Massachusetts Eye and Ear Infirmary

# SAFETY AND FEASIBILITY OF CULTIVATED AUTOLOGOUS LIMBAL EPITHELIAL CELL TRANSPLANTATION IN THE TREATMENT OF LIMBAL STEM CELL DEFICIENCY

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## List of Abbreviations

| | |
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| AE | Adverse Event |
| AIDS | Acquired Immune Deficiency Syndrome |
| AMT | Amniotic Membrane Transplantation |
| AS-OCT | Anterior Segment Optical Coherence Tomography |
| BCVA | Best Corrected Visual Acuity |
| BUN | Blood, Urea, Nitrogen |
| CALEC | Cultivated Autologous Limbal Epithelial Cells |
| CLAU | Conjunctival Limbal Autograft |
| CMCF | Cell Manipulation Core Facility |
| CRF | Case Report Form |
| eCRF | Electronic Case Report Form |
| DFCI | Dana-Farber Cancer Institute |
| eGFR | Estimated Glomerular Filtration Rate |
| FDA | Food and Drug Administration |
| GA | General Anesthesia |
| HIPAA | Health Insurance Portability and Accountability Act |
| HIV | Human Immunodeficiency Virus |
| ICF | Informed Consent Form |
| IND | Investigational New Drug |
| IRB | Institutional Review Board |
| IVCM | In Vivo Confocal Microscopy |
| LSC | Limbal Stem Cells |
| LSCD | Limbal Stem Cell Deficiency |
| MEEI | Massachusetts Eye and Ear Infirmary |
| MIVA | Monitored Intravenous Anesthesia |
| MM | Medical Monitor |
| MOP | Manual of Procedures |
| MRSA | Methicillin-resistant Staphylococcus aureus |
| NEI | National Eye Institute |
| OSDI | Ocular Surface Disease Index |
| PACT | Production Assistance for Cellular Therapies |
| PHRC | Partners Human Research Committee |
| QC | Quality Control |
| SANDE | Symptom Assessment iN Dry Eye |
| SOP | Standard Operating Procedures |

# Contents

## 1. BACKGROUND

The cornea is a transparent, avascular tissue covered by non-keratinized stratified epithelium that is responsible for maintaining a smooth ocular surface for normal vision as well as for providing a barrier against environmental and external stress. The entire ocular surface is covered by corneal, limbal, and conjunctival epithelial cells that, together with a stable pre-ocular tear film, maintain its integrity.

Corneal epithelial stem cells are adult somatic stem cells located at the limbus and represent the ultimate source of transparent corneal epithelium (Schermer et al., 1986, Tseng et al., 1996). When these limbal stem cells (LSC) become dysfunctional or deficient, the cornea is unable to maintain its surface epithelial integrity and phenotype and a disease called corneal limbal stem cell deficiency (LSCD) develops.

Corneal scarring and opacity is the 5$^{th}$ commonest cause of blindness worldwide, accounting for 5.1% of blindness (Resnikoff et al., 2008). LSCD, a major cause of corneal scarring, arises from a variety of congenital or acquired causes that are infectious (like trachoma), immunologic, oncologic or iatrogenic in nature and in turn lead to severe ocular surface dysfunction. The LSCD can be caused by Stevens-Johnson syndrome, ocular cicatricial pemphigoid, aniridia, chemical or thermal burns, contact lenses, a variety of microbial infections, long-term use of topical (including antiglaucoma) medications, irradiation, tumors, or multiple surgical procedures or cryotherapy involving the ocular surface. LSCD afflicts thousands of people in North America (Holland and Schwartz, 1999) and is particularly prevalent in chemical and thermal burns of the ocular surface.

The clinical hallmark of LSCD is conjunctivalization of the corneal surface (or replacement of normal and transparent corneal epithelium by opaque conjunctival epithelium), neovascularization, recurrent or persistent epithelial defects, ocular surface inflammation, and scarring all of which can lead to decreased vision, pain, and impaired quality of life (Puangsricharern and Tseng, 1995) (Kruse et al., 1990) (Figure 1).

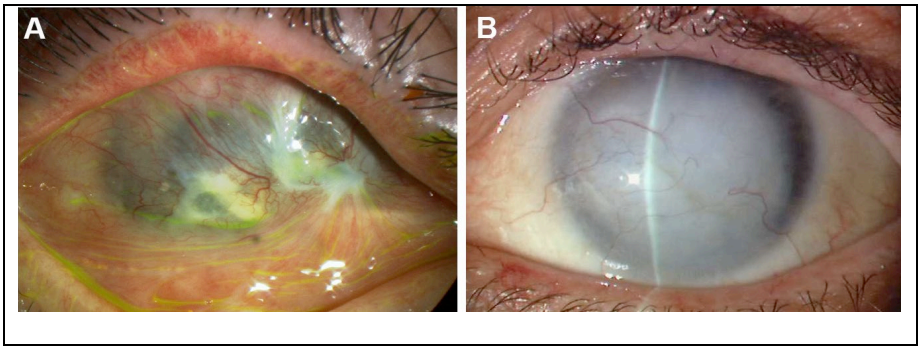


*Figure 1 A and B: In limbal stem cell deficiency (LSCD), the cornea becomes vascularized, scarred, and opaque; the demarcation between cornea and conjunctiva is lost. Patients experience loss of vision, photophobia, irritation and pain.*

Conventional corneal transplantation replaces only central cornea and cannot satisfactorily treat the corneal surface with extensive or complete LSCD. As a result, therapeutic strategies have been developed at replacing limbal epithelium with or without corneal transplantation surgery (Tseng, 1996, Kenyon and Tseng, 1989, Copeland and Char, 1990, Morgan and Murray, 1996, Holland and Schwartz, 1996). Several techniques have been reported for limbal stem cell transplantation or keratoepithelioplasty. In unilateral cases of LSCD, donor tissue is obtained

from the fellow eye, called limbal autograft; in bilateral cases of LSCD tissue is obtained from cadaver donors, called limbal allograft (Yalcindag et al., 2008, Dua and Azuara-Blanco, 1999, Kenyon and Tseng, 1989). Both procedures seek to provide therapeutic benefit through transplantation of a new source of epithelium onto a diseased ocular surface after the removal of the recipient's scarred and diseased epithelium (Dua and Forrester, 1990, Dua and Azuara-Blanco, 1999, Clinch et al., 1992, Jenkins et al., 1993, Kenyon and Tseng, 1989). Since limbus is vascularized and contains antigen presenting cells, limbal allografts carry a high risk of rejection and require use of lifelong systemic immunosuppression. Limbal allograft survival is limited to 50% at 5 years despite the use of systemic immunosuppression and survival is much lower if concurrent penetrating keratoplasty is performed to aid in visual rehabilitation (Smolin, 2005).

Autologous limbal transplantation, such as conjunctival limbal autograft (CLAU), is a viable option in unilateral cases and it circumvents the use of immunosuppression. Traditionally, two large pieces of the limbus are removed from the donor eye. The main limitation of this technique is that it carries risks of inducing limbal stem cell deficiency to the donor eye when harvesting limbal autografts (Haamann et al., 1998, Tan et al., 1996, Kenyon and Tseng, 1989). Cases of corneal haze, pseudopterygium, epithelial defects and conjunctivalization in the donor eye have been reported (Dua et al., 2010, Morgan and Murray, 1996, Fogla and Padmanabhan, 2005, Yao et al., 2002). Although recently some attempts have been made to use a smaller graft, such as Simple Limbal Epithelial Transplantation (SLET), there are only limited data, especially regarding long-term follow-up on the new procedure (Sangwan, 2012).

In order to circumvent risks of allograft rejection (as seen in limbal allografts) and damage to the donor eye (as seen in limbal autografts, such as CLAU), a technique has been developed to cultivate autologous limbal epithelial cells (CALEC) for transplantation. This technique is advantageous because of its ability to utilize a small amount of the participant's own tissue. The risk of damage to the donor eye is bypassed by expanding limbal epithelial (stem) cells from a small biopsy (2-3 $mm^2$) onto the substrate, such as amniotic membrane, in culture before transplantation. The success of using such a new surgical approach has been reported in several human studies (Koizumi et al., 2001, Rama et al., 2010, Sangwan et al., 2005, Schwab et al., 2000, Shimazaki et al., 2007, Shortt et al., 2007, Basu et al., 2012, Baradaran-Rafii et al., 2010, Nakamura et al., 2006, Pellegrini et al., 1997).

The general advantages of CALEC transplantation are that 1) it provides an amplified source of epithelial (stem) cells as compared to transplantation of the similar size of limbal biopsy, 2) it is an autologous source of cells and does not carry risk of immunologic rejection, 3) it carries significantly lower risk of inducing LSCD in the donor eye, and 4) it is possible to repeat the biopsy and transplant owing to the non-invasiveness of the procedures to both donor and recipient eyes. Currently there are no good options for treatment of LSCD in the U.S and patients have a significant burden from the disease. That, along with recent advances in stem cell research provide compelling reasons for conducting a study aimed at assessing first, the safety and feasibility of CALEC grafts, and additionally, their effectiveness in treating LSCD.

The most common substrate utilized for ex vivo expansion of limbal epithelial cells has been amniotic membrane. Amniotic membrane transplantation (AMT) has extensive history in ocular surface reconstructive surgery and its utility alone or in combination with limbal stem cell transplantation has been reported (Holland and Schwartz, 2000, Koizumi et al., 2000, Kolli et al., 2010, Nakamura et al., 2003, Prabhasawat et al., 2001, Shimazaki et al., 2002). In cases of partial LSCD, AMT alone has been shown to provide a useful substrate for regeneration of the remaining epithelial cells by enhancing epithelial cell migration and creating a hospitable

microenvironment mimicking limbal stem cell niche (Grueterich et al., 2003, Tseng, 1989, Dietrich-Ntoukas et al., 2012, Shahdadfar et al., 2012, Tsai and Tsai, 2010, Tseng et al., 2002). In addition, use of amniotic membrane as a substrate for limbal epithelial cell growth has added the benefits of amnion's ability to facilitate epithelialization (Lee and Tseng, 1997, Tsai and Tseng, 1988), reduce inflammation and scarring (Fernandes et al., 2005, Prabhasawat et al., 2001), and act as a new and natural basement membrane when the underlying stromal tissue has been destroyed.

Combining the expansion of cells ex vivo with cultivation on an amniotic membrane has the advantage of ensuring a compatible extracellular matrix for the graft, thus increasing its durability and manipulability (Zakaria et al., 2010). This method is an improvement over earlier attempts at the use of engineered corneal surfaces, in which fragile sheets of epithelial cells, with no substantial underlying stromal support, were transplanted (Nishida et al., 2004, Pellegrini et al., 1997).

There is no CALEC product available in the U.S., and we have thoroughly reviewed efforts to produce such a product in other countries. As a result, our first goal has been to optimize and standardize the techniques of CALEC preparation for the clinical trial in the U.S. We have significantly improved upon the quality of the cell therapy product and process of preparation by using completely defined reagents, preforming additional testing on reagents used (for example bovine adventitious virus testing), using autologous starting material, removing antibiotics and murine feeder cells, employing highly reproducible methods for cell isolation, and expansion and quality control of the resultant CALEC sheets. These modifications and standardizations were aimed at enhancing safety and predictability of the patients' clinical outcomes. Based on the pre-clinical testing performed in the Center for Human Cell Therapy Laboratory the CALEC constructs are ready for the phase I/II clinical trial. We have received Investigational New Drug Application (IND #16102) approval from the FDA for our CALEC graft to perform the clinical trial at the Massachusetts Eye and Ear Infirmary (MEEI) for unilateral LSCD. The FDA has advised us in the setting of aims, inclusion and exclusion criteria, and study design.

