Effect of Corneal Preservation Time on Long-Term Graft Success
Cornea Preservation Time Study (CPTS)
Protocol

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Study Chair: Jonathan Lass MD
Department of Ophthalmology & Visual Sciences
Case Western Reserve University
University Hospitals Eye Institute
11100 Euclid Ave; 4129 Lakeside
Cleveland, OH 44106

Data Management and Analysis Center (DMAC):
Director: Allison Edwards MS
Jaeb Center for Health Research
15310 Amberly Drive, Suite 350
Tampa, FL 33647

Coordinating Center (CC):
Director: Loretta Szczotka-Flynn OD, PhD
Department of Ophthalmology & Visual Sciences
Vision Research Coordinating Center
Case Western Reserve University
University Hospitals Eye Institute
11100 Euclid Ave; 4126 Lakeside
Cleveland, OH 44106

Cornea Image Analysis Reading Center (CIARC):
Director: Beth Ann Benetz, M.A.
Department of Ophthalmology & Visual Sciences
Case Western Reserve University
University Hospitals Eye Institute
11100 Euclid Ave; Wearn 646
Cleveland, OH 44106

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Chapter 1
BACKGROUND AND RATIONALE

1.1 Background

The Cornea Donor Study (CDS) was conceived in 1997 when a real threat to the donor pool was present based on the concerns of an emerging HIV and hepatitis epidemic, the impact of refractive surgery procedures, an aging population with a rise in the number of Fuchs' Endothelial Corneal Dystrophy (FECD) and pseudophakic bullous keratopathy (PBK) cases in the United States, and a worldwide demand for corneal tissue. This was most prescient, since the number of corneas provided by eye banks in the United States subsequently rose from 43,492 cases in 1997 to 59,271 in 2010, a 37% increase in demand.(1) This demand has been met by our phenomenal success in changing the perception among patients, surgeons, and the eye bank community that older donor tissue (> 65 years of age to 75) is as suitable and successful in keratoplasty as younger tissue, at least for the management of FECD and PBK cases. The use of older donor tissue has resulted in opening up a whole pool of tissue that would not have been used by many surgeons in the United States. This change in perception is the result of a simple, but powerful, evidence-based prospective, masked clinical trial, which showed 86% graft success following penetrating keratoplasty (PKP) for endothelial dysfunction conditions at 5 years in both the ≥ 65 to 75 year donor group and the <65 donor group. As an aside, the CDS also demonstrated the tremendous value of an academic and community network of well trained corneal surgeons and their ability to work with a coordinating center and corneal endothelial image analysis reading center to produce high quality and reliable data with a tremendous impact on practice patterns in corneal transplantation.

Several factors which had the potential to impact the donor supply in 1997 still remain, new issues have arisen, and all impact the future efficient provision donor tissue and need for increasing the donor supply:

1. The donor pool is impacted by a continued threat from common viral infections, in particular Hepatitis B, with 2,698 donors alone rejected in 2008 and 3,631 donors in 2010 (a 34% increase in unusable tissue from the previous year) based on a positive Hepatitis B Core (HBcAB) antibody test.(1)
2. With increasing FDA regulations towards testing for emerging infections, such as West Nile Virus (from 6 cases in 2008 to 97 cases in 2010, a more than 15 fold increase,(1) and Chagas Disease,(2.3) more tissue will be rejected, or delays in test results will result in cancellation of transplants. In addition, as additional serologic testing is added, the test could delay tissue release or result in false positives that could even decrease tissue supply.
3. For serious potentially transmissible diseases, such as Prion agents, no reliable laboratory test currently exists and only historical screening can be used to safeguard transplant recipients. This strategy is justified only as long as the general population prevalence rate of Prion disease is small. If testing for potential slow virus diseases disqualifies a substantial number of donors, this could have a tremendous negative impact on the donor pool.
4. The impact of an expanding aging population over 65 with the addition of the baby boomers will result in a greater number of FECD and PBK cases, and increased demand for tissue.
5. The phenomenal growth of endothelial keratoplasty (EK), specifically Descemet Stripping Endothelial Keratoplasty (DSEK) or Descemet Stripping Automated Endothelial

Keratoplasty with the use of an automated microkeratome (DSAEK), in the past five years with a more than three-fold increase from 6,027 cases in 2006 to 19,159 in 2010(1) has opened up a new pool of patients in which endothelial dysfunction cases are being treated surgically before structural damage occurs.(4) This growth has been assisted by improved efficiency as a result of an increasing number of eye banks preparing the donor tissue for the EK procedure, rather than in the operating room by the surgeon. At the same time, the primary donor failure rate for EK has been reported as high as 5% on average(4) is 10x as high as in PKP (e.g. in the CDS, reference 5). As this procedure gains even wider acceptance, further impact on the donor pool will occur. Although donors that have anterior scars or have undergone refractive surgical procedures may now be used for EK,(6) this positive impact on the donor pool is offset by the earlier implementation of a surgical approach with EK and a higher primary donor failure rate.

6. More tissue will also be needed in the future due to a potentially higher % yield loss of tissue during the process of tissue cutting either for DSAEK or new procedures still under development, including Descemet Membrane Automated Endothelial Keratoplasty (DMAEK) and Descemet Membrane Endothelial Keratoplasty (DMEK).
7. An increasing number of donors are being rejected because of cataract surgery incisions that are too close to the central cornea. As the use of older donor tissue from previous cataract surgery patients becomes more commonplace, prior endothelial damage from these cataract incision wounds will impact the use of the larger donor EK grafts which are commonly up to 9 mm in diameter (personal communication, Gerald Cole, Tissue Banks International).
8. Unlike Canada, where transplant recipients must be on a long waiting list, American surgeons and their patients have been fortunate to generally have their transplant surgical cases on a scheduled basis. With all the issues above, cancelled surgeries due to the lack of tissue could occur frequently, resulting in lost revenues and added cost to the facility, the surgeon, and in some cases the patient and families who have taken off work for the procedure.

With all these concerns listed above, flexibility with the use of longer preserved corneas within the FDA guidelines will help increase the donor tissue used domestically and help to obviate these concerns as they may arise.

The status of the national donor supply remains dynamic with fluctuations on a daily, weekly, and monthly basis depending on donor, surgeon, and patient supply and demand. It is these dynamic fluctuations and the various threats that loom to the donor supply listed above, including emerging infections, which have driven the eye banking community to continually find better ways to have a more orderly donor tissue supply with maximum flexibility within FDA limits for tissue usage. This continuing desire has led to the strong reaffirmation of this proposal by the eye bank and surgeon community to help assure that there will be an adequate supply of transplantable donor tissue when the study would be completed and results publicized in 2016-17.

1.1.1 Studies of Preservation Time

Most studies have examined either death to surgery or time from preservation in medium to surgery (preservation time) as a possible confounding variable or potential factor influencing corneal clarity without it being the primary variable of interest; thus, the clinical outcome of

graft success and its relation to preservation time has been unclear. In fact, there have been no prospective masked trials that have randomized donor groups on this basis.

PKP Findings:

- Chang et al(7) and Abbott et al(8) indicate that death-to-surgery times were not positively correlated with graft clarity following PKP.
- Wagoner et al(9) showed in a retrospective study of 234 PKPs utilizing donor tissue ranging in preservation time from 168 to 348 hours in Optisol GS that the likelihood of graft survival was not statistically significantly affected by progressively longer periods of donor storage time with no primary donor failures.
- Doganay et al(10) with a group of 48 patients undergoing PKP for keratoconus, FECD, and PBK, examined preservation time in one group up to an average of 233 hours in Optisol GS (n=18) compared to another group on average of 21 hours. No difference on graft survival was noted in this small series.
- Sugar et al(11) noted that an increase in stromal edema and Descemet folds increased with higher death-to-preservation time following PKP in the CDS, but death-to-surgery was not a variable of interest and the time from death to use was limited to 5 days.

EK Findings:

- Guttman(12) in a small, non-peer reviewed report, found greater cell loss at six months following EK in those corneas that were used over 96 hours with the correlation lost at 12 months. The highest death to use time observed in the study was 182 hours (7.6 days).
- Price et al(13) showed with EK that ECD was not found to be significantly correlated with death to preservation or death to use time.
- Chen et al(14) and Terry et al(15) found no influence of the time from death to implantation on graft success following EK, but mean time was approximately 95 hours. This lack of correlation of storage time with graft success was also emphasized in an editorial by Terry.(16)
- Terry et al(17) in 362 eyes following EK with storage time averaging 99 hours (range 21 to 186 hrs), showed no difference in cell loss at 2 years for those stored up to 4 days compared to those stored up to 8 days.

1.2 Rationale

This study addresses an important public health issue related to the utilization of donor tissue for corneal transplantation and the need to increase and secure the donor pool. Similar to the bias which existed regarding donor age prior to the initiation of the CDS, the majority of corneal surgeons in the United States do not accept tissue with preservation time longer than 7 to 8 days, even though the FDA approval of Optisol GS, since its introduction in the early 90's, is for a preservation time of up to 14 days. Instead these corneas are exported to the international community where they are routinely transplanted up to 14 days of storage. A lack of information, particularly on a preservation time over 7 days, has likely contributed to a bias against using corneal tissue beyond this time. This study will address this bias by examining two parameters of long-term success: recipient corneal (stromal) clarity and endothelial cell density following EK (4) for the endothelial dysfunction conditions that have moderate risk for failure, FECD and PBK. Demand for corneas has substantially increased with the advent of EK.(1) In addition to the increased demand, there are additional areas of concern which may impact the

future donor supply including potential changes in the cornea evaluation process such as increased regulations and more extensive laboratory requirements to test for emerging infections, e.g. Hepatitis B and C, West Nile Virus, Chagas Disease.(2.3) By changing the practice pattern and increasing utilization of tissue beyond 7 days up to the FDA approved, 14 days from preservation to surgery, this will facilitate increasing the domestic donor supply enabling easier distribution of tissue and more time for tissue evaluation to rule out emerging infections with suspected donors.

1.3 Study Objectives

The primary objectives of the "Effect of Corneal Preservation Time on Long-Term Graft Success" (CPTS) study are:

- To determine if the 3-year graft failure rate following EK performed with donor corneas with a preservation time of 8 to14 days is non-inferior to the failure rate when donor corneas with a preservation time of 7 or fewer days are used.
- To determine if the central corneal endothelial cell density 3-years after EK is related to preservation time.
- To evaluate the effect of donor, operative and postoperative factors on graft failure and endothelial cell density three years following EK.

