 2013;27(2):141-149.

41. Monnier VM, Sun W, Gao X, et al. Skin collagen advanced glycation endproducts (AGEs) and the long-term progression of sub-clinical cardiovascular disease in type 1 diabetes. *Cardiovasc Diabetol*. 2015;14:118.

42. Sell DR, Sun W, Gao X, et al. Skin collagen fluorophore LW-1 versus skin fluorescence as markers for the long-term progression of subclinical macrovascular disease in type 1 diabetes. *Cardiovasc Diabetol*. 2016;15:30.

43. Williams RS, Mayko ZM, Friend DF, Straiko MD, Clay RD, Stoeger CG. Descemet membrane endothelial keratoplasty (DMEK) tissue preparation: a donor diabetes mellitus categorical risk stratification scale for assessing tissue suitability and reducing tissue loss. *Cornea*. 2016;35:927-931.

44. Eye Bank Association of America. 2020 Eye Banking Statistical Report. 2021.

45. Price MO, Feng MT, Price FW, Jr. Endothelial Keratoplasty Update 2020. *Cornea*. 2021;40(5):541-547.

46. What is the Uniform DRAI. American Association of Tissue Banks. https://www.aatb.org/standards/uniform-drai. Published 2021. Accessed April 20, 2021, 2021.

47. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes—2020. American Diabetes Association. https://care.diabetesjournals.org/content/43/Supplement_1/S14. Accessed April 20, 2021.

48. Bennett A, Mahmoud S, Drury D, et al. Impact of Donor Age on Corneal Endothelium-Descemet Membrane Layer Scroll Formation. *Eye Contact Lens*. 2015;41(4):236-239.

49. Stulting RD, Lass JH, Terry MA, et al. Factors associated with graft rejection in the Cornea Preservation Time Study. *Am J Ophthalmol*. 2018;196:197-207.

50. Benetz BA, Lass JH, Gal RL, et al. Endothelial morphometric measures to predict endothelial graft failure after penetrating keratoplasty. *JAMA Ophthalmol*. 2013;131(5):601-608.

51. Lass JH, Benetz BA, Verdier DD, et al. Corneal Endothelial Cell Loss 3 Years After Successful Descemet Stripping Automated Endothelial Keratoplasty in the Cornea Preservation Time Study: A Randomized Clinical Trial. *JAMA Ophthalmol*. 2017;135(12):1394-1400.

52. DRCR.net Public Website. DRCR Retina Network https://public.jaeb.org/drcrnet/view/Home_Page. Accessed April 20, 2021, 2021.

53. Advanced Research and Diagnostics Laboratory. Advanced Research and Diagnostics Laboratory | Medical School - University of Minnesota (umn.edu) Published 2021. Accessed June 23, 2021, 2021.

54. Rosenwasser GO, Szczotka-Flynn LB, Ayala AR, et al. Effect of Cornea Preservation Time on Success of Descemet Stripping Automated Endothelial Keratoplasty: A Randomized Clinical Trial. *JAMA Ophthalmol*. 2017;135(12):1401-1409.

55. Price MO, Feng MT, Scanameo A, Price FW, Jr. Loteprednol Etabonate 0.5% Gel Vs. Prednisolone Acetate 1% Solution After Descemet Membrane Endothelial Keratoplasty: Prospective Randomized Trial. *Cornea*. 2015;34(8):853-858.

56. Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring Guidance for Industry. Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring | FDA Published 2013. Accessed June 23, 2021, 2021.