In summary, LSCD is a common cause of serious and prevalent corneal blindness, and there are significant limitations to the current available treatment options and standards of care. Taking into consideration prior experience with CALEC in other countries, we have set a goal to optimize and standardize the techniques of CALEC preparation for the clinical trial in the U.S. We have significantly improved upon the quality of the cell therapy product and process of preparation; such modifications and standardizations are aimed at maximizing safety and predictability of the participants' clinical outcomes. In this application, we will evaluate the safety and feasibility of our CALEC product as well as its efficacy in treating the LSCD.

## 2. STUDY AIMS

The main aim of the study is to determine preliminary estimates of the safety and feasibility of cultivated autologous limbal epithelial cell (CALEC) transplantation in the treatment of unilateral LSCD. Secondarily, efficacy of CALEC will be investigated. This secondary objective is intended to be exploratory rather than conclusive.

### 2.1. PRIMARY OBJECTIVES

1. **Safety:** To establish the safety of CALEC transplantation by determining the incidence of primary ocular adverse events through 18 months of follow-up.

2. **Feasibility:** To establish feasibility of manufacturing CALEC for corneal surface reconstruction.

### 2.2. SECONDARY OBJECTIVES

1. **Efficacy:** To investigate whether CALEC transplantation is efficacious in treatment of LSCD participants by comparing pre-operative to post-operative clinical parameters at months 3, 12, and 18.

### 2.3. STUDY CONCLUSIONS

The results of this Phase I/II study will provide guidance on whether to continue to a larger Phase III study and will also provide preliminary data to help determine sample size for future trials. Generally, if the study is not halted for feasibility issues, and there are no major safety or efficacy concerns, a larger study of CALEC could be considered. However, caveats of the study data and other potential factors outside of the trial will need to be considered and weighed in the ultimate decision of whether to proceed.

## 3. STUDY TIMELINE

The total duration of the study, from funding start date to final analysis and manuscript, will be approximately 72 months. This will include study startup, initiation and completion of recruitment (according to procedures detailed in Section 5), completion of 18 months of follow-up for all participants completing a transplant, and study closeout. Details of the study timeline and milestones can be found in the CALEC Study Policy Document.

## 4. STUDY ORGANIZATION

This is an open label, single-center study to assess safety and feasibility of CALEC transplantation in participants with unilateral LSCD. This study will be performed at the Clinic Center, Massachusetts Eye and Ear Infirmary in Boston, MA. The CALEC will be manufactured in the Connell and O'Reilly Families Cell Manipulation Core Facility (CMCF) at the Dana-Farber Cancer Institute (DFCI) in Boston, MA, which will be the Resource Center for this study (CMCF DFCI). The Coordinating Center for this study will be Jaeb Center for Health Research located in Tampa, FL.

We will also have two Committees for this study, the Operations Committee and the Data and Safety Monitoring Committee (DSMC). The Operations Committee, which also functions as both Executive and Editorial Committees, has the overall responsibility for administering the study and for making day-to-day operational decisions including the recruitment plan, data capture and maintenance, protocol compliance, participant retention, and planning of investigator and other committee meetings. The DSMC will be responsible for reviewing the ethical conduct of the study and for monitoring the data for evidence of adverse or beneficial treatment effects. Details of the study organization can be found in the CALEC Study Policy Document.

## 5. POPULATION AND RECRUITMENT

This study includes participants with unilateral LSCD. All participants will be recruited at the Massachusetts Eye and Ear Infirmary according to Section 5.3. All study participants will be screened and must fit all criteria described in Sections 5.1 and 5.2. Should a participant's first biopsy fail, the participant can be rescreened at a later date for possible participation (Section 5.6).

### 5.1. PARTICIPANT INCLUSION CRITERIA

- Male or female participants age 18 to <90 years old at time of enrollment with a minimum life expectancy of 18 months
- Ability of a participant or guardian/legal representative to provide written informed consent and to comply with study assessments for the full duration of the study
- Participants with unilateral limbal stem cell deficiency (LSCD) as determined by conjunctivalization of the cornea defined by fibrovascular pannus more than 2 mm from the limbus into the cornea for ≥6 clock hours
    - Ideal candidates will also meet the following additional criteria, but these will not be required:
        - Lack of limbal palisades of Vogt for ≥9 clock hours

### 5.2. PARTICIPANT EXCLUSION CRITERIA

#### 5.2.1. GENERAL EXCLUSION CRITERIA

*Confirmed none of the following are present via blood draw at screening visit:*

- Uncontrolled diabetes, defined as most recent HbA1c >8.5% (does not need to be repeated at screening visit if done within the last 3 months prior to screening visit)
- Decreased renal function, defined as eGFR below 60 mL/min per 1.73 $m^2$
- Aspartate aminotransferase or alanine aminotransferase levels >3x institutional upper limit of normal (Institutional upper limit: AST 10-40 U/L ALT 10-55 U/L)
- Total bilirubin >2.0x institutional upper limit of normal (except participants with known Gilbert's syndrome) (Institutional upper limit: Total Bilirubin 0.0-1.0 mg/dL)
- Platelet levels <100,000 or >450,000 per microliter
- Hemoglobin levels <11.0 g/dL in men <10.0 g/dL in women
- Prothrombin time >16 seconds or activated partial thromboplastin time >35 seconds in participants not taking warfarin or an international normalized ratio >3 in participants taking warfarin
- Human Immunodeficiency Virus (HIV) infection or Acquired Immune Deficiency Syndrome (AIDS)
- Active hepatitis B or C

*Other criteria:*

- Inability to tolerate monitored anesthesia
- Current pregnancy (positive urine test) or lactation, or intent to become pregnant between enrollment and the first 3 months after transplant
- Participation in another simultaneous medical investigation or trial
- Any medical, psychiatric, debilitating disease/disorder or social condition that in the judgment of the investigator would interfere with or serve as a contraindication to adherence to the study protocol or ability to give informed consent
- Signs of current infection, including fever and current treatment with antibiotics
- Presence of potential allergy to the CALEC graft or any of the chemical components within its formulation

### 5.2.2. EXCLUSION BASED ON EITHER EYE

- Prior corneal surgery within 30 days prior to study entry except placement of amniotic membrane
- Corneal or ocular surface infection within 30 days prior to study entry or CALEC type transplantation
- Ocular surface malignancy
- Severe cicatricial eye disease

### 5.2.3. EXCLUSION BASED ON DONOR EYE

- Conjunctivalization of the cornea defined by fibrovascular pannus more than 2mm from the limbus into the cornea for ≥3 clock hours
- Lack of limbal palisades of Vogt for ≥3 clock hours
- History of allo-limbal transplantation
- Severe dry eye disease as determined by Schirmer's test ≤5 mm

### 5.2.4. EXCLUSION BASED ON RECIPIENT EYE

- Severe dry eye disease as determined by Schirmer's test ≤2 mm

## 5.3. RECRUITMENT

This study will be conducted in accordance to the Food and Drug Administration's (FDA) regulations. In addition, this study will follow the guidelines set forth in the Health Insurance Portability and Accountability Act (HIPAA), which are written in 45 CFR 160-164.

Potential study participants will be identified by the study investigators and their research team at the Cornea and Refractive Surgery Service, Massachusetts Eye and Ear Infirmary. A Research Technician, Study Coordinator, Research Fellow and/ or Investigator will identify the patients in their clinic who have unilateral LCSD. Once identified, the study team will evaluate whether the patient may be able to participate in the study based on their medical record and the study eligibility criteria. If no definitive reasons to exclude the patient are found during the review, the patient will be approached by their physician (investigator) to review the study.

Those patients who volunteer to participate will go over the study details and the consent form with study staff. If he or she understands the study, including its risks, and agrees to participate, they will be asked to sign a written consent document. Subsequently, the screening procedures will be performed to confirm the eligibility of the study participant. Once it is determined that the participant qualifies to enroll in the study, he or she will have another visit to provide the baseline data. After this baseline visit, the participant will be assigned into a study arm, as described below in Section 5.5. The schedule of post-corneal reconstruction visits is standard for participants undergoing these types of procedures.

This study does not exclude, or intentionally recruit, participants based on gender, race, or ethnicity.

## 5.4. INFORMED CONSENT PROCESS

Participants are required to sign an informed consent form (ICF) which includes a Health Insurance Portability and Accountability Act (HIPAA) authorization form before participating in the study. Given that the Investigators and study staff are responsible for maintaining detailed knowledge of the study protocol, safety profile, and previous work with the procedure, these individuals will discuss the protocol with potential participants and obtain informed consent. The

study team will review the study procedures, visit schedule, known risks, potential benefits (if any), alternative treatments and financial responsibilities with all potential participants. Each participant will be informed of their right to withdraw at any time from the study without affecting their care or relationship with the treating physician and participating institution. A study member will also explain and discuss with the participant their confidentiality rights as described in the HIPAA form. Participants will be given the opportunities to ask questions. An Investigator will obtain written consent from each participant prior to any study procedures performed by the study team.

A note will be made on the study record that the ICF was signed by the participant. The ICF will follow the guidelines set forth by the FDA and Partners Human Research Committee (PHRC), which is the Institutional Review Board (IRB) for this study. A copy of the signed consent form will be given to the participant.

We do not anticipate a need to obtain on-going consent over the course of the study. If protocol amendments result in consent form changes, participants will be re-consented if the changes affect data integrity, or affect the participants' rights, safety, or well-being. Legally Authorized Representatives (LAR) can consent on behalf of participants who cannot provide their own consent. Consent by the LAR will be done in accordance with Massachusetts laws and PHRC requirements.

### 5.5. ENROLLMENT PROCEDURES

There will be two sequential phases of enrollment – Phase A followed by Phase B.

**Phase A – Staggered Enrollment**

During Phase A, eligible participants completing the screening and baseline visits will be enrolled in a staggered fashion (described below) to receive CALEC. This will be the procedure until three participants in Phase A complete a CALEC transplant (or until halting guidelines apply, see Section 9.4). During Phase A, the following will apply:

- If a participant enrolls during Phase A but withdraws from the study prior to transplant, the participant will not count towards the recruitment goal of three participants required for Phase A
- If a biopsy is done for a participant in Phase A, and the biopsy is unable to generate a viable cell sheet for transplant, a second biopsy may be attempted according to the procedure in Section 5.6.
- Once a given Phase A biopsy attempt has either (1) failed or (2) succeeded and participant has completed the 2-week visit, the study team can then proceed to either of the following:
  - Schedule a second biopsy attempt for any prior Phase A participant whose first biopsy failed (according to Section 5.6)
  - Consent and enroll a new participant in Phase A

- Phase A enrollment ends after three participants in Phase A have completed a CALEC transplant and the 2-week visit after transplantation. After this occurs, Phase B will begin. Some additional Phase A participants whose first biopsy failed may complete a second biopsy after Phase B begins, and would count towards the Phase A cohort.

**Phase B – Open Enrollment**

During Phase B, eligible participants completing the screening and baseline visits will be enrolled to receive CALEC, with no requirements to stagger the enrollments.

During Phase B, the following will apply:

- If a participant enrolls during Phase B but withdraws from the study prior to transplant, the participant will not count towards the recruitment goal.
- If a biopsy is done for a participant in Phase B, and the biopsy is unable to generate a viable cell sheet for transplant, a second biopsy can be attempted according to the procedure in Section 5.6.

**Study enrollment ends as soon as one of the following is met (whichever occurs first):**

- A total of 17 CALEC transplants (across Phase A and B) have been completed.
- The last transplant that can reasonably allow 18 months of follow up within the study timeline as noted in Section 3 has been completed.

The Statistical Analysis Plan documents how participants in the study cohort will be analyzed.