1.4 Synopsis of Study Design

1.4.1. Study Design

The CPTS is a randomized, controlled clinical trial examining the impact of preservation time on graft failure and endothelial cell density following EK. The study has been designed so that the surgeons and eye banks can follow and provide their usual surgical and post-operative procedures and care to study participants with the exception of assignment of donor tissue. Study eyes will be randomly assigned to receive a donor cornea from preservation date to surgery date of 8 to 14 days or a donor cornea from preservation date to surgery date of 7 or fewer days, with a comparable death to preservation time for both groups. Surgeons and study participants are masked to time from preservation to surgery.

1.4.2. Major Eligibility Criteria

1.4.2.1. Study Participants

Major eligibility criteria include:

- Study participant age between 30 and <91 years with a minimum life expectancy of 3 years and at least one eligible eye
- Study eye is a candidate for EK due to one of two conditions related to endothelial dysfunction:
  - Fuchs' Endothelial Corneal Dystrophy (FECD)
  - Aphakic/pseudophakic corneal edema
- Eyes with anterior chamber intraocular lens (IOL) are excluded

Specific eligibility criteria are listed in Section 3.1. The determination of eye eligibility is performed at the time EK surgery is planned. A participant can have two study eyes if both eyes are eligible. The eligibility of the second eye would be assessed at the time surgery on the

second eye is being scheduled. Surgery on the second study eye can be performed no sooner than 6 weeks after EK on the first study eye.

1.4.2.2. Donor Corneas

Eye banks will follow their procedural routine for procurement of tissue and determining its suitability for EK, including prior LASIK or photorefractive keratectomy donors, in accordance with the Medical Standards and Procedure Manual of the EBAA(19,20). This includes standard serologic testing, specular microscopy, and slit lamp examination.

The following major eligibility criteria will apply to all donor tissue assigned to study eyes:

- Meets current EBAA and eye bank standards for human transplantation
- Age of donor at time of death 10-75 years
- If the donor body was refrigerated or eyes on ice within 10 hours of death, the body or eye may stay refrigerated up to $\leq$20 hrs; if no refrigeration then the death to preservation time should be $\leq$10 hrs
- Eye bank determined minimum ECD of $\geq$ 2300 cells/mm^2 (upon the initial screening determination of ECD)
- Polymorphism/Polymegethism: None to no more than mild changes (slight)
- Guttae: no true guttae present
- No evidence of central endothelial cell damage/trauma or dystrophy, such as FECD

1.4.2.3. Treatment Groups

Enrolled eyes of study participants will be randomly assigned to receive either a donor cornea with a preservation time of 8 to 14 days or a donor cornea with a preservation time of 7 or fewer days.

1.4.2.4. Sample Size

1,330 study eyes from up to 1,330 study participants, depending on what proportion of participants elect and are eligible to enroll both eyes.

1.4.2.5. Visit Schedule and Procedures

Enrolled eyes of study participants will be examined at a baseline/enrollment visit, at the time of EK surgery, and at post-operative visits at 1 day, 1 week, 1 month, 6, 12, 24, and 36 months in addition to any routine care visits. Participants with two study eyes will follow a modified visit schedule to minimize return visits for participants that had bilateral EK as part of the study.

Procedures at each protocol visit will follow the surgeon's standard of care in addition to detailed and standardized measurements of recipient and donor corneal stroma clarity, pachymetry, and central endothelial cell density as outlined in the Table in Section 4.3. Non-protocol visits will follow the surgeon's standard of care.

1.4.2.6. Outcomes

***Primary Outcome Measure*:** Graft failure, defined as the occurrence of one of the following within 3 years of surgery:

- Regrafting of the study eye for any reason
- Cornea which remains cloudy without clearing, according to the following:

  (1) cloudy cornea on the first postoperative day which does not clear within 8 weeks

  OR

  (2) cloudy cornea which was initially clear postoperatively but becomes and remains cloudy for 3 months without clearing.

Secondary Outcome Measure: Endothelial cell density at 3 years from surgery, conditional on graft survival at 3 years from surgery.

1.5 General Considerations

The study is being conducted in accordance 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. The CPTS Procedures Manuals provide details of the procedures followed by the eye banks and by the clinical sites. Data will be directly collected in electronic case report forms, which will be considered the source data.

Chapter 2
ELIGIBILITY AND ENROLLMENT
CLINICAL SITES

2.1 Eligibility Assessment

Eligibility is assessed during a routine examination by an investigator, as there are no examination procedures required to assess patient eligibility other than those that are part of standard patient care.

A participant can have two study eyes if both eyes are eligible at the time of enrollment or if the second eye becomes eligible at a later time. The determination of eye eligibility is performed at the time EK surgery is planned, 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 can be performed no sooner than 6 weeks after EK on the first eye. Participants with two eligible eyes will have the option of deciding whether to include one or both eyes in the study.

2.2 Eligibility Criteria

To be eligible, a study participant must meet the participant inclusion criteria and have at least one eye meeting the study eye inclusion criteria and none of the exclusion criteria.

2.2.1. Study Participant Eligibility Criteria

2.2.1.1 Study Participant Inclusion Criteria

1) Age range between 30 and <91 years with minimum life expectancy of at least 3 years.
2) Willingness to return for follow-up study visits at 1 day, 1 week, 1 month, 6 months, 1 year, 2 years and 3 years.
3) Fluent in English or Spanish.

2.2.1.2 Study Participant Exclusion Criteria

1) Decisionally and/or cognitively impaired

2.2.2 Study Eye Inclusion Criteria

1) EK is scheduled between 10 and 60 days after enrollment
    - *The 10-day requirement relates to the need to be able to randomly assign the eye to either intervention group.*
    - *The 60-day requirement relates to the need to have current eligibility and enrollment data at the time of surgery. If surgery is postponed to >60 days after the initial enrollment visit, a new Baseline Visit and eligibility assessment will have to be performed.*
2) Presence of a condition related to endothelial dysfunction which will be treated by EK.
    - Eligible indications for EK include:
        a. Presence of FECD meeting at least one of the following:
            - Phakic FECD
            - Phakic FECD with cataract
                - Triple procedure including EK for FECD, cataract extraction and posterior chamber intraocular lens implantation (IOL) is allowed

- Aphakic FECD
- Pseudophakic FECD with posterior capsule supported, suture-fixated, or sulcus-supported posterior chamber IOL

b. Aphakic or pseudophakic corneal edema with posterior capsule supported, suture-fixated, or sulcus-supported posterior chamber IOL without FECD

2.2.3 Study Eye Exclusion Criteria

1) Prior EK
2) Indication for surgery that is not suitable for EK (e.g, keratoconus, stromal dystrophies and scars)
3) Presence of a condition that has a very high probability for failure (e.g., failed EK or PKP, heavily vascularized cornea, uncontrolled uveitis)
4) Other primary endothelial dysfunction conditions including posterior polymorphous corneal dystrophy and congenital hereditary corneal dystrophy
5) Anterior chamber IOL in study eye prior to or anticipated during EK
6) Planned intraocular lens exchange of an anterior chamber IOL with a posterior chamber IOL in study eye at time of study EK
7) Pre-operative central sub-epithelial or stromal scarring that the investigator believes is visually significant and could impact post-operative stromal clarity assessment
8) Stromal vascularization that is visually significant (by investigator's judgment)
9) Presence of anterior synechiae (iris to cornea)
10) Peripheral anterior synechiae (iris to angle) in the angle greater than a total of three clock hours
11) Hypotony (Intraocular pressure <10 mm Hg)
12) Uncontrolled (defined as intraocular pressure > 25mm Hg) glaucoma with or without prior filtering surgery or shunt or mini-shunt placement.

A shunt or mini-shunt is any device implanted to lower intraocular pressure through an external route (e.g Ahmed) or internal route (e.g. Glaukos) that is present in the anterior chamber angle or extends into the anterior chamber.

13) Controlled glaucoma with prior shunt or mini-shunt placement for glaucoma
    - Note: FECD or pseudophakic/aphakic corneal edema with posterior chamber IOL that also have undergone filtering surgery (without shunt or mini-shunt) in which glaucoma is currently considered under control will be eligible
14) Fellow eye visual acuity < 20/200 that is not correctable with EK

2.3 Eligibility Criteria for Second Study Eye

1) Study participant has already enrolled one eye
2) The second eye meets all study eye inclusion and exclusion criteria (2.2.2 and 2.2.3)
3) EK surgery in second eye is not planned within 6 weeks of EK on first study eye

2.4 Screening Evaluation and Baseline Testing

2.4.1 Historical Information

A history will be elicited from the potential study participant and extracted from available medical records. It is anticipated that potential participants will be patients within the practices

of the site investigator who are deciding to undergo EK with the respective surgeon. Thus, obtaining histories relevant to the CPTS eligibility criteria will be part of routine care.

2.4.2 Baseline Testing

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

In addition to the usual assessment for candidates for EK for the two acceptable conditions for the CPTS, the surgeons will grade disease severity in those study participants who have FECD employing the FECD Genetics Multi-center Study Grading Assessment Guide (18). Family history of FECD will be solicited.

2.5 Subject Enrollment

A maximum of 1330 participants will be enrolled, depending on the number of participants who enroll both eyes into the study to reach the recruitment goal of 1330 study eyes with a goal to enroll an appropriate representation of minorities. As the enrollment goal approaches, sites will be notified of the end date for recruitment. Study participants who have signed an informed consent form can be randomized up until the end date.

After the informed consent form is signed, enrollment will be accomplished using the study website. Enrollment must be completed at least 10 days prior to the date of surgery. The study participant is then managed according to the investigator's usual routine without regard to the fact that the study participant is participating in the trial.

2.6 Randomization

Randomization of participant eyes will occur via an automated computer program. The randomization schedule will be stratified by surgeon using a permuted blocks design. The randomization groups are as follows:

- Preservation Time Group: $\leq$7 days
- Preservation Time Group: 8-14 days

A study participant may have both eyes enrolled in the study. The eye scheduled for surgery which will occur first will be assigned randomly to a preservation time group, and the eye scheduled for surgery which will occur second will be assigned to the alternate group.

Chapter 3
DONOR ELIGIBILITY AND CORNEA ASSIGNMENT
EYE BANKS

3.1 Eye Bank Procedures

With the exception of procedures related to assignment of a cornea to a participant eye, specular microscopy external calibration and technician certification procedures and study procedures for obtaining specular images, eye bank procedures will mimic standard procedures as closely as possible to minimize disruption to their normal routine. Eye banks will be able to use any FDA-approved media for intermediate term storage up to 14 days at 4^{o}C.

3.1.1 Donor Eligibility

All eye banks will follow their procedural routine for procurement of tissue and determination of suitability for EK, including prior LASIK or PRK donors, in accordance with the Medical Standards and Procedure Manual of the EBAA(19,20). This includes standard serologic testing, specular microscopy, and slit lamp examination.

The following eligibility criteria will apply to all donor tissue assigned to participant eyes:

- Obtained from an EBAA accredited eye bank
- Meets current EBAA and eye bank standards for human transplantation
- Age of donor at time of death 10-75 years
- If the donor body was refrigerated or eyes on ice within 10 hours of death, the body or eye may stay refrigerated up to $\leq$20 hrs; if no refrigeration then the death to preservation time should be $\leq$10 hrs
- Eye bank determined minimum ECD of $\geq$ 2300 cells/mm^2 (upon the initial screening determination of ECD)
- Polymorphism/Polymegethism: None to no more than mild changes (slight)
- Guttae: no true guttae present
- No evidence of central endothelial cell damage/trauma or dystrophy, such as FECD.

3.1.2 Assignment of Donor to Study Eyes

The primary eye bank, as designated by the clinical site, will receive a notification when there is a pending donor assignment. The Eye Bank Procedures Manual provides details regarding the assignment process. When the eye bank submits an assignment request, a computer program will use a minimization algorithm to evaluate all available donors and ensure a balance of subgroups (0-4 days, 5-7 days, 8-11 days, 12-14 days from preservation to surgery) within the primary randomization groups. Time from preservation to scheduled surgery date will be calculated as whole days for the purpose of assignment into the appropriate randomized preservation time group. As surgeons will be masked to all donor information, the label and report that accompanies the donor will be generated from the study website in such a way to maintain masking.

After the donor assignment procedure is completed, the eye bank will complete a donor information form on the study website, which includes information about the retrieval of the cornea (date/time of death, date/time of retrieval, aspects of the processing), cause of death, age of the donor, and ECD.

For those assignments where pre-cut tissue has been requested, the eye bank will cut and prepare the tissue on the same day that they would cut tissue if the donor was being assigned to a non-study participant for that particular surgeon, and will complete a cutting information form.

3.1.2.1 Donor Not Available

If an eligible donor in the correct preservation time window is not available at the primary eye bank on the date of assignment, attempts will be made by the primary eye bank to import tissue from another EBAA accredited eye bank. If tissue in the correct preservation time window is still not available, it will be up to the surgeon to either (1) reschedule the surgery or (2) inform the participant that they will receive a donor that is otherwise healthy but does not meet criteria to be in the study and that they will be discontinued from the study.

3.1.2.2 Rescheduled Surgery and/or Reassignment of Tissue

If the surgeon rejects the assigned donor cornea for any reason, surgery will be rescheduled, and a new assignment will be completed.

If surgery is rescheduled for any reason, and an already assigned donor cornea is no longer in the correct preservation time window, a new assignment will be completed.

3.1.3 Eye Bank Procedures for Study Images

Detailed procedures for obtaining best image quality and transmission to the DMAC will be provided in the CPTS-CIARC Calibration, Certification, and Study Imaging Clinical Procedure Manual and the technician(s) performing this procedure will be certified by the Cornea Image Analysis Reading Center (CIARC).

3.1.3.1 Screening Images

Up to 3 screening images of the central donor corneal endothelium obtained according to the eye bank's usual procedure should be submitted to the DMAC.

3.1.3.2 Pre-Operative Images

Three pre-operative images of the central donor corneal endothelium should be obtained in a viewing chamber by a certified technician, and submitted to the DMAC. If the eye bank is performing the cutting, the eye bank should obtain these pre-operative images after the tissue has been cut. If the surgeon is performing the cutting, the eye bank should obtain these pre-operative images as close as possible prior to shipment to the surgeon following appropriate warming to room temperature.

Chapter 4
TRANSPLANTATION AND FOLLOW UP CLINICAL SITES

4.1 Endothelial keratoplasty procedure

Surgery (DSEK, DSAEK) 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 usage, other procedures (e.g. cataract surgery), and operative complications (e.g., difficult donor preparation, difficult placement). *As a reminder, the DMAEK and DMEK procedures will not be acceptable endothelial keratoplasty procedures for the CPTS nor will be PKP.*

The surgeon will be masked to donor parameters (e.g. donor age, donor ECD), except the FDA-approved 4^0C preservation medium being employed (Optisol GS, Life 4^0C, etc.) and parameters needed to perform the surgery (e.g. post-cut thickness). Most importantly the surgeon and study participant will be masked to preservation time.

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) will be collected on the data forms.

4.3 Follow-up visit schedule

Protocol-specified follow-up visits (and visit windows) for the first eye, established to conform to the usual practice and timed from surgery date, will be as follows:

- Day 1 (1-2 days)
- Day 7 (5 – 9 days)
- Day 30 (20-40 days)
- 6 months (4-8 months)
- 12 months (10-14 months)
- 24 months (20 - 28 months)
- 36 months (35 – 42 months)

Additional visits can be performed more often at the discretion of the investigator. A data form will be completed for each protocol and non-protocol visit. For example, if graft failure is determined and a regraft is required on a non-protocol visit, the appropriate follow-up visit form and graft failure form should be completed if and when this occurs.

If the second eye of an active participant is enrolled, a modified visit schedule will be allowed to minimize return visits for participants that had bilateral EK as part of the study. For example, sites will be allowed to follow standard of care practices to avoid unnecessary visits linked to targeting each eye within its respective windows as listed above. Sites are encouraged to schedule both eyes within their respective windows when the windows overlap, but at minimum at least one eye must be within its target visit window at each visit. The only exception to this is the 36 month visit which must be completed for each eye within its respective window, even it an extra return visit is required.

4.4 Testing procedures

The Table below shows the key elements of data collection at each study visit. Additional visits may occur as needed for the usual care of the participant.

	Pre-op	1 Day	1 Week	1 Month	6 Months	12 Months	24 Months	36 Months
Parameters								
Medication History	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Slit lamp examination	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Intraocular pressure	**X**		**X**	**X**	**X**	**X**	**X**	**X**
Ultrasonic pachymetry			**X**	**X**	**X**	**X**	**X**	**X**
Endothelial imaging					**X**	**X**	**X**	**X**
Post-op complications and other untoward events		**X**	**X**	**X**	**X**	**X**	**X**	**X**

4.5 Definition of testing procedures

4.5.1 Slit Lamp Examination

The slit lamp examination should be performed per the investigator's usual routine. Specific details of the data collected during the slit lamp examination are found in the site procedures manual.

4.5.1.1 Recipient corneal stroma clarity

The recipient corneal stroma clarity will be assessed by slit lamp examination using the following 3-level classification:

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

Specific details regarding the grading of recipient corneal stroma clarity 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.

4.5.1.2 Donor corneal stroma clarity

Donor corneal stroma clarity will be assessed by slit lamp examination. Specific details regarding the grading of donor corneal stroma clarity are found in the Site Procedures Manual.

4.5.1.3 Graft rejection assessment

Graft rejection will be assessed during the slit lamp examination using a modification of the Collaborative Corneal Transplantation Studies (CCTS) classification (21,22). 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 prerogative, but documented in the medication history.

4.5.2 Intraocular pressure

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

4.5.3 Ultrasonic pachymetry

Corneal thickness will be measured by a CPTS-provided ultrasonic pachymeter to ensure standardization of this measurement across sites. Technical staff acquiring this measurement will be trained and certified on study pachymeter use. If no measurement can be obtained (e.g. if the cornea is too thick), this will be noted on the data form. Measurements taken on other pachymeters will only be allowed if the study pachymeter is temporarily not functional.

4.5.4 Specular/confocal microscopy

Specular or confocal microscopy of the central endothelium will be obtained on all participants that have not experienced graft failure to determine ECD by CIARC. Detailed procedures for obtaining best image quality and transmission to the DMAC will be provided in the CPTS-CIARC Calibration, Certification, and Study Imaging Clinical Procedure Manual and the technician(s) performing this procedure will be certified by the CIARC.

4.6 Additional procedures

Data on all additional procedures performed on the study eye will be collected, including:

- 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)

Additionally, data on donor tissue rim cultures may be collected if performed as part of standard of care.

4.7 Graft Failure

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 on the first postoperative day which does not clear within 8 weeks

  OR

  (2) cloudy cornea which was initially clear postoperatively but becomes and remains cloudy for 3 months without clearing.

  - *A study participant whose cornea becomes cloudy (clouded recipient central stroma, based on the modified CDS 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 on the first postoperative day which does not clear or requires a regraft within 8 weeks), associated with surgical complications
- Primary donor failure (cloudy cornea on the first postoperative day 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).

4.8 Final Status

A Participant Final Status Form will be completed if the participant dies, withdraws, or is deemed to be lost to follow-up by the CC staff. An Eye Final Status Form will be completed if a study eye is re-grafted, receives an AC IOL during surgery, experiences a suprachoroidal hemorrhage during surgery, receives a non-study donor cornea, will no longer have surgery, or experiences enucleation, phthisis, graft failure due to blunt trauma, or graft failure due to penetrating trauma,.

CHAPTER 5
ADVERSE EVENT REPORTING

5.1 Adverse Event Reporting and Review

Adverse event information will be captured on the electronic case report forms or separate adverse event forms completed after surgery and at all post-op visits. Adverse events can be systemic or ocular. Death will be reported whether study related or not, with cause of death if known; systemic events will be reported only if related or possibly related to study procedures. Related and unrelated ocular adverse events will be reported only in the study eye as there is no plausible reason to believe that the EK procedure could affect a non-study eye.

Each site will be responsible for informing the CC of any reportable adverse events as outlined in the Site Procedures Manual. The study chair will be responsible for abiding by reporting requirements within the necessary time frames to the UHCMC IRB, NEI program officer, and FDA, as required. Each Principal Investigator is responsible for abiding by reporting requirements specific to his/her IRB.