In both Phases, investigators, participants, and those involved in the study assessments will all be aware of (unmasked to) the study intervention received and appropriate protocol procedures.

### 5.6. SECOND BIOPSY

If a participant experiences failure of the biopsy to generate a viable cell sheet, the reasons for failure will be reviewed. The participant can be rescreened for a second attempt at biopsy and CALEC construct if the following are met: (1) the reason for failure is not likely to re-occur for that participant, (2) a repeat biopsy does not present a significant risk to the participant, and (3) study enrollment has not ended. Failure is considered not likely to re-occur when initial failure is deemed secondary to culture condition not cell growth (such as evidenced by normal growth of P0 cells). Second biopsy attempts will be scheduled at least 30 days after the initial biopsy which is medically appropriate for this type of biopsy procedure. The screening visit and baseline visit will be repeated, unless the second biopsy is scheduled to occur within 55 days of the original screening visit date, in which case only the baseline visit will be repeated. The second biopsy must occur within 25 days of the second baseline visit. (Note: The second baseline assessments will not be used as primary baseline values, but rather will be used to document any changes, and may be considered for sensitivity analyses.)

Each participant will be limited to a total of two biopsies. If a second attempt will not be pursued or the second biopsy also fails to generate a viable cell sheet, the participant may or may not continue in follow up, as defined by the criteria in Section 5.7.

### 5.7. DISCONTINUATION OF STUDY FOLLOW UP AND PARTICIPANTS WHO DO NOT COMPLETE A TRANSPLANT

A study participant has the right to withdraw from the study at any time. If a study participant is considering withdrawal from the study, the principal investigator should personally speak to the individual about the reasons, and every effort should be made to accommodate him or her. Participants that decide to withdraw after transplantation will be asked to return to the clinic for an Early Termination Visit as described in Section 6.8.12. After discontinuation of study follow up for any reason, the subject will continue care with their non-study clinician.

Other considerations for study discontinuation are as follows:

- **Prior to biopsy**

- Study participants who are determined to be ineligible for any reason (including pregnancy) prior to biopsy will not proceed to biopsy or transplant, and will either discontinue or postpone and re-screen at a later time.

- **<u>After biopsy but prior to transplant</u>**
  - Study participants who become pregnant will consult with the study doctor and their Obstetrician/Gynecologist or primary care physician before determining if it is safe for them to continue with the transplant procedure. If the decision is made not to continue with the transplant procedure, the participant will either discontinue or postpone and re-screen at a later time.
  - Study participants who are determined to be ineligible for any other reason will consult with the study doctor before determining if it is safe for them to continue with the transplant procedure. If the decision is made not to continue with the transplant procedure, the participant will either discontinue or postpone and re-screen at a later time.
  - Study participants for whom the biopsy fails to generate a viable cell sheet may be evaluated for a second biopsy according to Section 5.6 above. If the participant does not proceed to second biopsy for any reason, or a second biopsy also fails to generate a viable cell sheet, the participant will discontinue.
  - <u>Additionally: Regardless of whether a participant will discontinue, all participants who had a biopsy and do not proceed to transplant will be monitored by the study team for 30 days to check for any adverse events related to the biopsy procedures.</u> If the donor eye after biopsy has a complication or adverse event that does not resolve after 30 days, the study team will also monitor the donor eye until the complication resolves. Those meeting criteria to discontinue, as detailed in the scenarios above, will discontinue after this time.

- **<u>After transplant</u>**
  - In general, all possible efforts will be employed to ensure participants who completed transplant are retained through the duration of follow up.

### 5.8. TERMINATION OF STUDY

The DSMC can recommend to terminate or temporarily suspend the clinical trial at any time point. Halting guidelines are described in Section 9.4. Should the study be terminated or suspended, the investigators, FDA, and IRB will be notified. The research staff and the IRB will determine the best method to notify participants of study termination or suspension to ensure participant safety, confidentiality, and data integrity. The investigators will notify all participants of the termination or suspension and the reasons for such action.

# 6. STUDY PROCEDURES, MEASUREMENTS, AND VISIT SCHEDULE

## 6.1. CALEC MANUFACTURING

Details of CALEC manufacturing have been provided in the Manual of Procedures Section 7.1.

## 6.2. PREPARATION AND ADMINISTRATION OF STUDY TREATMENTS

Biopsies and reconstruction CALEC procedures will be performed by the study PI, Dr. Ula Jurkunas, to minimize surgeon variability. In extraneous circumstances where Dr. Ula Jurkunas is not available to perform CALEC biopsy and/or reconstruction procedure, the study co-investigators Dr. Reza Dana and Dr. Jia Yin will perform the procedures.

### 6.2.1. PREPARATION (CALEC)

#### 6.2.1.1. LIMBAL BIOPSY

Two days prior to biopsy of the donor eye, all participants will be started on a topical fluoroquinolone. Some participants may also start on vancomycin in the eye to be biopsied if the participants are Methicillin-resistant Staphylococcus aureus (MRSA) positive or are considered to be part of high-risk populations (e.g., health care personnel).

#### 6.2.1.2. TRANSPLANTATION

Two days prior to transplantation, the participant will be started on a topical fluoroquinolone (all participants) and potentially vancomycin drops (in participants that are MRSA positive or in high-risk populations, e.g., health care personnel) in the recipient eye.

### 6.2.2. ADMINISTRATION

#### 6.2.2.1. CALEC PROCEDURE – BIOPSY

1. Participant will be taken to the preoperative area of the ambulatory surgery center where standard operating procedures (SOP) will be employed in preparing the donor eye for the surgery.
2. Type of anesthesia will be decided depending upon the age and overall functioning of the participant. If the procedure is to be performed under general anesthesia (GA), GA consent will be obtained from the participant or guardian. Otherwise, monitored intravenous anesthesia (MIVA) will be performed after the consent.
3. The eye that is to be biopsied will be marked as such and the other eye will be covered.
4. Fluoroquinolone and proparacaine drops will be administered 3 times prior to the procedure.
5. Lidocaine 1% gel will be administered into the eye and the eye will be closed with tape.
6. The participant will be brought to the operating suite and positioned in a supine position under the operating microscope in the manner typical for ophthalmic surgery.
7. The operative eye will be cleaned using 5% Betadine solution per standard surgical protocol. Both the cul-de-sac and the eyelashes will be cleaned.
8. Under sterile conditions, a limbal biopsy of 3mm-by-3 mm (1 clock hour) will be dissected from superior or inferior portion of the eye, at the discretion of the operating surgeon. The actual size of the graft will be measured and captured for data collection.
9. The biopsied material will be placed into the container with HypoThermosol® FRS for transfer to the Dana-Farber Cancer Institute.
10. The biopsied site in the conjunctiva will be closed using interrupted sutures and/or fibrin glue per the surgeon’s discretion..
11. Maxitrol or Tobradex ointment or drops will be placed onto the eye, if patient has no known allergy.
12. Either a patch or shield will be placed over the eye.

#### 6.2.2.2. CALEC PROCEDURE – TRANSPLANTATION

1. The participant will be taken to the preoperative area of the ambulatory surgery center where standard operating procedures (SOP) will be employed in preparing the recipient eye for the surgery.
2. Type of anesthesia will be decided depending upon the age and overall functioning of the participant. If the procedure is to be performed under general anesthesia (GA), GA consent will be obtained from the participant or guardian. Otherwise, monitored intravenous anesthesia (MIVA) will be performed after the consent.
3. In cases of MIVA anesthesia, peribulbar block will be injected in the operated eye.
4. The recipient eye that is to be operated on (i.e., the eye with LSCD) will be marked as such and the other eye will be covered.
5. Fluoroquinolone and proparacaine drops will be administered 3 times prior to the procedure.
6. Peribulbar or retrobulbar block with 50:50 mixture of lidocaine and bupivacaine will be injected into the recipient eye.
7. The participant will be brought to the operating suite and positioned in a supine position under the operating microscope in the manner typical for ophthalmic surgery.
8. The operative eye will be cleaned using 5% Betadine solution per standard surgical protocol. Both the cul-de-sac and the eyelashes will be cleaned.
9. If excessive ocular surface bleeding is detected, topical epinephrine (1:10,000) will be used to constrict the blood vessels and minimize bleeding prior to or during the procedure. A conjunctival peritomy may be performed per investigator discretion. The fibrovascular tissue will be dissected from the limbus and the cornea. Hemostasis will be achieved by wet-field cautery. Mitomycin C may be placed underneath the conjunctiva per the surgeon's discretion.
10. The transwell with CALEC graft inside will be removed from the original container and will be placed on a sterile silicone platform and rinsed with BSS® Sterile Irrigation Solution. A free-held trephine, size depending on the operated eye, will be used to punch the graft. The size of trephine used will be recorded in the operative note. The transwell with the remnants of the graft will be lifted off. The trephined CALEC graft on the transwell membrane will be lifted with the forceps and transferred to the surgical field where the CALEC graft will be peeled from the transwell membrane and centered onto the ocular surface with epithelium side up. CALEC will be secured with sutures and/or fibrin glue per the surgeon's discretion. The conjunctiva will be closed using sutures and/or fibrin glue per the surgeon's discretion. At the end of the procedure, fluorescein will be used to assess epithelial integrity. Lack of fluorescein uptake in the central cornea will indicate that epithelium of the CALEC graft is intact.
11. Bandage contact lens will be placed over the graft.
12. Subconjunctival injection of Kefzol and Decadron will be given, if patient has no known allergy.
13. Maxitrol or Tobradex ointment or drops will be placed onto the eye, if patient has no known allergy.
14. A patch and/or shield will be placed over the eye.

### 6.3. MODIFICATION OF STUDY INTERVENTION FOR A PARTICIPANT

If a participant has an allergy to any of the medications listed in the protocol, that medication will not be used and/or will be substituted with an appropriate alternative based on the investigators' discretion. If participant has a latex allergy, standard precautions will be employed to avoid latex products during the peri- and intra-operative periods.

## 6.4. ADDITIONAL SURGERIES

If a participant requires any additional surgeries for visual rehabilitation in the recipient eye, including cataract surgery or corneal transplantation, the surgeries will be performed at least 3 months after corneal reconstruction with CALEC.

## 6.5. MEDICATIONS AND TREATMENTS

### 6.5.1. CONCOMITANT MEDICATION/TREATMENTS

Participants who enter the study taking anti-glaucoma medication will be able to continue that medication throughout the duration of the study. If participants develop increased intraocular pressure (IOP) during the study, anti-glaucoma medications may be prescribed that include one or more or in combination formulations.

### 6.5.2. ACCEPTABLE CONCOMITANT MEDICATIONS

Participants will be able to use anti-glaucoma medications. Participants will be able to have increased dose of topical corticosteroids throughout the course of the study if determined to be necessary by the treating ophthalmologist. Participants who develop infections will be treated with appropriate antimicrobial agents such as antibiotics, anti-viral and/or anti-fungal agents. Participants will be allowed to use ointments that have antibiotic and/or steroid components.

Whenever possible, non-preserved medications will be prescribed to reduce epithelial toxicity.

Current medication regimens will not be changed, if possible.

### 6.5.3. PROHIBITED CONCOMITANT MEDICATIONS

- Other investigational treatments

### 6.5.4. STUDY MEDICATION REGIMEN

The pre-study topical ocular medication regimens of all participants will be reviewed at study enrollment, and may be changed based on investigator's clinical judgement. All participants will be prescribed a topical fluoroquinolone, a topical steroid 1%, Vancomycin 14 mg/mL (see below), 20% autologous serum eye drops, and artificial tears in both eyes as follows. If participants have or develop an allergy to the drops, the investigators will prescribe an appropriate substitute. The regimen applies to only those participants who complete the biopsy and/or the transplant, as applicable.

<u>Screening Visit</u>

If the conjunctival culture taken at screening reveals the presence of MRSA bacteria or the participant is in a high-risk population for MRSA (i.e. hospital employee), the participant will be treated with Vancomycin 14 mg/mL, four times per day in both eyes.

<u>Day 1 Post-Biopsy Visit</u>

The participant will start a topical fluoroquinolone and a topical steroid 1% in the donor eye on Day 1 after biopsy until time of corneal reconstruction (approximately two weeks). The topical steroid will be started at four times per day and adjusted based on clinical judgement. If previously using Vancomycin to treat MRSA, the participant will continue using it four times per day in both eyes.

<u>Day 1 Post-Transplant Visit (Corneal Reconstruction)</u>

- The participant will start a topical fluoroquinolone and a topical steroid 1% in recipient eye. S/he will continue regimen until 2-week visit. If previously using Vancomycin to treat MRSA, the participant will continue using four times per day in both eyes.

- The participant will start 20% autologous serum eye drops in the recipient eye after corneal reconstruction and will continue it for at least 1 month. Dosage will be adjusted based on clinical judgement. In addition the participant will start artificial tears four times per day in both eyes and continue throughout the course of the study.

1-Week Visit after Transplant

The participant will continue all medications as prescribed at Day 1 Post-Graft visit. Dosage will be adjusted based on clinical judgement.

2-Week Visit after Transplant

- The participant may start taper of topical fluoroquinolone and topical steroid 1% in the recipient eye; changes to dosage will be made for individual participants based on clinical judgement. The participant will be able to stop the steroid and fluoroquinolone in the donor eye, unless inflammation persists in which case usage will continue up to 4 weeks.
- The participant will continue autologous serum eye drops and artificial tear use four times per day in both eyes. Vancomycin will be tapered according to the same schedule as the fluoroquinolone and the topical steroid 1%.

1-Month Visit after Transplant

The participant will continue all medications as described at the 2-week visit. Around the time of this visit, participants will be starting their daily dose of the topical fluoroquinolone and the topical steroid 1% as described in the taper regimen at the 2-week visit. If the participant was previously using Vancomycin, it will be decreased along the same schedule. The autologous serum eye drops may be stopped at investigator discretion based on participant symptoms of discomfort and epithelial healing.

3-Month Visit after Transplant

From this point forward, the participant's medication regimens will no longer follow a protocol-dependent schedule and will be adjusted as medically necessary. Participants who have been MRSA positive will undergo another conjunctival culture and Vancomycin dosing will be adjusted based on findings. If participant has to continue bandage contact lens, as determined by investigator discretion based on participant symptoms of discomfort and epithelial healing, the fluoroquinolone will be continued for the duration of lens wear.

### 6.5.5. OTHER TREATMENTS

At least one ocular punctal plug will be placed in each eye as needed for which the Schirmer's Test is below 10 mm and the patient does not have an existing plug or had punctual cautery in the past. The plug(s) will be placed at the screening visit and then replaced at subsequent visits if they fall out. Punctal plugs may also be initiated at any visit per usual clinical practice. A bandage contact lens will be placed on the treated eye of all subjects up until 30 days post procedure. The bandage contact lens may continue to be worn past 30 days post procedure if there is evidence of epithelial defect or if deemed necessary by the treating physician.

## 6.6. CLINICAL EVALUATIONS

The following is a summary of how each clinical evaluation will be performed. Section 6.8 summarizes the study visit schedule and all assessments performed at each visit. The MOP provides more details regarding the order of testing and by whom each assessment is performed.

### 6.6.1. SYMPTOM ASSESSMENT

The Ocular Surface Disease Index (OSDI) and Symptom Assessment iN Dry Eye (SANDE) questionnaires will be administered by certified study personnel prior to initiating the ophthalmic exam.

#### 6.6.1.1. OCULAR SURFACE DISEASE INDEX (OSDI)

This disease-specific questionnaire includes three subscales: ocular discomfort (OSDI symptoms), which includes symptoms such as gritty or painful eyes; functioning (OSDI-function), which measures limitation in performance of common activities such as reading and working on a computer; and environmental triggers (OSDI-triggers), which measures the impact of environmental triggers, such as wind or drafts, on dry eye symptoms (Manual of Procedure (MOP), Section 21, Vitale et al., 2004).

#### 6.6.1.2. SYMPTOM ASSESSMENT IN DRY EYE (SANDE)

The SANDE questionnaire uses a horizontal visual analog technique to quantify each participant's symptomatology with regard to dryness and/or irritation (MOP, Section 22). Each index will use a 100mm line to individually assess both the average frequency and the average severity of symptoms of ocular discomfort or dryness experienced by the participant. The participant will be asked to put a mark on two given lines to depict the extent of their symptoms separately in terms of frequency and severity – the mark will be measured and recorded by the study team.

### 6.6.2. VISUAL ACUITY

At each visit, participants' best corrected visual acuity (BCVA) of both eyes will be assessed by Snellen visual acuity and recorded (MOP, Section 23). Manifest refraction will be performed if pinhole improves BCVA more than 1 line.

### 6.6.3. INTRAOCULAR PRESSURE (IOP) EVALUATION

IOP will be evaluated by one or a combination of the available modalities noted below depending on the cooperation of the participant and ocular appearance on the exam as well as ability to obtain the reading (MOP, Section 24).

- Tonopen: To measure IOP using tonopen applanation, the Tono-Pen XL (Mentor, Santa Barbara, CA) will be calibrated daily. Following administration of 0.5% proparacaine hydrochloride in the cul-de-sac, the pen tip (with disposable cover) will be touched to the central cornea until a reading is measure. Only measurements with a 5% standard error will be accepted. If error is greater than 5%, the measurement will be repeated.
- Pneumotonometry: Pneumotonometer (Reichart) measurements will be taken perpendicularly to the cornea, at the center of the cornea after calibration according to the manufacturer's instructions in the event Tonopen is unable to provide a reading
- Palpation: The IOP of the donor eye will be determined by palpation for one week post biopsy. The IOP of the recipient eye will be determined by palpation until the thirteen week visit after transplant.

### 6.6.4. SLIT LAMP EXAMINATION

At each visit, participants will undergo assessment of eyelid, conjunctiva, cornea, and all intraocular structures of both eyes, by a certified study investigator <u>who was not the investigator performing the biopsy or transplant for that participant.</u> This may include a research associate or research fellow who does not perform surgeries but functions as a clinical examiner investigator only. The number of such investigators will be limited and each will complete sample assessments on the primary efficacy endpoints (both the corneal epithelial defect surface area and NEI scale gradings noted below) as part of their study training and certification.

#### 6.6.4.1. CORNEAL OPACIFICATION

The cornea will be examined and its opacification will be graded according to the Fantes Scale (MOP, Section 26) (Fantes et al., 1990).

#### 6.6.4.2. CORNEAL FLUORESCEIN STAINING

For the clinical exam with corneal fluorescein staining, a single Akorn FUL-GLO Fluorescein Sodium strip will be wetted with a drop of sterile saline and applied to the inferior fornix and examination and photography (Section 6.6.7.2 below) will be performed between 2-5 minutes after the instillation of fluorescein.

The entire cornea will be examined using slit lamp evaluation with a yellow barrier filter (#12 Wratten) and cobalt blue illumination (staining is more intense when it is observed with a yellow filter). Staining with fluorescein will be used to determine presence of corneal epithelial defects. The extent of corneal fluorescein staining will be evaluated using the National Eye Institute (NEI) grading scale (MOP, Section 25). Each of five corneal zones (superior, nasal, central, inferior, and temporal) will be graded from 0 (normal) to 3 (severe) and staining score of the cornea will range from 0 and 15 points. Epithelial defects (as defined as confluent epithelial staining of >1 $mm^2$) will be measured at the greatest horizontal and vertical dimensions, and surface area will be calculated in $mm^2$.

### 6.6.5. SCHIRMER'S TEST

The Schirmer's test will be performed with anesthesia by placing a narrow filter-paper strip (5mm × 35mm strip of Whatman #41 filter paper) in the inferior cul-de-sac (MOP, Section 27). This test is to be conducted in a dimly lit room. The participant will be instructed to gently close their eyes until five minutes have elapsed. The strips will be removed. Since the tear front will continue advancing a few millimeters after it has been removed from the eyes, it is important to mark the tear front with a ball-point pen at precisely five minutes. Aqueous tear production will be measured by the length in millimeters that the strip wets in 5 minutes.

### 6.6.6. IMPRESSION CYTOLOGY

At 12, 15, and 18 months after transplantation, if there is clinical suspicion of limbal stem cell deficiency in the recipient eye, an impression cytology of the ocular surface will be performed to confirm the diagnosis. Impression cytology will be performed in the recipient eye after application of the topical anesthetic. Nitrocellulose membranes will be firmly pressed onto the ocular surface (as shown in MOP) for 5 seconds. Each membrane will cross the limbus so as to collect cells from the cornea, the limbus, and the conjunctiva. The membranes will then be removed and fixed in methanol solution immediately. Samples will then be taken to the pathology laboratory for further staining and analysis.

### 6.6.7. SLIT LAMP PHOTOGRAPHY

Digital corneal photography to measure neovascular area and epithelial defect area will be done by a certified study photographer or research associate, using a slit lamp with a digital camera attachment and a flash-through-the-slit illumination system.

#### 6.6.7.1. PHOTOGRAPHY FOR NEOVASCULAR AREA

The entire cornea will be pictured using diffuse illumination and 10x magnification. If lids are drooping, the photographer will attempt to gently remove them from area of focus with a cotton swab. Participants with dark irises, which may prohibit a clear image, may be dilated with tropicamide only (without use of an adrenergic agent that may induce vascular 'blanching') to enhance visualization of corneal blood vessels. Digital photographs will be taken and the

images will be uploaded into the graphics editing software (ImageJ). The neovascular area will be calculated as described in MOP. Digital slit lamp corneal pictures will be analyzed using graphics-editing software (Photoshop) and a mathematical program (Matlab script). After the total area is delineated, the blood vessels will be isolated using Photoshop. Neovascular area (in pixels) is computed using a Matlab script to assist a qualified technician to measure the area of the vessels themselves.

**6.6.7.2. PHOTOGRAPHY FOR EPITHELIAL DEFECT AREA**

For photography with corneal fluorescein staining, a single Akorn FUL-GLO Fluorescein Sodium strip will be wetted with a drop of sterile saline and applied to the inferior fornix and examination (Section 6.6.4.2 above) and photography will be performed between 2-5 minutes after the instillation of fluorescein.

Following staining, the entire cornea will be imaged using cobalt blue filter of the slit lamp. A magnification of 10x with diffuse illumination will be used. Digital photographs will be taken and the images will be uploaded into the graphics editing software. The epithelial defect area will be calculated. Digital slit lamp corneal pictures will be analyzed using ImageJ. The total area of the epithelial defect(s) will be measured in pixels. Then, total corneal area will also be measured in pixels. Then, the ratio of the total corneal area which is covered by epithelial defect(s) will be calculated in percentage.

**6.6.8. LID MARGIN AND CONJUNCTIVA CULTURES**

Conjunctival cultures will be performed to evaluate the microbiologic colonization at the screening visit in all participants and at the 3- and 12-month visits only if MRSA was detected at screening. A moistened swab will be used to get specimen from the lower fornix in each eye, and will be inoculated above the “R” and “L” on the blood and chocolate plates in the shape of “C.” The plates will be placed in an incubator set to 35°C with 5% $CO_2$. All plates will be observed daily for seven days for the formation of microbial colonies. The bacteria will be identified by using standard microbiologic techniques.