Certain adverse events may require expedited reporting. Since this study does not involve investigational drugs or devices and participants in this study would have undergone EK regardless of study participation, expedited reporting of serious adverse events will be limited to unanticipated and/or serious events in the study eye that are related or possibly related to preservation time. A list of events that require expedited reporting was determined in conjunction with the DSMC. The following events require separate adverse form completion and expedited reporting by the site to the CC within 1 working day of learning of the event, and then subsequently by the CC to the Medical Monitor on the same working day of notification and to the NEI Program Office and designated DSMC member(s) within 1 week of notification:

- Endophthalmitis
- Microbial keratitis (bacterial, fungal, parasitic) within 3 months of EK
- Other unexpected, serious adverse events related or possibly related to preservation time

Operative and post-operative complications and all other adverse ocular findings will be recorded on the case report forms and tabulated in semi-annual DSMC reports. Adverse events presumed related to preservation time, study follow-up procedures of specular microscopy and pachymetry, or systemic events related to EK will be captured on separate adverse event forms. The DSMC will be provided the expedited adverse event reports and the tabulated semi-annual reports in a manner that will enable them to unmask the treatment group if desired.

5.2 Data and Safety Monitoring Committee Review of Adverse Events

The DSMC has approved the protocol and template informed consent form; they will also approve substantive amendments and will provide independent monitoring of adverse events. Cumulative adverse event data are semi-annually tabulated for review by the DSMC. Following each DSMC data review, a summary will be provided to IRBs. A list of specific adverse events to be reported to the DSMC expeditiously is given in section 5.1

5.3 Risks and Discomforts

The risks and discomforts for patients undergoing EK are the same regardless of study participation. Potential risks 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 the topical drops can cause an allergic reaction, seizures, and an irregular heartbeat.
- other potential risks include developing glaucoma, additional surgery due to healing problems or movement out of position of the donor cornea, retinal swelling or detachment, or loss of vision.
- rejection reactions occur approximately 10% of the time (23), but are usually reversible if treated promptly with topical corticosteroids, but sometimes it leads to failure of the transplant.
- measurement of intraocular pressure involves a topical anesthetic and fluorescein dye carries a small risk of corneal abrasion and temporary corneal discomfort. There is the rare possibility of allergic reaction to the dye or anesthetic drops.
- Other risks of EK include:
    - Endophthalmitis: a serious infection inside the eye that needs 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 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.

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

- The anesthetics or instruments that touch the eye to check corneal thickness (pachymeter) or image the endothelium (confocal or specular microscope) could cause minor irritation and rarely breakdown of the surface corneal cells. There is the rare possibility of allergic reaction to the anesthetic drops or feeling faint from the procedure.

CHAPTER 6
MISCELLANEOUS CONSIDERATIONS

6.1 Potential Benefits to Subjects

Study participants will not benefit directly from participation in this study. If longer preservation time up to the FDA limit of 14 days can be shown to not adversely impact graft success and endothelial cell density at 3 years, more donor tissue will be available for efficient distribution within the United States for all keratoplasty procedures. In the future, if a study participant requires another EK in either the same eye or their other eye, the information obtained from this study might benefit them.

6.2 Alternative(s) to Participation

Because of the nature of the study, the only other alternative is to not participate in the study. The potential participants' standing with his or her physician and/or hospital will not change.

6.3 Special Considerations in Follow-up

In a long-term trial such as this in which outcome is not assessed for several years after enrollment, special measures are necessary to assure that the participants will remain in follow up and return for the outcome assessment examination. Detailed contact information will be collected at the time of enrollment and updated regularly.

The Coordinating Center will maintain contact with each patient. Permission for such contacts will be included in the Informed Consent Form. The principal purpose of the contacts will be to develop and maintain rapport with the participant and to update contact information. The initial phone contact will occur about one month following the EK surgery. Subsequent phone contacts will occur on a semi-annual basis. Based on the experience with these calls by the Jaeb Center in the CDS, the CC will similarly maintain this type of contact with participants throughout their 3 year time in the study which the CC and DMAC believe is critically important for the validity of the study. The purpose of these calls is <u>not</u> to collect study data to be used for monitoring or in analyses or to provide medical information. Nor is it intended to schedule the participants for their study visits; that is up to the local study coordinator. This plan has been reviewed by the IRB at UH Case Medical Center and felt to be feasible as long as incorporated into the consent form at all our IRBs monitoring the study.

For participants who move out of the area of their study physician or whose medical insurance coverage changes, an attempt will be made to have their care transferred to another study physician. When this is not possible, the CC will locate an ophthalmologist in the participant's new area to arrange for follow-up and the participant will be asked to sign a medical record release form to provide the ophthalmologist with information as well as to obtain the results of examinations performed by the ophthalmologist.

6.4 Women and Minorities

We anticipate that study enrollment will be representative of the U.S. population of subjects who undergo corneal transplantation for these endothelial conditions. Both males and females are

enrolled into each protocol. All ethnic and racial groups are eligible for participation in this study, with the goal of having appropriate minority representation of those that undergo corneal transplantation in the United States.

6.5 Financial Information

All visits, including but not limited to, pre-operative, post-operative, surgery, and any standard of care follow-up appointments will be charged to the participant or his/her insurance carrier. The participant will be responsible for any deductible or co-payments as defined by their particular insurance carrier.

The costs for pachymetry, and specular or confocal microscopy are considered research and the costs will not be incurred by the participant.

Study participants will be given a reimbursement of $25 for each study visit for travel costs. This payment will be processed by the Jaeb Center.

6.6 Confidentiality

The investigators will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants in this clinical study. Medical and research records will be maintained in the strictest confidence. However, as part of the quality assurance and legal responsibilities of an investigator, the site must permit authorized representatives of the CC to examine (and when permitted or required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. Unless required by law, no copying of records with personally identifying information will be permitted. Only the coded identity associated with documents or other participant data may be copied (obscuring any personally identifying information) or transmitted to the CC. Authorized representatives as noted are bound to maintain strict confidentiality of medical and research information that may be linked to identified individuals.

6.7 Privacy of Protected Health Information

The Health Insurance Portability & Accountability Act (HIPAA) is a Federal law that helps to protect the privacy of the study participant's health information and to whom this information may be shared. The Authorization forms used for this research study will tell the study participant what health information (called Protected Health Information or PHI) will be collected for this research study, who will see the study participant's PHI and in what ways they can use the information. The researchers and staff must agree to protect the study participant's health information by using and disclosing it only as permitted by the subject in their Authorization and as directed by state and Federal law.

Chapter 7
STATISTICAL METHODS

The approach to sample size and statistical analyses are summarized below. A detailed statistical analysis plan will be written and finalized prior to the completion of the study. The analysis plan synopsis in this chapter contains the framework of the anticipated final analysis plan.

7.1 Sample Size

The sample size of 1330 is calculated based on a non-inferiority (a single one-sided test) design with the goal to determine that the graft failure rate of the recipients of donor tissue transplanted 8 to 14 days after preservation is not worse than the graft failure rate of recipients of donor tissue transplanted ≤ 7 days after preservation.

Non-Inferiority Limit	Power = 90%				
	Failure Rate				
	12%	10%	8%[a]	6%	4%
10%	362	310			
8%	566	482	394		
6%	1006	858	702	538	
4 %	2262	1928	1576	**1208**	824
2%	9044	7708	6304	4832	3290

Note: Numbers in table are total sample size for both treatment groups combined (crossover and lost to follow up are not accounted for). Half would be randomized to each group.

[a] CDS data: 3-year failure rate

In the Cornea Donor Study (CDS), the 3-year failure rate was 8%. Clinical expectations suggest that the graft failure rate from the EK procedure will be smaller than the failure rate from the PKP procedure that was used in CDS; therefore, a 3-year failure rate of 6% has been assumed. Based on equal allocation of recipients to each group and type I error of 5%, a sample size of 1208 will provide 90% power for a non-inferiority limit of 4%. Based on information from the CDS, approximately 10% of subjects will have incomplete follow up (due to death, withdrawal or lost to follow up) by the end of year 3. Increasing the calculated sample size by this amount gives a total of 1330 subjects (665 per group).

- The inclusion of participants with two study eyes, one in each treatment group, will tend to reduce the variance and as a result increase statistical power. To be conservative, this has not been accounted for in the sample size estimation, since the correlation of outcome with two eyes is not known.

7.2 Data Analysis

7.2.1 Primary Analysis of Graft Failure

Participant study eyes that did not have surgery, received a non-study donor, had an AC IOL implanted during surgery, or experienced a suprachoroidal hemorrhage will be excluded from the primary analysis. It is highly unlikely these events could be related to preservation time, thus inclusion of them could actually bias *towards* concluding non-inferiority. Therefore, the primary

analysis will deviate from the principle of intent-to-treat, but this approach is conservative for a non-inferiority analysis.

In addition, the following will be censored at the last visit prior to occurrence: lost to follow-up, withdrawn from study, death, enucleation, phthisis, or graft failures due to a blunt or penetrating trauma.

7.2.1.1 Unadjusted Analysis

- Three year Kaplan-Meier graft failure estimates with 95% confidence intervals (variance estimated using the Greenwood method) will be calculated separately for the two treatment groups (≤7 days and 8 to 14 days from preservation to surgery). A one-sided 95% confidence interval will be constructed for the difference in 3 year graft failure rates between the two groups. The bootstrap re-sampling technique will be used to account for potentially correlated data from donors who donated both corneas in this study and potentially correlated data from 2 study eyes of the same study participant. The two treatment groups will be declared equivalent if the one-sided 95% confidence interval for the difference in proportions excludes the pre-defined non-inferiority limit of 4%.

7.2.1.2 Adjusted Analysis

- Multivariate analysis will be performed using Cox proportional hazards regression model. The primary multivariate model will include the corneal diagnosis regardless of statistical significance, in addition to time from preservation to surgery. In additional models, potential confounders including recipient and donor age, recipient and donor race, presence of glaucoma, presence of corneal vessels, history of smoking, and certain aspects of the retrieval and processing of the donor tissue (including multiple types of storage media, if more than one is used in preservation of the corneal tissues) will be screened by assessing the change in the preservation time effect when the potential confounder is controlled for in the Cox model. Variables that do not contribute significantly ($P > 0.05$) will be removed from the model.
- Random surgeon effects will be tested using a generalized linear model via the SAS GLIMMIX procedure. This marginal model produces a robust standard error (RSE) by use of a sandwich estimator, which corrects for correlated data.
- Potential effect modifiers of donor tissue preservation time such as recipient age or corneal diagnosis will be screened by including first-order interaction terms. Variables that exhibit modification of the donor tissue preservation time effect with an associated P value < 0.05 will be retained in the model.