**6.6.9. FUNDUS EXAMINATION**

A dilated fundoscopy (fundus examination) will be performed to evaluate the fundus for abnormalities. If the fundoscopy cannot be performed, a B-scan ultrasound will be performed instead to evaluate the fundus for abnormalities.

**6.6.10 ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY**

Anterior segment optical coherence tomography (AS-OCT) is a non-contact imaging modality that provides high-resolution cross-sectional images of ocular structures. The subject will be positioned in front of the AS-OCT machine (RTVue-100, OptoVue, Freemont, CA). One scan of the central cornea and one scan of the limbus in each quadrant (superior, inferior, nasal, and temporal) will be obtained using an automated scanning algorithm. AS-OCT imaging of both eyes will be performed at the baseline, 12 and 18-month post-operative visit. Central epithelial thickness is calculated by an automatic algorithm. The deepest limbal epithelial thickness in each quadrant will be measured manually on cross-sectional images. The outcomes are central corneal epithelial thickness and limbal epithelial thickness in superior, inferior, nasal, and temporal quadrants. There may be future measurements using these images that are currently unknown. AS-OCT will be done by a certified study photographer or research associate.

**6.6.11 IN VIVO CONFOCAL MICROSCOPY**

In vivo confocal microscopy (IVCM) is an imaging method that allows visualization of the corneal structures at the cellular level. A drop of topical anesthetic will be instilled in each eye prior to

the procedure and a temporary bandage contact lens may be applied prior to imaging for patient comfort and removed after imaging. One drop of hypromellose 2.5% is applied to the patient's eye and one drop of hypromellose 0.3% will be placed on the objective lens of the microscope according to the manufacturer's instructions. IVCM will be performed on each eye with a Heidleberg retina tomograph (HRTII)/Rostock cornea module (RCM) (Heidelberg Engineering GmbH, Dossenheim, Germany) with a water-immersion objective lens. One scan of the central cornea and one scan of each quadrant of the limbus (superior, nasal, temporal, and inferior) will be obtained. IVCM of both eyes will be performed at the baseline visit, 12 and 18-month post-operative visits unless patient reports eye pain, discomfort, irritation and cannot tolerate the procedure, or there is clinical contraindication in the case of corneal epithelial defect, hemorrhage, or inflammation. Images will be analyzed to determine the presence or absence of the following cell types: corneal epithelial cells are defined as polygonal cells with bright, well-defined borders, dark cytoplasm and no visible nuclei; conjunctival cells are defined as cells with bright nuclei and ill-defined borders. Limbal palisades of Vogt are defined as hyper-reflective double contoured linear structures that alternate with islands of epithelial cells. The outcomes are: presence (or absence) of corneal epithelial cells in the central cornea and limbus, presence (or absence) of conjunctival epithelial cells in the central cornea and limbus, and the presence (or absence) of limbal palisades of Vogt. There may be future analysis of the images that are currently unknown. IVCM will be done by a certified study photographer or research associate.

## 6.7. LABORATORY EVALUATIONS

Participants will have blood drawn at the Massachusetts Eye and Ear Infirmary Clinical Laboratory at screening (see list of tests run at screening).An additional blood draw will be collected within 7 days before or after biopsy to test for Hepatitis B, Hepatitis C, and HIV, and results communicated to CMCF prior to release of the product.

Additionally, female participants of child-bearing potential will undergo a urine pregnancy test at screening for study eligibility determination and then again at the visit before CALEC transplantation.

Participants with uncontrolled diabetes, as defined by the most recent HbA1c >8.5% (at the screening visit or within 3 months prior to screening visit) and with decreased renal function, as defined by eGFR (estimated glomerular filtration rate) below 60 mL/min per 1.73 $m^2$, will be excluded from the study. Similarly, we will check lab values for liver enzymes, hemoglobin, platelets, prothrombin time, partial thromboplastin time, and international normalized ratio. Aspartate aminotransferase or alanine aminotransferase levels >3x institutional upper limit of normal will be considered abnormal. Total bilirubin >2.0x institutional upper limit of normal (except participants with known Gilbert's syndrome) will be considered abnormal. Further, platelet levels <100,000 or >450,000 per microliter, and male hemoglobin of <11.0 g/dL, and female hemoglobin levels of <10.0 g/dL, will also be consider abnormal. Finally, prothrombin time >16 seconds or activated partial thromboplastin time >35 seconds in participants not taking warfarin, and an international normalized ratio >3 in participants taking warfarin, will be considered abnormal. Participants testing positive for HIV or AIDS or active hepatitis B or C will also be excluded. If any of these lab values are found to be abnormal, the participants will be excluded and referred to their primary care physicians for further workup and treatment.

## 6.8. STUDY SCHEDULE

Below is a summary of the study visit schedule. The following sections summarize all assessments performed at each visit. The MOP provides more details regarding the order of testing. For a summary table of study visit schedule and all assessments performed, see

Appendix VII. All visits occur after the participant has been enrolled (after obtaining informed consent).

- **Screening Visit**
- **Baseline Visit** –required within 30 days of Screening Visit
- **Biopsy** - required within 25 days of Baseline Visit
- **Post-Biopsy Visit** – required 1 day after biopsy
- **Pre-operative Visit** - required 1 to 5 days prior to transplant
- **Transplant** – anticipated to occur 10 to 30 days after biopsy
- **Day 1, Week 1, Week 2*, and Month 1 Visits**
    - Same for Phase A and B participants
        - Only required for participants who complete a transplant
        - Visit schedule is timed from transplant date
- **Month 3, 6, 9*, 12, 15*, and 18 Visits**
    - Same for Phase A and B participants
        - Only required for participants who complete a transplant
        - Visit schedule is timed from transplant date

* The 2 week, 9 month, and 15 month visits will be optional for participants for whom travel is prohibitive. For participants who opt out of these visits, the clinical site will work with the participant's usual care ophthalmologist on the following:

(1) Advise that the participant follows the protocol's post-operative medication regimen, although the regimen will not be enforced by the protocol in these participants.
(2) Request adverse events be reported to the clinical site, under a medical release from the participant.

### 6.8.1. STUDY SCREENING

Prospective participants, as defined by the inclusion/exclusion criteria, will be considered for entry into this study. The study design and treatment regimen will be discussed with each participant. Written informed consent will be obtained before any study-specific screening evaluations are performed. The following evaluations and procedures will be performed for all participants during the screening period:

- Informed consent process culminating in signed consent document
- Eligibility Assessment
- Record current ocular and systemic medications
- Record medical/surgical history in the past 5 years
- Record demographic data, including date of birth, sex, and race/ethnicity
- Review of systems
- Blood draw for testing for confirmation of eligibility criteria, including:
    - Hepatitis B
    - Hepatitis C
    - HIV, AIDS
    - CBC (including platelet and hemoglobin levels)

    - eGFR
    - HbA1c, if needed (does not need to be repeated at screening visit if done within the last 3 months prior to screening visit)
    - Aspartate aminotransferase and alanine aminotransferase levels
    - Bilirubin levels
    - Prothrombin and thromboplastin time
- Urine pregnancy test for women of childbearing potential
- Slit lamp examination (both eyes), including
    - Corneal opacification (as defined by Fantes Scale) (both eyes)
    - Staining with fluorescein (extent of staining and epithelial defect area) (both eyes)
- Visual Acuity BCVA (both eyes)
- Conjunctival swab and culture (both eyes)
- Intraocular pressure (both eyes)
- Fundus examination or B-scan ultrasound (both eyes)
- Slit lamp photographs (both eyes) by certified technician, including
    - Photography for neovascular area
    - Photography (with fluorescein) for epithelial defect
- Schirmer's test with anesthesia (both eyes)
- Referral to primary care physician to confirm general health prior to surgery, as needed according to MEEI hospital SOPs
- Punctal plugs, if needed (Section 6.5.5) (both eyes)

In addition, blood draw will be performed for autologous serum eye drops prior to transplantation per CMCF guidelines.

**6.8.2. BASELINE (WITHIN 30 DAYS OF SCREENING VISIT)**

A baseline visit will be conducted after a participant is considered eligible to participate as determined by the screening visit. Baseline visit procedures must occur within 30 days after the screening visit

Baseline procedures may be conducted over multiple visits after screening.

The following procedures will be conducted for the baseline visit:

- Review of pre-operative health screening results to confirm general health prior to surgery according to MEEI hospital SOPs
- Slit lamp examination (both eyes), including
    - Corneal opacification (as defined by Fantes Scale) (both eyes)
    - Staining with fluorescein (extent of staining and epithelial defect area) (both eyes)
- Visual acuity BCVA (both eyes)
- Intraocular pressure (both eyes)
- Slit lamp photographs (both eyes) by certified technician, including
    - Photography for neovascular area
    - Photography (with fluorescein) for epithelial defect
- Anterior Segment Optical Coherence Tomography
- In Vivo Confocal Microscopy
- Punctal plugs, if needed (Section 6.5.5) (recipient eye only)
- Assessment of changes in medical conditions since screening
- Symptom assessment

In addition, within seven days before or after limbal biopsy, blood draw will be performed for donor serology testing in CALEC participants only.

### 6.8.3. LIMBAL BIOPSY (WITHIN 25 DAYS OF BASELINE VISIT)

Following the baseline visit, a biopsy of the donor eye will be taken as an outpatient procedure for all participants receiving the CALEC. The biopsy must occur within 25 days after the baseline visit. Refer to section 6.2.

### 6.8.4. TRANSPORTATION OF TISSUE TO CMCF & PREPARATION OF CALEC

If participants are enrolled in the CALEC arm, the tissue taken from the donor eye during the biopsy will be taken to Connell and O'Reilly Families Cell Manipulation Core Facility (CMCF) at the Dana-Farber Cancer Institute (DFCI) per operating procedures (MOP, Section 7).

### 6.8.5. POST-BIOPSY ASSESSMENT OF DONOR EYE (1-2 DAYS AFTER CALEC BIOPSY)

- Slit lamp examination (donor eye) including
  - Staining with fluorescein (extent of staining and epithelial defect area)
- Visual acuity (BCVA) (donor eye)
- Intraocular pressure (donor eye)
- Adverse event assessment
- Slit lamp photos (donor eye) by certified technician, including:
  - Photography (with fluorescein) for epithelial defect
- Punctal plug placement, if needed (Section 6.5.5)

### 6.8.6. TRANSPORTATION OF CALEC FROM CMCF TO MEEI

The CALEC is anticipated to be released 10 to 30 days after biopsy, upon which it will be transported from CMCF to MEEI (MOP, Sections 7.1.3 and 7.1.4).

### 6.8.7. PREOPERATIVE ASSESSMENT OF RECIPIENT EYE (1 TO 5 DAYS PRIOR TO TRANSPLANT)

A preoperative assessment will be evaluated prior to reconstruction to confirm fitness for reconstruction of the recipient eye. All participants will receive this preoperative assessment 1 to 5 days prior to the day of corneal reconstruction (transplant).

The following procedures will be performed at these visits:

- Urine pregnancy test for women of childbearing potential
- Slit lamp examination (recipient eye) including
  - Corneal opacification (as defined by Fantes Scale)
  - Staining with fluorescein (extent of staining and epithelial defect area)
- Visual acuity BCVA (recipient eye)
- Intraocular pressure (recipient eye)
- Adverse event assessment
- Punctal plugs, if needed (Section 6.5.5) (both eyes)

Participants will be asked to report any pregnancies that occur within three days prior to surgery.

### 6.8.8. CORNEAL RECONSTRUCTION WITH CALEC (DAY 0)

Participants will undergo corneal reconstruction within 24 hours of release of CALEC.

A detailed description of the procedures can be found in Section 6.2.2. All participants will be discharged the day of surgery, unless unforeseen complications prohibit discharge. If a participant is admitted for observation, the Day 1 post-operative visit will occur while the participant is in the hospital. All other visits will follow as originally scheduled.