7.2.2 Secondary Analyses of Graft Failure

7.2.2.1 Preservation Time

The time from preservation to surgery is treated as a binary variable in the primary analysis (see above section). Secondary analyses will look at the time from preservation to surgery as a categorical variable with multiple levels and as a continuous variable:

- Kaplan-Meier estimates of graft failure with 95% confidence interval will be calculated for each of the following donor groups: 0 – 4, 5 – 7, 8 – 11, and 12 – 14 days from preservation to surgery(counting partial days as whole days).

- A Cox proportional hazards model will be constructed treating the time from preservation to surgery as a continuous variable (using time of day to calculate hours from preservation to surgery). Polynomial terms will be added to assess any curvilinear, J, or U shaped relationship between time from preservation to surgery and graft failure. If no significant departure from a linear relation is detected, a one sided 95% confidence interval will be computed for the hazard ratio per day of time from preservation to surgery.

- The proportional hazards assumptions will be tested through the use of time-dependent variables with a logarithm transformation of time. If this assumption is violated then hazard ratios will be presented separately for different periods following transplant.

7.2.2.2 Predictive Factors

A Cox model will be constructed including preservation time group regardless of statistical significance. Additional recipient/donor factors (see some examples listed below) will be considered for the model and included, if significantly associated with graft failure ($p< 0.05$). The proportional hazards assumptions will be tested as described above.

- Recipient factors
  - preoperative diagnosis
  - gender
  - age
  - race
  - prior use of glaucoma medication
  - prior glaucoma surgery (trabeculectomy, laser procedure)
  - current smoker (at time of surgery)
  - lens status (phakic, posterior chamber intraocular lens)
  - Intraocular pressure (IOP) treated as a binary variable: < 25 vs. ≥ 25 mmHg

- Donor/graft factors
  - eye bank determined screening ECD
  - pre-operative CIARC determined ECD
  - age
  - gender
  - race
  - history of diabetes
  - cause of death
  - type of storage medium

7.2.2.3 ECD as Time Dependent Predictor of Graft Failure

The relationship between endothelial graft failure (graft failure due to endothelial decompensation) and ECD will be addressed paralleling the methods used in the CDS. A Cox model will be fit with ECD as a time dependant covariate. This analysis will be limited to

subjects with at least one gradable follow up image. The rate of change will also be calculated as a time dependent variable defined as the least squares slope over all previous measurements starting at 6 months (e.g., the rate of change at one year would be the slope fit to the 6 month and 1 year ECD values). Missing values will be imputed by Rubin's method. If significant departure from linearity is detected, then ECD will be treated as a categorical variable. The proportional hazards assumptions will be tested as described above.

To check whether results are sensitive to how missing data are handled, a second model will be fit with a time dependent indicator for missing ECD.

7.2.3 Graft Rejection

Associations of baseline recipient and donor factors with the occurrence of a graft rejection will be assessed in univariate and multivariate proportional hazards models. Life-table analyses will be used to compute the probability of a first rejection event within intervals defined by the study exam schedule. Data will be censored at the time of a non-rejection graft failure or at the last visit.

7.2.4 Endothelial Cell Density (ECD)

7.2.4.1 Included Subjects

The primary analysis will include all study participants with a gradable 3-year image, who have not experienced graft failure 3 years after transplantation. Study participants with a missing cell count at 3 years will be included in a secondary analysis using Rubin's method of multiple imputation.

7.2.4.2 Outcome Measures

The primary outcome will be the ECD at 3 years, conditional on graft survival at 3 years. All other ECD measurements during follow up will be considered as a secondary outcome.

7.2.4.3 Descriptive Statistics

- Summary statistics (mean ± SD and/or median/quartiles as appropriate to the distribution) will be given for the ECD by the 2 treatment groups (≤7 and 8 to 14 days) and 4 treatment groups (0 – 4, 5 – 7, 8 – 11, and 12 – 14 days).
- Change from eye bank determined screening ECD will be summarized in a similar manner.
- Boxplots of ECD and change from eye bank determined screening ECD will be given for the 2 randomization groups.
- A scatter plot will be constructed of eye bank determined screening ECD vs. 3 year ECD with a symbol used to denote the two randomization groups.

7.2.4.4 Primary Analysis

The primary analysis will be limited to subjects with gradable 3 year images, who have not experienced graft failure 3 years after transplantation. An ANCOVA model with 3 year ECD as the dependent variable adjusting for eye-bank-determined screening ECD will be used to assess the effect of preservation time. The time from preservation to surgery will be treated as a binary

variable. If residual values from the models above are highly skewed then a transformation (e.g., square root or logarithm) or non-parametric methods will be used instead.

- Random effects will be modeled to account for any correlated data from the same donor and any correlated data from 2 study eyes of the same study participant.
- Additional ANCOVA models will also adjust for other recipient/donor risk factors, (if $p< 0.05$. Random surgeon effects will also be explored using a mixed effects model.

7.2.4.5 Secondary Analyses

7.2.4.5.1 Sensitivity Analysis

Sensitivity analysis will also be performed to check whether results change meaningfully depending on how missing data are handled. The missing 3 year ECD values for subjects with surviving grafts at 3 years will be imputed and included in an analysis as described in the previous section. The data imputation will be performed by using Rubin's method of multiple imputation.

7.2.4.5.2 Analysis with donor tissue preservation time as continuous/multi-categorical variable

The analyses described above will be repeated with time from preservation to surgery treated as continuous (using time of day to calculate hours from preservation to surgery) or multi-category variable (0 – 4, 5 – 7, 8 – 11, and 12 – 14 days from preservation to surgery) in separate models.

7.2.4.5.3 Longitudinal analysis

This analysis also will be limited to subjects with a surviving graft at 3 years. A repeated measures least squares regression model will be fit using all available images at baseline, 6 months, 1, 2, and 3 years. This analysis will be performed *with* and *without* imputation of missing data. Rubin's method of data imputation will be used to impute the ECD values for all missing time points. The time from preservation to surgery will be modeled as both continuous and categorical as described above. If residual values have a skewed distribution then transformation (e.g., square root or logarithm) or non-parametric analysis will be used.

7.2.5 Course of Cornea Changes After Endothelial Keratoplasty

The association of donor, operative and postoperative related factors with ECD will be evaluated and assessed in univariate and multivariate ANCOVA models , adjusting for the reading center grading of pre-operative ECD (imaged post-cut if the eye bank was performing the cutting and imaged just prior to shipping if the surgeon was performing the cutting). This ECD value will be considered the baseline for these analyses.

7.2.6 Safety Analysis Plan

The main safety analysis will involve tabulation of data by treatment group of events that could be considered possibly related to the preservation time such as post-op infection. The efficacy analyses related to graft failure, corneal thickness, and ECDs also could be viewed as safety analyses. Operative and post-operative complications will also be tabulated.

Further details of the analytic approach will be provided in the detailed statistical analysis plan.

7.2.7 Additional Tabulations and Analyses

The following will be tabulated according to treatment group:

- Baseline demographic and clinical characteristics
- Visit completion rate for each visit
- Protocol deviations

7.2.8 DSMC Interim Analysis Plan

No formal interim analyses are planned towards demonstrating non-inferiority before the end of the study since the recruitment period is planned to be short compared with the follow-up period and since we believe it is imperative to have three years of follow up to assess non-inferiority.

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In addition to semi-annual review described in Section 5.2, the following plan for interim monitoring for a potential recommendation of early stopping of enrollment has been established in conjunction with the DSMC. This plan is based on early donor failure rate and on the progress of recruitment.

- Rate of failure within the first 8 weeks: Upon enrollment of the first 100 eyes, and then quarterly thereafter (i.e., one review between each DSMC semi-annual meeting) the DMAC will evaluate the failure rate within the first 8 weeks (i.e. both the early failures and the primary donor failures, as defined in Section 4.6) in each group and notify the DSMC who will have the option of requesting additional information between the semi-annual reviews. The DSMC may also request more frequent reviews at any time.
- Recruitment Progress: Recruitment progress will be evaluated at the first two DSMC meetings following initiation of recruitment. If based on the current recruitment total and recruitment trend over the previous 3 months, the projected timeline for the remaining recruitment is more than 16 months at the 1st review or more than 12 months at the 2nd review, the DSMC will discuss whether the study timeline can be met.

Following each DSMC data review, a summary will be provided to the IRBs.

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APPENDIX A

PROTOCOL AMENDMENT #1

EXTENSION OF FOLLOW-UP TO COMMON END DATE

July 1, 2015

1. Overview: Extension of follow-up after 3 years.

Participants who re-consent to additional follow-up will continue annual protocol visits past the 3-year time point, until at least 2017. The same data will be captured from annual visits as well as interim unscheduled visits as was done previously. The same study specific tests – pachymetry and specular/confocal microscopy - will continue to be performed at the annual visits. Visits will continue to be captured until approximately the 2nd quarter of 2017, therefore all participants will have a last possible common endpoint rather than exiting individually when they reach their 3 year post-operative visit.

Rationale: Continued follow-up of CPTS participants provides an excellent opportunity to gain more information about many factors affecting DSAEK outcomes, including donor age and preservation time. This additional longitudinal data will provide important information on longer term DSAEK outcomes that are not necessarily impacted by preservation time, although we will continue to assess that variable as well.

2. Eligibility and Informed Consent

a. Eligibility

All active study participants will be eligible for extended follow-up to a common end date, until at least 2017. The exact end date will be determined by the Operations Committee based on funding and data analysis requirements.

b. Informed Consent

Active study participants will be asked to sign a new informed consent form or addendum prior to the post-3 year visits or as soon thereafter as feasible. The new informed consent form (or addendum) may be signed during the next scheduled visit (either Protocol Visit or Unspecified Visit) or by mail if the governing IRB approves that process. Until the new informed consent form or addendum is signed, the study participant will not be examined post-3 year for study purposes. If the new informed consent form or addendum is not signed, follow-up for that participant will end upon completion of the 36-month visit.

3. Follow-Up Visits

a. Visit Schedule and Windows

The post-3 year visit schedule will vary by the participant's enrollment date. Some participants will be eligible for Year 4 and Year 5 visits if the windows below fall within the extended follow-up period.

Additional protocol-specified follow-up visits (and visit windows) for the first eye, will be as follows:

- 48 months (44 - 52 months)
- 60 months (56 – 64 months)

Additional visits may be performed more often as needed. A data form will be completed for each protocol visit and any non-protocol visits where the intent of the visit was to examine the study eye by a study investigator. Additional non-protocol visits by non-study investigators may also be uploaded by the clinical site to the study website, as was done during the initial 3-year follow-up phase. For example, if graft failure is determined and a regraft is required on a non-protocol visit, the appropriate follow-up visit form and graft failure form should be completed if and when this occurs.

If the second eye of an active participant was enrolled, a modified visit schedule will be allowed to minimize return visits for participants that had bilateral EK as part of the study. For example, sites will be allowed to follow standard of care practices to avoid unnecessary visits linked to targeting each eye within its respective windows as listed above. Sites are encouraged to schedule both eyes within their respective windows when the windows overlap, but at minimum at least one eye must be within its target visit window at each visit.

b. Testing Procedures

The Table below shows the key elements of data collection at each study visit. Additional visits may occur as needed for the usual care of the participant.

	48 Months	60 Months
Parameters		
Medication History	**X**	**X**
Slit lamp examination	**X**	**X**
Intraocular pressure	**X**	**X**
Ultrasonic pachymetry	**X**	**X**
Endothelial imaging	**X**	**X**
Post-op complications and other untoward events	**X**	**X**

c. Detailed Testing Procedures

Procedures for testing at each follow-up visit are identical as listed in Chapter 4.

d. Adverse Events

Adverse event reporting remains identical to Chapter 5.

e. Other Considerations in Follow-up

All other retention and follow-up procedures including central contract from the Coordinating Center will continue as in the original study and as outlined in Chapter 6.

4. Statistical Analyses

The statistical methods for all 3 study objectives completed up to the 3 year primary endpoint will be extended to the 4 and 5 year endpoints. Additional analyses on the impact of missing data will be evaluated, including comparison of baseline characteristics for those who agreed versus declined to consent to continue.

CPTS Statistical Analysis Plan

Version: 4.0
Date: 6-21-16
In sync with Protocol Version 4.0

Version History

Version Number	Author	Approver	Effective Date	Revision Description
1.0	Allison Ayala	Craig Kollman	9-4-14	Revisions prior to 9-4-14 were not versioned according to new JCHR standards. Changes were clarifications from DSMC meetings and were documented in DSMC meeting minutes.
2.0	Allison Ayala	Craig Kollman	8-12-15	Added analyses related to extension of CPTS to capture 4 and 5 year visits, which was incorporated into protocol version 4.0.
3.0	Allison Ayala	Craig Kollman	1-15-16	The DSMC, OC, and EC approved the clarification of how to handle cases that were cloudy at the 3 year visit. Details of the original discussion are in the November 3, 2015 DSMC minutes and details of the discussion/proposal that followed (and final decision) are in the following folder: Analysis Notes to Save\3 year failure cutoff discussions
4.0	Allison Ayala	Craig Kollman	6-21-16	1. Clarify that "surgical trauma" (like blunt and penetrating trauma) is also a severe event, if leading to failure, that is unrelated to PT and should be censored prior to the event. 2. Clarify that out of window 3 year visits can be used up to 44 months for determining 3 year failures. Decision/discussion saved in the following folder: Analysis Notes to Save\3 year visit window

CPTS Statistical Analysis Plan

1.0 Study Objectives

The objectives of the "Effect of Corneal Preservation Time on Long-Term Graft Success" (CPTS) study are:

Objective 1 (Primary Objective): To determine if the 3-year graft failure rate following EK performed with donor corneas with a preservation time of 8 to14 days is non-inferior to the failure rate when donor corneas with a preservation time of 7 or fewer days are used.

Objective 2: To determine if the central corneal endothelial cell density 3-years after EK is related to preservation time.

Objective 3: To evaluate the effect of donor, operative and postoperative factors on graft failure and endothelial cell density three years following EK.

The purpose of this document is to describe the analysis plan for these 3 objectives, as well as some pre-planned secondary analyses. There may be additional secondary analyses performed that are not described in this document.

2.0 Sample Size

The sample size of 1330 is calculated based on a non-inferiority (a single one-sided test) design with the goal to determine that the graft failure rate of the recipients of donor tissue transplanted 8 to 14 days after preservation is not worse than the graft failure rate of recipients of donor tissue transplanted ≤ 7 days after preservation.

Non-Inferiority Limit	**Power = 90%**				
	Failure Rate				
	12%	**10%**	**8%[a]**	**6%**	**4%**
10%	362	310			
8%	566	482	394		
6%	1006	858	702	538	
4 %	2262	1928	1576	**1208**	824
2%	9044	7708	6304	4832	3290

Note: Numbers in table are total sample size for both treatment groups combined (crossover and lost to follow up are not accounted for). Half would be randomized to each group.

[a] CDS data: 3-year failure rate

In the Cornea Donor Study (CDS), the 3-year failure rate was 8%. Clinical expectations suggest that the graft failure rate from the EK procedure will be smaller than the failure rate from the PKP procedure that was used in CDS; therefore, a 3-year failure rate of 6% has been assumed. Based on equal allocation of recipients to each group and type I error of 5%, a sample size of 1208 will provide 90% power for a non-inferiority limit of 4%. Based on information from the CDS, approximately 10% of subjects will have incomplete follow up (due to death, withdrawal or lost to follow up) by the end of year 3. Increasing the calculated sample size by this amount gives a total of 1330 subjects (665 per group).

- The inclusion of participants with two study eyes, one in each treatment group, will tend to reduce the variance and as a result increase statistical power. To be conservative, this has not

been accounted for in the sample size estimation, since the correlation of outcome with two eyes is not known.

3.0 Graft Failure (Primary Outcome)

3.1 Primary Analysis of 3 Year Graft Failure - *(Analysis plan for objective 1)*

3.1.1 Formal Statistical Hypothesis

The primary objective of the study is to determine if the 3-year graft failure rate following EK performed with donor corneas with a preservation time of 8 to14 days is non-inferior to the failure rate when donor corneas with a preservation time of 7 or fewer days are used. In terms of formal statistical hypothesis testing, the null and alternative hypotheses are:

$$\text{Ho: } p_{8\text{-}14} - p_{0\text{-}7} \geq 4\%$$
$$\text{Ha: } p_{8\text{-}14} - p_{0\text{-}7} < 4\%$$

where $p_{8\text{-}14}$ and $p_{0\text{-}7}$ is the probability of graft failure by 3 years in the 8-14 day group and the <=7 day group, respectively.

3.1.2 Analysis Cohort

Participant study eyes that did not have surgery, received a non-study donor, had an AC IOL implanted during surgery, or experienced a suprachoroidal hemorrhage will be excluded from the primary analysis. It is highly unlikely these events could be related to preservation time, thus inclusion of them could actually bias *towards* concluding non-inferiority. Therefore, the primary analysis will deviate from the principle of intent-to-treat, but this approach is conservative for a non-inferiority analysis.

3.1.2.1 Censoring of Data for Occurrence of a Severe Event Unrelated to Preservation Time

In order to minimize bias towards concluding non-inferiority, severe events not expected to be related to preservation time will be censored at the last examination prior to the occurrence of the event that severely impacts the study eye, if the eye was not on the path to failure at the last visit prior to the occurrence of the severe unrelated event (see 3.1.5 for the 'rules'). These events include:

- Enucleation (eye will be dropped)
- Phthisis (eye will be dropped)
- Failure due to surgical (unrelated to the initial DSEK), blunt or penetrating trauma (eye will be followed until failure criteria met)

3.1.2.2 Analyzing Preservation Time Group Crossovers As-Treated

If a study eye receives a study donor cornea in the wrong preservation time group, it will be analyzed as treated, as this will minimize bias towards concluding non-inferiority. A secondary intent-to-treat analysis will also be completed if this occurs.

3.1.3 Definition of Graft Failure (per protocol, section 4.7):

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 on the first postoperative day which does not clear within 8 weeks
  OR
  (2) cloudy cornea which was initially clear postoperatively but becomes and remains cloudy for 3 months without clearing.
  Note: graft failure is based on cloudy recipient stroma. Any reference to cloudy cornea when defining graft failure is with regard to the recipient stromal clarity.

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 or equivocal recipient cornea on the first postoperative day which does not clear or requires a regraft within 8 weeks), associated with surgical complications, including immediate peri-operative complications such as acute angle closure noted on the 1 day post-operative visit
- Primary donor failure (cloudy or equivocal recipient cornea on the first postoperative day 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).

3.1.4 Definition of Graft Failure Date

A cornea that is "**on the path to failure**" means the cornea has met criteria to initiate the path to failure and it has not been removed from that path, defined as follows:

1. Definition of how to initiate "on the path to failure" :
   - Initiating At 1 Day Visit: A cornea may be classified as either cloudy ***or*** equivocally cloudy to start a count of how many days "on the path to failure".
   - Initiating After 1 Day Visit: A cornea must be initially classified as cloudy to start a count of how many days "on the path to failure".
2. A cornea classified as equivocal after the "on the path to failure" count begins is still considered "on the path to failure."
3. A cornea classified as clear after the "on the path to failure" count begins is no longer "on the path to failure"; the count restarts the next time the cornea is classified as cloudy.

The date of graft failure is defined as follows

1. If "on the path to failure" (per 3.1.4 above) initiates at the 1 Day Visit:
    a. If the cornea remains "on the path to failure" (per 3.1.4 above), is classified cloudy at least once during those consecutive visits, and is classified cloudy at least 56 days after surgery date, then the cornea meets the failure definition and the date of failure will be the date of the 1 day visit. (Note: this means it must be classified cloudy at least twice during the path to failure, and at least one of those >= 56 days after surgery)
    b. If the cornea remains "on the path to failure" (per 3.1.4 above) and a regraft occurs within 56 days or after 56 days (but prior to being classified cloudy after 56 days), the date of failure will equal the date of the 1 day visit.
2. If "on the path to failure" (per 3.1.4 above) initiates after the 1 Day Visit:
    a. If a cornea is classified as cloudy, remains "on the path to failure" (per 3.1.4 above), and is classified cloudy at least 90 days after the initial cloudy classification, then the cornea meets the failure definition and the date of failure will be the first date at which cornea is indicated as cloudy.
    b. If a cornea is "on the path to failure" (per 3.1.4 above) for <90 days and a regraft occurs, the date of failure will equal the first exam date where the cornea is cloudy.
3. If a cornea is clear and then a regraft occurs, the date of failure will be equal to the date of regraft.