**6.8.9. FOLLOW-UP PERIOD**

Each participant will be followed for a period of 18 months from the time of the transplantation.

Participants will be asked to report any pregnancies that occur within seven days after transplant.

**6.8.10. POST-TRANSPLANT (DAY 1)**

- Slit lamp examination (both eyes) including
  - Corneal opacification (as defined by Fantes Scale) (both eyes)
  - Staining with fluorescein (extent of staining and epithelial defect area) (donor eye required; recipient eye at discretion of investigator )
- Visual acuity (BCVA)(both eyes)
- Intraocular pressure (both eyes)
- Slit lamp photographs (both eyes) by certified technician, including
  - Photography for neovascular area
    - Photography (with fluorescein) for epithelial defect (donor eye required; recipient eye at discretion of clinician)
- Punctal plugs, if needed (Section 6.5.5) (both eyes)
- Application of bandage contact lens, if needed (Section 6.5.5)
- Adverse event assessment

**6.8.11. POST-OP FOLLOW-UP: WEEKS 1, 2, MONTH 1 (4 WEEKS ± 3 DAYS) AND MONTHS 3, 6, 9, 12 (13, 26, 39, 52 WEEKS ± 1 WEEK), AND MONTHS 15, AND 18 (65 AND 78 WEEKS ±2 WEEKS)**

- Symptom assessment (every visit except week 1, week 2, month 1)
- Slit lamp examination (both eyes) including
  - Corneal opacification (as defined by Fantes Scale) (both eyes)
  - Staining with fluorescein (extent of staining and epithelial defect area) (donor eye required; recipient eye at discretion of investigator at week 1 and 2, and required at month 1 and thereafter.)
- Visual acuity BCVA (both eyes)
- Intraocular pressure (both eyes)
- Slit lamp photographs (both eyes) by certified technician, including
  - Photography for neovascular area
  - Photography (with fluorescein) for epithelial defect (donor eye required; recipient eye at discretion of investigator at week 1 and 2, and required at month 1 and thereafter)
- Anterior Segment Optical Coherence Tomography (months 12 and 18)
- In Vivo Confocal Microscopy (months 12 and 18)
- Punctal plugs, if needed (Section 6.5.5)
- Application of bandage contact lens, if needed (Section 6.5.5)
- Conjunctival swab and culture (months 3 and 12, only if MRSA was detected at screening visit)
- Impression cytology (in the recipient eye will be done as needed to confirm clinically suspected LSCD months 12, 15, and 18)

- Adverse event assessment

### 6.8.12. EARLY TERMINATION VISIT

Participants who withdraw from the study or are unable to complete all of the study visits will be asked to complete an early termination visit prior to discontinuing their participation. The early termination visit will include the following:

- Symptom Assessment
- Visual acuity (BCVA) (both eyes)
- Intraocular pressure (both eyes)
- Slit lamp examination (both eyes) including
    - Corneal opacification (as defined by Fantes Scale) (both eyes)
    - Staining with fluorescein (extent of staining and epithelial defect area) (both eyes)
- Slit lamp photographs (both eyes) by certified technician, including
    - Photography for neovascular area
    - Photography (with fluorescein) for epithelial defect
- Punctal plugs, if needed (Section 6.5.5)
- Bandage contact lens, if needed (Section 6.5.5)
- Adverse event assessment

### 6.8.13. UNSCHEDULED VISIT

Should a study participant need to be seen for medical reasons at a time point outside of the study protocol, the visit will follow the Week 1 schedule. Assessments may include, but are not limited to:

- Visual acuity BCVA (both eyes)
- Intraocular pressure (both eyes)
- Slit lamp examination (both eyes) including
    - Corneal opacification (as defined by Fantes Scale) (both eyes)
    - Staining with fluorescein (extent of staining and epithelial defect area) (each eye at discretion of investigator)
- Punctal plugs, if needed (Section 6.5.5)
- Bandage contact lens, if needed (Section 6.5.5)
- Adverse event assessment

## 7. OUTCOME MEASURES

The following is a summary of the definition of the outcome measures. The Statistical Analysis Plan details the analysis approach for all outcomes.

### 7.1. PRIMARY OUTCOME MEASURES

#### 7.1.1. SAFETY MEASURES

The occurrence of the following adverse events at any time during the 18 months of follow-up in the recipient eye will serve as the **primary safety events of interest**:

1. Ocular infection (defined as endophthalmitis or microbial keratitis [bacterial, fungal, parasitic])
2. Corneal perforation
3. Graft detachment ≥50%

In addition to the primary safety events, all adverse events (systemic and ocular in donor and recipient eyes) will be captured. The severity of each adverse event and the relationship of the event to the cell therapy procedure will be assessed by an independent Medical Monitor(s) (MM). The independent Medical Monitor's (see Section 9.3) coding of the adverse event and designation of severity and relatedness to treatment will serve as final to use for adverse event reporting and safety outcome analysis.

#### 7.1.2. FEASIBILITY MEASURES

Manufacturing feasibility will be evaluated for each biopsy attempt. At least one CALEC construct will be attempted to be manufactured from each biopsy, as detailed in the MOP. Each attempted construct will undergo Quality Control (QC) testing to determine product conformity to CMCF release criteria per MOP, which includes assessments of cell growth, cell viability, and culture contamination. If all of these QC release criteria are met for at least one CALEC construct, the biopsy attempt is considered a feasibility success.

In the event that a construct met all QC release criteria, but surgery was not performed for a reason unrelated to the development of construct for transplant, the case would still be considered a feasibility success for the biopsy attempt.

Up to two biopsy attempts of a single participant may be performed according to Section 5. Feasibility analysis will explore both the number of biopsies per participant and the number of construct attempts per biopsy, and whether those construct attempts met QC release criteria. Each of the individual QC testing and acceptance criteria will be documented to further describe constructs that did not meet the criteria for release.

### 7.2. SECONDARY OUTCOME MEASURES

Efficacy Measures: The primary efficacy outcome will be a binary "Complete Success" of the graft, as defined below. Secondarily, "Partial Success" will also be considered according to a 3 category outcome defined below.

1. "Complete Success" will be defined as *improvement* in corneal surface integrity
2. "Partial Success" will be defined as
    a. No improvement in corneal surface integrity and
    b. Improvement in either
        i. Extent of corneal vascularization or
        ii. Participant symptomatology
3. Otherwise, not a success

Improvement in each area is defined as follows, where changes are measured relative to the Baseline visit:

- Corneal surface integrity
  - If epithelial defect surface area (based on clinical assessment) at the Baseline visit is > 0 $mm^2$, then *improvement* will be defined as:
    - Decrease in epithelial defect surface area (based on clinical assessment by an independent investigator, not the treating surgeon, as described in the MOP) by ≥75%
  - If epithelial defect surface area (based on clinical assessment) at the Baseline visit is = 0 $mm^2$, then *improvement* will be defined as:
    - Epithelial defect surface area of 0 $mm^2$ and
    - Decrease in corneal surface staining (based on clinical assessment by an independent investigator, not the treating surgeon, using NEI grading scale) by ≥50%
- Extent of corneal vascularization
  - Decrease in neovascular area (based on digital slit lamp photographs, using mathematical software to calculate as described in the MOP) by ≥25%
- Participant symptomatology
  - Decrease in Ocular Surface Disease Index (OSDI) score by ≥25% or
  - Decrease in Symptom Assessment in Dry Eye (SANDE) score by ≥25%

The primary efficacy time points will be at 3 months (to assess early success), as well as 12 and 18 months (to assess late success). Efficacy outcomes at all interim post-3 month time points will also be evaluated secondarily. The pattern of efficacy outcomes for an eye at these and all interim time points 3 months and later will be evaluated secondarily to assess the degree of stability of each level of success over time.

### 7.3. OTHER OUTCOME MEASURES

In addition to the primary and secondary outcome measures defined above, distribution of data and summary statistics will be evaluated on the following at each time point where relevant data are collected: IOP, impression cytology, corneal opacification, visual acuity, AS-OCT outcomes (central corneal epithelial thickness and limbal epithelial thickness in superior, inferior, nasal, and temporal quadrants), and IVCM outcomes (presence of corneal epithelial cells, presence of conjunctival epithelial cells, and the presence of limbal palisades of Vogt.) All reported adverse events will also be tabulated.

## 8. MONITORING STUDY PROGRESS

Study progress will be monitored weekly or bi-weekly on Operations Committee calls. Recruitment reports will be available on the study website to monitor recruitment progress and ultimately follow up completion relative to the projected timeline. Operations Committee calls will also serve to keep all units of the Coordinating Center, Clinical Center, and PIs informed and engaged with the day-to-day progress of whether study goals are being met, including successful study oversight (including safety monitoring and protocol adherence monitoring of the monitoring plan) and eventually plans for closeout, manuscripts, and dissemination of study results. The Operations Committee will also monitor study progress with regards to timeliness and achievement of study goals.

# 9. ASSESSMENT OF SAFETY

## 9.1. METHODS AND TIMING FOR ASSESSING, RECORDING, AND REPORTING OF SAFETY PARAMETERS

All adverse events will be assessed by investigators and recorded at each visit following the baseline visit.

Details of recording and reporting adverse events have been provided in MOP.

Details of monitoring and reporting adverse events can be found in the Monitoring Plan.

## 9.2. SAFETY OVERSIGHT

Independent Medical Monitors (MMs) will be appointed to this study and will review any reported adverse events on a periodic basis to independently evaluate all adverse events. The MMs will be designated from the EMMES Corporation, Rockville, MD and will be unaffiliated with the CALEC study in any other way. The MMs are responsible for periodic review of all adverse events and within 24 hours of notification for cases that require any expedited reporting (for which an auto-email notification alert at the time of data entry will be generated). Details of the adverse event review by the MM can be found in the Monitoring Plan. The MM coding of the adverse event and designation of severity and relatedness to treatment will serve as final to use for adverse event reporting and safety outcome analysis.

The DSMC will be responsible for reviewing the ethical conduct of the study and for monitoring the data for evidence of adverse or beneficial treatment effects. Adverse events will be reported to the DSMC according to the following:

- All adverse events will be reviewed with the full DSMC on a periodic basis.
- A subset of adverse events will be reported expeditiously to designated DSMC members, as defined by the DSMC in the DSMC Charter.
- During Phase A, designated DSMC members will also be provided with a full report of all adverse events for each Phase A participant who completed CALEC transplant, upon completion of their 2 week visit, to review prior to the consenting and enrolling of the next study participant (or prior to proceeding to a second biopsy for a Phase A participant).
- Also, if criteria for considering temporary or permanent suspension of the trial are met (Section 9.3), the DSMC will be notified expeditiously.

Details of safety oversight by the DSMC can be found in the Monitoring Plan and the DSMC Charter.

## 9.3. STUDY HALTING GUIDELINES

The DSMC may recommend at any time whether the study should continue per protocol, be further investigated, be discontinued, or be modified and then proceed. The FDA may also suspend additional enrollment and study interventions/administration of study product for the entire study, if applicable.

There will be no formal halting guidelines for safety, as the DSMC will receive full safety reports on Phase A participants as noted in Section 9.2 above, and will also expeditiously receive some events as noted in Section 9.2 above throughout the duration of the study.