Examples

Example 1
equivocal (1 day) ← failure date
equivocal
cloudy (>56 days after surgery)
cloudy (need the second cloudy to confirm)

Example 2
equivocal (1 day) ← failure date
equivocal
regraft

Example 3
clear
equivocal
cloudy* ← failure date
equivocal
cloudy (>90 days after *)

Example 4
clear
cloudy
clear
cloudy* ← failure date
cloudy (<90 days after *)
regraft

Example 5
clear
clear
regraft ← failure date

3.1.5 Censoring and Non-Protocol Graft Failures

3.1.5.1 Eyes with Incomplete Follow-Up

In eyes that dropped prior to the 3 year visit without meeting criteria for graft failure the following 'rules' will apply:

1. A cornea that is "on the path to failure" (per 3.1.4 above) at the last completed visit AND was cloudy at least once during the "on the path to failure" period will be flagged for **'non-protocol' graft failure review by the Executive Committee**. If confirmed as a non-protocol graft failure, the failure date will be determined as it is in 3.1.4 above.
2. All others *will NOT* be considered a graft failure and the data will be **censored** at the last completed visit

3.1.5.2 Eyes with 3 Year Visit Not Meeting Failure Criteria

In eyes that complete the 3 year visit without meeting criteria for graft failure the following 'rules' will apply:

1. If the cornea is "on the path to failure" (per 3.1.4 above) at the 3 year visit, data beyond the 3 year visit up to 42 months will be used (if available) to determine whether the cornea will be considered a failure up to 3 years. Data beyond 42 months will not be used for this assessment.
    - If the cornea clears at a subsequent follow-up visit (within 42 months) prior to meeting confirmation of failure criteria, the cornea will not be classified as a graft failure and data will be censored at the 3 year visit.
    - If additional follow-up data (within 42 months) confirm a failure (via regraft or 90 days confirmed cloudy), then the cornea will be classified as a graft failure up to 3 years and date of failure will be determined per 3.1.4 above.
    - If data beyond the 3 year visit are not available, OR the cornea remains "on the path to failure" beyond the 3 year visit but the follow-up data that are available within 42 months still do not confirm failure, the case will be flagged for **'non-protocol' graft failure review by the Executive Committee**. (Note all of these cases will have been cloudy at least once during the "on the path to failure" period.) If confirmed as a non-protocol graft failure, the failure date will be determined per 3.1.4 above.
2. If the cornea is not "on the path to failure" (clear or equivocal following clear) at the 3 year visit, it *will NOT* be considered a graft failure and the data will be **censored** at the 3 year visit

3.1.5.3 Severe Events Unrelated to Preservation Time

In eyes that met failure due to a severe event unrelated to preservation time (listed in section 3.1.2.1), the following 'rules' will apply:

1. A cornea that is "on the path to failure" (per 3.1.4 above) at the last completed visit prior to the severe event leading to failure AND was cloudy at least once during that "on the path to failure" period prior to

the event will be flagged for **'non-protocol' graft failure review by the Executive Committee**. If confirmed as a non-protocol graft failure, the failure date will be determined as it is in 3.1.4 above.

2. All others *will NOT* be considered a graft failure and the data will be **censored** at the last completed visit prior to the severe event leading to failure.

Examples

Example 1
clear
clear
cloudy ← 'non-protocol' failure date
equivocal
lost to followup

Example 2
clear
clear
equivocal
equivocal ← censored date
lost to followup

Example 3
clear
clear
cloudy ← 'non-protocol' failure date
blunt trauma
cloudy
regraft

Example 4
clear
cloudy
clear ← censored date
blunt trauma
cloudy
regraft

Example 5
equivocal (1 day)
equivocal ← censored date
lost to followup

Example 6
equivocal (1 day) ← 'non-protocol' failure date
cloudy
lost to followup

3.1.6 Clarifications Regarding 3 Year Visit Windows

The visit that the site designates as the "3 year visit" is the one that drives whether a 3 year failure has occurred. If the eye is dropped without meeting failure and prior to completion of the 3 year visit, the rules in 3.1.5.1 apply. If failure definition is met prior to a 3 year visit, then this is counted as a 3 year failure according to 3.1.4. If failure is not met prior to a 3 year visit, the rules in 3.1.5.2 are followed. The following clarifications regarding visit windows will apply to these rules:

- The window for the 3 year visit, per protocol, is 35-42 months. The 3 year visit will be permitted to occur late out of window, up to 44 months. For a 3 year visit occurring late out of window (between 42-44 months) the same rules above apply relative to this designated 3 year visit.
- If there is no designated 3 year visit completed, data (i.e. unspecified visits) up to 44 months can be used to determine if failure met. The same rules above will apply.
- NOTE: This means that data beyond 42 months (up to 44 months) CAN be used to determine 3 year failure status if and only if the visits occur prior to or including a designated 3 year visit late out of window, OR in the absence of a designated 3 year visit.

3.1.7 Unadjusted Analysis

Three year Kaplan-Meier graft failure estimates with 95% confidence intervals (variance estimated using the Greenwood method) will be calculated separately for the two treatment groups (≤7 days and 8 to 14 days from preservation to surgery). A one-sided 95% confidence interval will be constructed for the difference in 3 year graft failure rates between the two groups. The bootstrap re-sampling technique will be used to account for potentially correlated data from donors who donated both corneas in this study and potentially correlated data from 2 study eyes of the same study participant. The technique will sample with replacement from the observed dataset. For each bootstrap sample the effect of preservation time will be estimated using the same method as in the primary analysis. Confidence intervals will be calculated using the bias-corrected and accelerated method. The number of bootstraps will be 100,000.

The two treatment groups will be declared equivalent if the one-sided 95% confidence interval for the difference in proportions excludes the pre-defined non-inferiority limit of 4%.

3.1.8 Adjusted Analysis

Multivariate analysis will be performed using Cox proportional hazards regression model. The primary multivariate model will include the corneal diagnosis regardless of statistical significance, in addition to time from preservation to surgery (treated as a binary variable).

In additional models, potential confounders including recipient and donor age, recipient and donor race, presence of glaucoma, presence of corneal vessels, history of smoking, and certain aspects of the retrieval and processing of the donor tissue (including multiple types of storage media, if more than one is used in preservation of the corneal tissues, observations during or after cutting, time from cut to surgery, and donor rim culture results) will be screened by assessing the change in the preservation time effect when the potential confounder is controlled for in the Cox model. Univariate models will be evaluated first, and factors from those models with a p value <0.10 will then be evaluated in a multivariate model. A final model will be constructed consisting of factors with a p value <0.01 following a backwards selection process.

3.1.9 Surgeon Effect

Random surgeon effects will be tested using a generalized linear model via the SAS GLIMMIX procedure. This marginal model produces a robust standard error (RSE) by use of a sandwich estimator, which corrects for correlated data.

3.1.10 Analysis of Potential Interaction

Potential effect modifiers of donor tissue preservation time such as recipient age or corneal diagnosis will be screened by including first-order interaction terms. Variables that exhibit modification of the donor tissue preservation time effect with an associated P value < 0.10 will be added to the model, and the final model will be constructed consisting of terms with a p value <0.01 following a backwards selection process.

3.2 Secondary Analyses of 3 Year Graft Failure

3.2.1 Preservation Time

The time from preservation to surgery is treated as a binary variable in the primary analysis (see above section). Secondary analyses will look at the time from preservation to surgery as a categorical variable with multiple levels and as a continuous variable:

- Kaplan-Meier estimates of graft failure with 95% confidence interval will be calculated for each of the following groups: 0 – 4, 5 – 7, 8 – 11, and 12 – 14 days from preservation to surgery (counting partial days as whole days).
- A Cox proportional hazards model will be constructed treating the time from preservation to surgery as a continuous variable (using time of day to calculate hours from preservation to surgery). Polynomial terms will be added to assess any curvilinear, J, or U shaped relationship between time from preservation to surgery and graft failure. If no significant departure from a linear relation is detected, a one sided 95% confidence interval will be computed for the hazard ratio per day of time from preservation to surgery.
  - The proportional hazards assumptions will be tested through the use of time-dependent variables with a logarithm transformation of time. If this assumption is violated then hazard ratios will be presented separately for different periods following transplant.

3.2.2 Predictive Factors – (*Analysis plan for objective 3, graft failure outcome*)

The association of factors potentially related to graft failure will be evaluated in univariate and multivariate Cox models , adjusting for preservation time group regardless of statistical significance. The proportional hazards assumptions will be tested as described above. Univariate models will be evaluated first, and factors from those models with a p value <0.10 will then be evaluated in a multivariate model. A final model will be constructed consisting of factors with a p value <0.01 following a backwards selection process.

Potential factors to evaluate include:

- Recipient factors
  - preoperative diagnosis

- gender
- age
- race
- prior use of glaucoma medication
- prior glaucoma surgery (trabeculectomy, laser procedure)
- current smoker (at time of surgery)
- lens status (phakic, posterior chamber intraocular lens)
- Intraocular pressure (IOP)

- Donor/graft factors
  - eye bank determined screening ECD
  - pre-operative CIARC determined ECD
  - age
  - gender
  - race
  - history of diabetes
  - cause of death
  - type of storage medium
  - death to preservation time
  - surgeon cut vs eye bank cut
  - postcut thickness
  - cut to surgery time
  - observations during or after cutting

- Surgical factors
  - Insertion method
  - Incision location
  - Incision site
  - Graft size

- Postoperative factors
  - Dislocation
  - Rebubbling
  - IOP
  - Graft rejection
  - Corneal thickness

3.2.3 ECD as Time Dependent Predictor of Graft Failure

The relationship between endothelial graft failure (graft failure due to endothelial decompensation) and ECD will be addressed paralleling the methods used in the CDS. A Cox model will be fit with ECD as a time dependant covariate. This analysis will be limited to subjects with at least one gradable follow up image. The rate of change will also be calculated as a time dependent variable defined as the least squares slope over all previous measurements starting at 6 months (e.g., the rate of change at one year would be the slope fit to the 6 month and 1 year ECD values). Missing values will be imputed by Rubin's method. If non-linear effects are detected, transformations will be used. For ease of interpretation, results will be presented as discrete categories with cutpoints chosen to display the trends identified from the transformed model. P-

values will still be from a continuous analysis although data are displayed as categories. The proportional hazards assumptions will be tested as described above.

To check whether results are sensitive to how missing data are handled, a second model will be fit with a time dependent indicator for missing ECD.

3.2.4 Secondary Outcome of Graft Rejection

Associations of baseline recipient and donor factors with the occurrence of a graft rejection will be assessed in univariate and multivariate proportional hazards models. Life-table analyses will be used to compute the probability of a first rejection event within intervals defined by the study exam schedule. Data will be censored at the time of a non-rejection graft failure or at the last visit.
A Kaplan-Meier approach will be considered for evaluating probability of first graft rejection, and time dependency of repeated rejection events will also be explored. Determination of the timing of a separate episode graft rejection will include a confirmation that the eye was off steroids at the visit where rejection was reported.

4.0 Endothelial Cell Density (ECD)

4.1 Primary Analysis of 3 Year ECD– *(Analysis plan for objective 2)*

4.1.1 Analysis Cohort

The primary analysis will include all study participants with a gradable 3-year image, who have not experienced graft failure 3 years after transplantation.

4.1.2 Outcome Measure

The primary outcome measure will be the ECD at 3 years, conditional on graft survival at 3 years.

4.1.3 Descriptive Statistics

- Summary statistics (mean ± SD and/or median/quartiles as appropriate to the distribution) will be given for the ECD by the 2 treatment groups (≤7 and 8 to 14 days) and 4 treatment groups (0 – 4, 5 – 7, 8 – 11, and 12 – 14 days).
- Change from eye bank determined screening ECD will be summarized in a similar manner.
- Boxplots of ECD and change from eye bank determined screening ECD will be given for the 2 randomization groups.
- A scatter plot will be constructed of eye bank determined screening ECD vs. 3 year ECD with a symbol used to denote the two randomization groups.

4.1.4 Analysis

An ANOVA model with 3 year ECD as the dependent variable will be used to assess the effect of preservation time.

- Confounding with regard to screening ECD is not expected to be an issue due to anticipated balance via randomization. Therefore, an ANCOVA model adjusting for eye-bank-determined screening ECD will only be used if this measurement is considered good enough to expect to have any impact on reducing variance.
    - Although all screening images are being collected, CIARC is not grading them other than a general quality assessment. CIARC will grade a sample of screening images within each eye bank. If more than 75% of the graded

ECDs are within 10% of the eye bank determined ECDs, the eye bank determined ECDs will be included in the model.

- The time from preservation to surgery will be treated as a binary variable.
- If residual values from the models above are highly skewed then a transformation (e.g., square root or logarithm) or non-parametric methods will be used instead.
- Random effects will be modeled to account for any correlated data from the same donor and any correlated data from 2 study eyes of the same study participant.
- Additional ANCOVA models will also adjust for other recipient/donor risk factors. Univariate models will be evaluated first, and factors from those models with a p value <0.10 will then be evaluated in a multivariate model. A final model will be constructed consisting of factors with a p value <0.01 following a backwards selection process
- Random surgeon effects will also be explored using a mixed effects model.
- Sensitivity analysis will also be performed to check whether results change meaningfully depending on how missing data are handled. The missing 3 year ECD values for subjects with surviving grafts at 3 years will be imputed and included in an analysis as described in the previous section. The data imputation will be performed by using Rubin's method of multiple imputation.

4.2 Secondary Analyses of 3 Year ECD

4.2.1 Preservation Time

The time from preservation to surgery is treated as a binary variable in the primary analysis of ECD (see above section). The analyses described above will be repeated with time from preservation to surgery treated as continuous (using time of day to calculate hours from preservation to surgery) or multi-category variable (0 – 4, 5 – 7, 8 – 11, and 12 – 14 days from preservation to surgery) in separate models.

4.2.2 Longitudinal analysis

This analysis also will be limited to subjects with a surviving graft at 3 years. A repeated measures least squares regression model will be fit using all available images at baseline, 6 months, 1, 2, and 3 years. This analysis will be performed *with* and *without* imputation of missing data. Rubin's method of data imputation will be used to impute the ECD values for all missing time points. The time from preservation to surgery will be modeled as both continuous and categorical as described above. If residual values have a skewed distribution then transformation (e.g., square root or logarithm) or non-parametric analysis will be used.

4.2.3 Predictive factors – *(Analysis plan for objective 3, ECD outcome)*

This analysis also will be limited to subjects with a surviving graft at 3 years. The association of factors potentially related to 3 year ECD will be evaluated in univariate and multivariate ANCOVA models , adjusting for preservation time group regardless of statistical significance, and the reading center grading of pre-operative ECD (imaged post-cut if the eye bank was performing the cutting and imaged just prior to shipping if the surgeon was performing the cutting). This ECD value will be considered the baseline for these analyses. Univariate models will be evaluated first, and factors from those models with a p value <0.10 will then be evaluated in a

multivariate model. A final model will be constructed consisting of factors with a p value <0.01 following a backwards selection process.

Potential factors to evaluate include:

- Recipient factors
  - preoperative diagnosis
  - gender
  - age
  - race
  - prior use of glaucoma medication
  - prior glaucoma surgery (trabeculectomy, laser procedure)
  - current smoker (at time of surgery)
  - lens status (phakic, posterior chamber intraocular lens)
  - Intraocular pressure (IOP)

- Donor/graft factors
  - eye bank determined screening ECD
  - pre-operative CIARC determined ECD
  - age
  - gender
  - race
  - history of diabetes
  - cause of death
  - type of storage medium
  - death to preservation time
  - surgeon cut vs eye bank cut
  - postcut thickness
  - cut to surgery time
  - observations during or after cutting

- Surgical factors
  - Insertion method
  - Incision location
  - Incision site
  - Graft size

- Postoperative factors
  - Dislocation
  - Rebubbling
  - IOP
  - Graft rejection
  - Corneal thickness

4.3 Effect of Preservation Time on Pre-Operative ECD – *(Secondary Non-Protocol Objective)*

An ANCOVA model with CIARC graded pre-operative ECD as the dependent variable, and adjusting for eye-bank-determined screening ECD, will be used to assess the effect

of preservation time. The time from preservation to surgery will be treated as a binary variable.

5.0 Safety Analysis Plan

All reported adverse events will be tabulated by treatment group. The main safety analysis will involve tabulation of data by treatment group of events that could be considered possibly related to the preservation time, including endophthalmitis, bacterial, fungal or parasitic corneal infection, or any other events designated by the study group as possibly related to preservation time.

Operative complications and procedures, post-operative complications and procedures (including dislocation of donor, interface fluid, air injection), and abnormalities noted on ocular exam will also be tabulated by treatment group to evaluate potential safety concerns.

The efficacy analyses already outlined in this document, related to graft failure and ECDs, also could be viewed as safety analyses. Occurrences of following additional events during follow-up will be tabulated by treatment group to assess potential safety concerns:

- IOP>25 mmHg (median and quartiles will also be presented)
- Corneal Thickness >750 microns (median and quartiles will also be presented)
- Definite signs of graft rejection
- Presence of stromal corneal vessels
- Presence of corneal scar or haze
- Epithelial defect >50%
- Donor stromal clarity = cloudy
- Recipient stromal clarity = cloudy

6.0 Additional Tabulations and Analyses

The following will be tabulated according to treatment group:

- Baseline recipient demographic and clinical characteristics
- Donor characteristics
- Visit completion rate for each visit
- Additional post-operative study eye procedures(not part of the safety analysis), at each visit
- Baseline characteristics in cases with graft failure, cases with incomplete follow up without graft failure, and cases with complete follow up without graft failure.
- Crosstabulation of immunizations or vaccinations versus signs of graft rejection, at each visit
- Protocol deviations

7.0 DSMC Interim Analysis Plan

No formal interim analyses are planned towards demonstrating non-inferiority before the end of the study since the recruitment period is planned to be short compared with the follow-up period and since we believe it is imperative to have three years of follow up to assess non-inferiority.

In addition to semi-annual review described in Section 5.2, the following plan for interim monitoring for a potential recommendation of early stopping of enrollment has been established in conjunction with the DSMC. This plan is based on early donor failure rate and on the progress of recruitment.

- Rate of failure within the first 8 weeks: Upon enrollment of the first 100 eyes, and then quarterly thereafter (i.e., one review between each DSMC semi-annual meeting) the DMAC

will evaluate the failure rate within the first 8 weeks (i.e. both the early failures and the primary donor failures, as defined in Section 4.6) in each group and notify the DSMC who will have the option of requesting additional information between the semi-annual reviews. The DSMC may also request more frequent reviews at any time.

- Recruitment Progress: Recruitment progress will be evaluated at the first two DSMC meetings following initiation of recruitment. If based on the current recruitment total and recruitment trend over the previous 3 months, the projected timeline for the remaining recruitment is more than 16 months at the 1st review or more than 12 months at the 2nd review, the DSMC will discuss whether the study timeline can be met.

8.0 Extension Analysis Plan

8.1 Background

CPTS Protocol Amendment V 4.0 7-1-15 extended follow up such that participants who are willing to re-consent will continue to be monitored for annual protocol visits past the 3-year timepoint, until at least 2017. The same data will be captured from annual visits as well as interim unscheduled visits as was done previously. The same study specific tests – pachymetry and specular/confocal microscopy - will continue to be performed on the annual visits. All participants will therefore have a last possible common endpoint rather than exiting individually when they reach their 3 year post-operative visit; all visits will continue to be captured until approximately the 2nd quarter of 2017.

Rationale: Continued follow-up of CPTS participants provides an excellent opportunity to gain more information about many factors affecting DSAEK outcomes, including donor age and preservation time. We estimate a maximum potential of 740 and 160 Year 4 and 5 visits, respectively, if most participants re-consent. This additional longitudinal data will provide important information on longer term DSAEK outcomes that are not necessarily impacted by preservation time, although we will continue to assess that variable as well.

Actual number of surgeries, spread into timing of when they occurred			84% of enrolled are expected to reach 3 year	90% of those reaching 3 year are expected to consent to CPTS extension		
Enrolled	CPTS Grant		Projected to reach 3 year visit	Projected to consent to CPTS extension	4 year visit	5 year visit
52	Y4	Q2 2015	44	39		
183		Q3 2015	154	138		
197	Y5	Q4 2015	165	149		
187		Q1 2016	157	141		
232		Q2 2016	195	175	**37**	
181		Q3 2016	152	137	**131**	
204	Y6	Q4 2016	171	154	**141**	
94		Q1 2017	79	71	**134**	
		Q2 2017			**167**	**35**
		Q3 2017			**130**	**125**

8.2 Objectives

The objective of the extension is to extend the 3 original study objectives to the 4 and 5 year endpoints.

8.3 Statistical Methods

The same statistical methods for all 3 study objectives as outlined above for analysis at 3 years will be extended to the 4 year and 5 year endpoints.

Baseline characteristics will be tabulated and compared between subjects who completed a 3 year visit and chose not to participate versus those who completed a 3 year visit and consented to participate, stratified by preservation time group. Subjects who dropped prior to the 3 year visit for any reason (regraft, death, LTF, withdrew) will be excluded from this comparison.