Halting guidelines for feasibility are as follows:

- A temporary suspension of enrollment will occur if four of the first eight CALEC biopsies, across Phases A and B, result in failure.
    - This could include two biopsies originating from one participant, (i.e., each biopsy would count separately)
    - Multiple construct attempts based on the same biopsy would be considered inclusive of a single biopsy
- If this criterion is met, the DSMC will be notified expeditiously and enrollment activities will be temporarily suspended until further direction from the DSMC. Starting from the time the criteria are identified as met, until the time the DSMC provides further direction, the temporary suspension of enrollment activities will be defined as follows.
    - No participants will be consented
    - Participants who have consented and are in some stage of completing screening or baseline visits will be on hold (labs values that have already been processed may be collected and entered into the CRF)
    - Participants who have not completed a biopsy will be on hold
    - Participants who had already completed transplant and thus in follow up will not be on hold

## 10. MONITORING PROTOCOL ADHERENCE

Details for monitoring clinical site for protocol adherence can be found in the Monitoring Plan. The Clinical Center will oversee personnel training and certification, IRB approval and reporting, participant recruitment and retention. The day-to-day local clinical procedures monitoring will be performed by the Clinical Research Supervisor with the support of study website reports provided by the Coordinating Center. The monitoring will be conducted to ensure human participant protection, study procedures, laboratory, study intervention administration, and data collection processes are of high quality and meet ICH E6 and MEEI regulatory guidelines. The Coordinating Center will be responsible for conducting the monitoring visits and ensuring that monitoring findings are reported to the PIs, who will then report to the IRB as necessary and are promptly addressed by the Study Coordinator.

The Coordinating Center will provide support via reports and queries for the Clinical Center oversight noted above, including reports for monitoring participant status and visit status (upcoming, pending, past due, missed). In addition, the Coordinating Center will develop and oversee a system for identifying via database queries, documenting, and reporting protocol and procedural deviations to the Clinical Center, PIs, IRB and DSMC. The Coordinating Center will be responsible for conducting on-site monitoring visits with support of the Clinical Center Clinical Research Supervisor. Additional accountability and oversight will be reinforced by Operations Committee monitoring of the activities noted above. This will include reports and review of recruitment, retention, and protocol deviations on at least a monthly basis.

## 11. QUALITY CONTROL OF DATA

Quality control details can be found in the Monitoring Plan. A major function of the Coordinating Center is to assure that high quality data are collected so that valid analyses can be conducted. The Coordinating Center will collaborate with the Clinical Center and PIs to provide the structure and support to enforce and confirm high quality data are being received. Data are verified on multiple levels across all stages of the study. In brief, this will include development of the following systems, as well as the monitoring and resolution of data issues identified via these systems:

1. Source Data / Data Entry Validations: Study data will be collected by completion of an electronic Case Report Form (eCRF), and via paper versions of the case report forms (CRF) as needed. eCRFs and CRFs serve as the primary source document for data collection on study participants and data can be readily entered on the study website directly into the Coordinating Center's database. Data entry validations include logic and contingency checks at the time of data entry on the website. Validations will also apply to edits made to the eCRFs. Edits are tracked via an audit table in the database.
2. Protocol Review: Study data will undergo near real time monitoring in which an automated regular (weekly or monthly) 'protocol review' program runs checks including cross form contingencies, write in fields, and data abnormalities, for review and feedback to the Clinical Center.
3. Data Cleaning: Data cleaning programs will be run periodically on frozen datasets for DSMC monitoring reports and to be conducted at the end when the database is locked.
4. Quality Control Reports: Reports will be generated for use by Coordinating Center, Clinical Center, and for review by oversight committees.

Site Monitoring and Protocol Adherence: Quality control of data also includes monitoring of protocol adherence as described in the prior section.

## 12. CONTINUED FOLLOW UP FOR CLAU PARTICIPANT ENROLLED PRIOR TO PROTOCOL AMENDMENT DISCONTINUING CLAU ARM OBJECTIVE

Prior to v5.0 of the CALEC Protocol, a secondary objective of the study was to compare CALEC efficacy and safety measures with the standard treatment alternative, CLAU. Phase B of the study was designed to randomize participants to receive either CALEC or the standard treatment alternative, CLAU. Due to recruitment challenges, v5.0 amended the Protocol to discontinue the CLAU comparison objective and enroll participants for CALEC treatment only. At the time of the protocol amendment, one participant had been randomized to the CLAU group and completed follow up through at least the 9 month visit. The prior versions of the protocol describe the biopsy, transplant, and medication regimen procedures that applied to this participant. The participant will continue to be followed with the remainder of the follow up visits and testing procedures that were required for the CLAU group.

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# 14. APPENDICES

## 14.1. APPENDIX I: SYMPTOM MEASUREMENT SCALES

**Ocular Surface Disease Index (OSDI) (**Vitale et al., 2004)

## Ocular Surface Disease Index© (OSDI©)[2]

Ask your patients the following 12 questions, and circle the number in the box that best represents each answer. Then, fill in boxes A, B, C, D, and E according to the instructions beside each.

| Have you experienced any of the following *during the last week?* | All of the time | Most of the time | Half of the time | Some of the time | None of the time |
|---|---|---|---|---|---|
| 1. Eyes that are sensitive to light? | 4 | 3 | 2 | 1 | 0 |
| 2. Eyes that feel gritty? | 4 | 3 | 2 | 1 | 0 |
| 3. Painful or sore eyes? | 4 | 3 | 2 | 1 | 0 |
| 4. Blurred vision? | 4 | 3 | 2 | 1 | 0 |
| 5. Poor vision? | 4 | 3 | 2 | 1 | 0 |

**Subtotal score for answers 1 to 5** (A)

| Have problems with your eyes limited you in performing any of the following *during the last week?* | All of the time | Most of the time | Half of the time | Some of the time | None of the time | N/A |
|---|---|---|---|---|---|---|
| 6. Reading? | 4 | 3 | 2 | 1 | 0 | N/A |
| 7. Driving at night? | 4 | 3 | 2 | 1 | 0 | N/A |
| 8. Working with a computer or bank machine (ATM)? | 4 | 3 | 2 | 1 | 0 | N/A |
| 9. Watching TV? | 4 | 3 | 2 | 1 | 0 | N/A |

**Subtotal score for answers 6 to 9** (B)

| Have your eyes felt uncomfortable in any of the following situations *during the last week?* | All of the time | Most of the time | Half of the time | Some of the time | None of the time | N/A |
|---|---|---|---|---|---|---|
| 10. Windy conditions? | 4 | 3 | 2 | 1 | 0 | N/A |
| 11. Places or areas with low humidity (very dry)? | 4 | 3 | 2 | 1 | 0 | N/A |
| 12. Areas that are air conditioned? | 4 | 3 | 2 | 1 | 0 | N/A |

**Subtotal score for answers 10 to 12** (C)

**Add subtotals A, B, and C to obtain D (D = sum of scores for all questions answered)** (D)

**Total number of questions answered (do not include questions answered N/A)** (E)

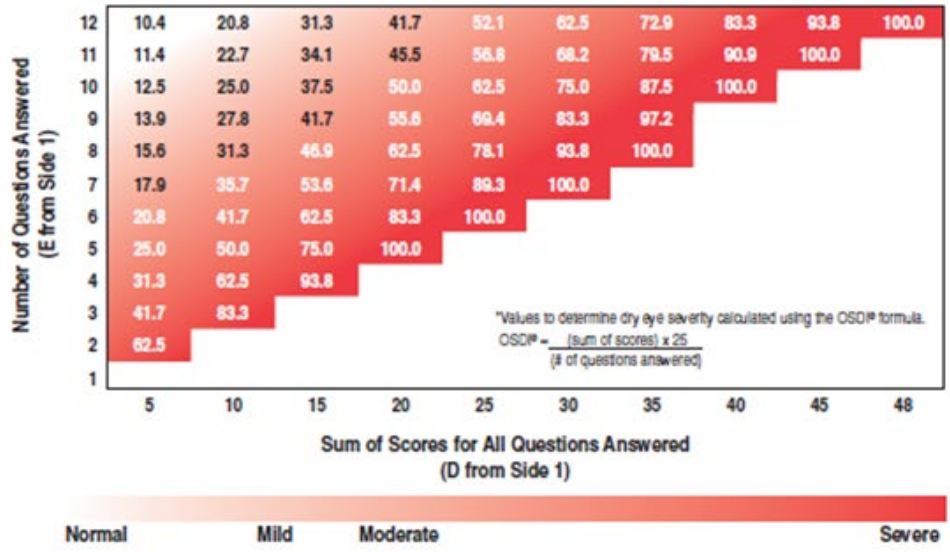
**Please turn over the questionnaire to calculate the patient's final OSDI© score.**

## Evaluating the OSDI© Score[1]

The OSDI© is assessed on a scale of 0 to 100, with higher scores representing greater disability. The index demonstrates sensitivity and specificity in distinguishing between normal subjects and patients with dry eye disease. The OSDI© is a valid and reliable instrument for measuring dry eye disease (normal, mild to moderate, and severe) and effect on vision-related function.

## Assessing Your Patient's Dry Eye Disease[1, 2]

**Use your answers D and E from side 1 to compare the sum of scores for all questions answered (D) and the number of questions answered (E) with the chart below.* Find where your patient's score would fall. Match the corresponding shade of red to the key below to determine whether your patient's score indicates normal, mild, moderate, or severe dry eye disease.**




Patient's Name: _____ Date: _____

How long has the patient experienced dry eye disease? _____

Eye Care Professional's Comments: _____

1. Data on file, Allergan, Inc.
2. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the Ocular Surface Disease Index. *Arch Ophthalmol.* 2000;118:615-621

## Symptom Assessment iN Dry Eye (SANDE) Questionnaire

(Schaumberg et al., 2007)

**Please complete the following questions regarding the frequency and severity of your dry eye symptoms.**

**1. Frequency of symptoms:**

Please place an 'X' on the line to indicate how often, on average, your eyes feel **dry and/or irritated**:

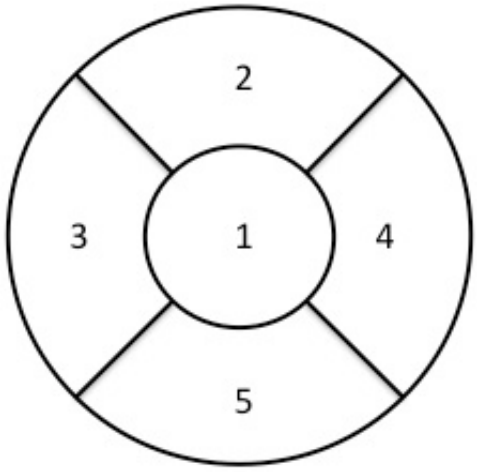
**Rarely** _____ **All of the Time**

**2. Severity of symptoms:**

Please place an 'X' on the line to indicate how severe, on average, your eyes feel **dry and/or irritated**:

**Very Mild** _____ **Very Severe**

## 14.2. APPENDIX II: NATIONAL EYE INSTITUTE CORNEAL FLUORESCEIN GRADING SCALE

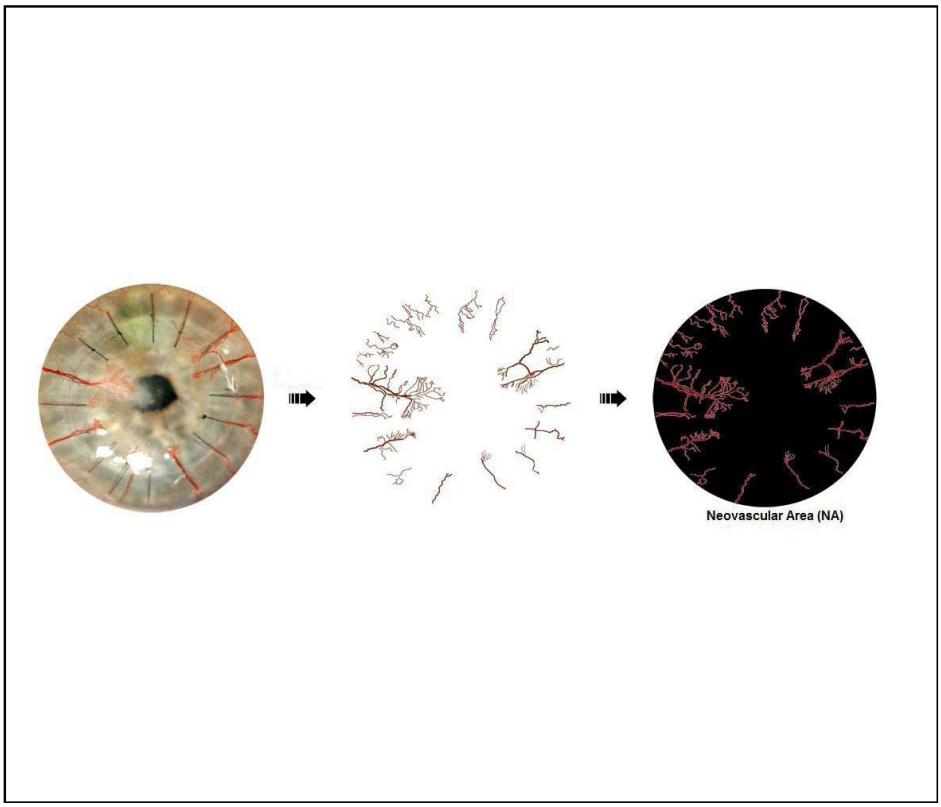



Score 0 – 3 for each of the 5 zones

For corneal fluorescein staining, saline-moistened fluorescein strips or 1% sodium fluorescein solution will be used to stain the tear film. The entire cornea will then be examined using slit lamp evaluation with a yellow barrier filter (#12 Wratten) and cobalt blue illumination. Each of five corneal zones (superior, nasal, central, inferior, and temporal) will be graded from 0 (normal) to 3 (severe) and total staining score of the cornea will be calculated by adding the scores of all 5 zones together. The scores will range r from 0 to 15 points.

## 14.3. APPENDIX III: VASCULARIZATION MEASUREMENT TOOL

Digital Quantification of Neovascularization

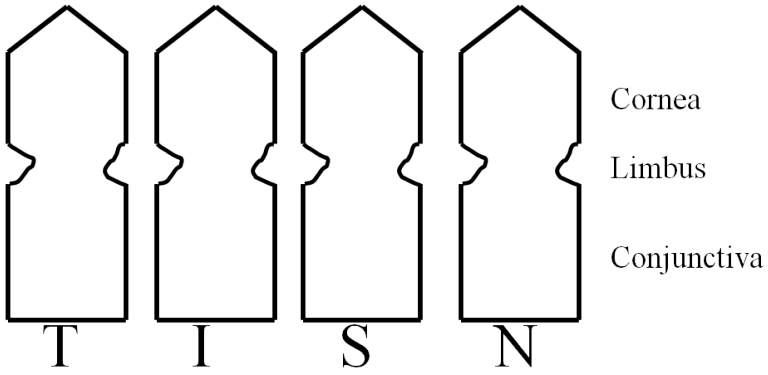


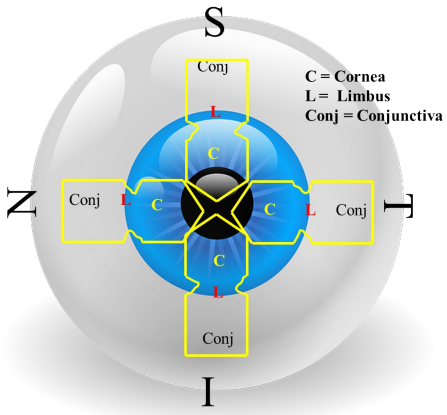

Digital slit lamp corneal images are analyzed using graphics-editing software (Photoshop) and a mathematical program (Java script from ImageJ). After the total area is delineated, the blood vessels are isolated using Photoshop. Neovascular area is computed using ImageJ to measure the area of the vessels in pixels.

## 14.4. APPENDIX IV: FANTES SCALE FOR CORNEAL OPACITY

| Grade | Description |
|---|---|
| 0 | Totally clear such that no opacity could be seen by any method of slit lamp microscopic examination |
| 0.5 | A trace or a faint corneal haze seen only by indirect broad tangential illumination |
| 1 | Haze of minimal density seen with difficulty with direct and diffuse illumination |
| 2 | A mild haze easily visible with direct focal slit illumination |
| 3 | A moderately dense opacity that partially obscured the iris details |
| 4 | A severely dense opacity that obscured completely the details of intraocular structures |

## 14.5. APPENDIX V: IMPRESSION CYTOLOGY




Reporting:

| Region | Goblet Cells Cornea |
|---|---|
| Superior | |
| Inferior | |
| Temporal | |
| Nasal | |

## 14.6. APPENDIX VI: SCHEDULES OF EVENTS & PROCEDURES FOR PARTICIPANTS COMPLETING TRANSPLANT

Key: **X=Donor Eye**; **X=Recipient Eye**; Eye procedures marked with a black **X** will be performed on both the recipient and donor eyes (or the participant).
[1]Perform only if needed and deemed appropriate by clinician. [2]Needed only if MRSA was previously detected. [3]Baseline procedures may be conducted over multiple visits after screening. [4]Portions of exam/photo that require staining in recipient eye are at investigator's discretion. [5]The 2 week, 9 month, and 15 month visits will be optional for participants for whom travel is prohibitive (see Protocol Section 6.8 for more details).

| Visit | 1 | 2 | 3 | 4 | | 5 | 6 | | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Time Period & Procedure post-transplant visits timed from transplant date)** | **Screening** | **Baseline[3]** | **Blood draw collected within 1-7 days before or after biopsy** | **Limbal Biopsy** (Within 25 days of Baseline) | **Transportation of Tissue to GMP lab and Preparation of Cell Sheets** | **Day 1 Post-Biopsy** | **Pre-Op** | **Transportation of CALEC to Mass. Eye and Ear– Release anticipated 10 to 30 days after biopsy** | **Corneal Reconstruction with CALEC (Day 0) – Within 24 hours of release** | **Day 1 Post-Graft** | **Week 1** | **Week 2[5]** | **Month 1 (4 weeks)** | **Month 3 (13 weeks)** | **Month 6 (26 weeks)** | **Month 9[5] (39 weeks)** | **Month 12 (52 weeks)** | **Month 15[5] (65 weeks)** | **Month 18 (78 weeks)** |
| **Timing Window** | | Within 30 days of Screening | | | | 1-2 days after biopsy | 1 to 5 days prior to transpl. | | | ±0 days | ± 3 days | ± 3 days | ± 3 days | ± 1 week | ± 1 week | ± 1 week | ± 1 week | ± 2 week | ± 2 week |
| Obtain Informed Consent | X | | | | | | | | | | | | | | | | | | |
| Eligibility Assessment | X | | | | | X | X | | | | | | | | | | | | |
| Past Medical History | X | X | | | | X | X | | | X | X | | | | | | | | |
| Pre-Op Health Screening | X | X | | | | | | | | | | | | | | | | | |
| Hematologic Lab Screening | X | | | | | | | | | | | | | | | | | | |
| Pregnancy Test | X | | | | | | X | | | | | | | | | | | | |
| Slit Lamp Examination | X | X | | | | X | X | | | X[4] | X[4] | X[4] | X | X | X | X | X | X | X |
| Visual Acuity BCVA | X | X | | | | X | X | | | X | X | X | X | X | X | X | X | X | X |
| Intraocular Pressure | X | X | | | | X | X | | | X | X | X | X | X | X | X | X | X | X |
| Fundus Examination or B-Scan Ultrasound | X | | | | | | | | | | | | | | | | | | |
| Schirmer's Test | X | | | | | | | | | | | | | | | | | | |
| AS-OCT | | X | | | | | | | | | | | | | | | X | | X |
| IVCM | | X | | | | | | | | | | | | | | | X | | X |
| Slit Lamp Photos | X | X | | | | X | | | | X[4] | X[4] | X[4] | X | X | X | X | X | X | X |
| Conjunctival Swab & Culture | X | | | | | | | | | | | | | X[2] | | | X[2] | | |
| Impression Cytology[1] | | | | | | | | | | | | | | | | | X[1] | X[1] | X[1] |
| Punctal Plugs[1] | X | X | | | | X | X | | | X | X | X | X | X | X | X | X | X | X |
| Bandage Contact Lens[1] | | | | | | | | | | X | X | X | X | X | X | X | X | X | X |
| Antibiotic | | | | | | X | X | | | X | X | X | X | X[1] | X[1] | X[1] | X[1] | X[1] | X[1] |
| Topical steroid and fluoroquinolone | | | | | | X | X | | | X | X | X | X | X[1] | X[1] | X[1] | X[1] | X[1] | X[1] |
| Vancomycin[2] | | X | | | | X | X | | | X | X | X | X | X[1] | X[1] | X[1] | X[1] | X[1] | X[1] |
| Artificial Tears | | | | | | | | | | X | X | X | X | X | X | X | X | X | X |
| 20% Autologous Serum Drops | | | | | | | | | | X | X | X | X[1] | X[1] | X[1] | X[1] | X[1] | X[1] | X[1] |
| Symptom Assessment | | X | | | | | | | | | | | | X | X | X | X | X | X |
| Adverse Event Assessment | | X (changes in medical status) | | | | X | X | | | X | X | X | X | X | X | X | X | X | X |

# 15. PROTOCOL ADDENDUM: SECOND TRANSPLANT

## 15.1. OVERVIEW

Some study participants who received CALEC and for whom epithelial defect and conjunctivalization has not resolved after 12 months may benefit from an additional transplant. Participants meeting study criteria (section 15.3) may be offered the opportunity to undergo a second procedure, either CALEC or CLAU. The following chapter outlines the protocol that will be followed for these participants. Up to three participants can receive a second transplant.

## 15.2. CRITERIA FOR SECOND TRANSPLANT

All of the following criteria must be met in order for a participant to be considered for a second transplant.

- Participant previously received CALEC (participants previously receiving CLAU will not be eligible)
- Second transplant cannot occur prior to the 12-month visit
- Presence of epithelial defect in recipient eye
    - Must have been present at 2 or more visits (>1 month between visits), any time on or after the 3-month visit
    - >1 mm in any dimension
    - (Note: Does not need to be present at the Screening/Baseline visit prior to second transplant)
- Presence of conjunctivalization / fibrovascular pannus in recipient eye
    - Must be present at the Screening/Baseline visit prior to second transplant
    - >2 mm from the limbus
    - ≥6 clock hours
- Investigator discretion that repeat transplant has therapeutic potential
- Absence of the following in the donor eye:
    - Conjunctivalization of the cornea defined by fibrovascular pannus more than 2mm from the limbus into the cornea for ≥3 clock hours
    - Lack of limbal palisades of Vogt for ≥3 clock hours
    - History of allo-limbal transplantation

NOTE: these criteria are independent of whether the participant meets the protocol definition of complete or partial success.

## 15.3. INFORMED CONSENT PROCESS

Participants meeting criteria and offered a second transplant option will undergo an informed consent process. The informed consent process will include a discussion with participants that they have the option of CLAU, CALEC, or no second transplant.

## 15.4. SCREENING AND BASELINE

Participants consenting to undergo a second transplant, either CLAU or CALEC, will undergo the same screening and baseline procedures in the protocol for the initial CALEC transplant (section 6.8.1-6.8.2), with the following exceptions:

- The screening visit will be combined with baseline visit since the subjects receiving the second graft will have already been followed for at least a year since their initial CALEC transplant
- The combined screening/baseline visit will include the following:
    - Evaluation of eligibility for a second graft

- Hematologic lab screening will include only the blood draw collected within 1-7 days as required for manufacturing of the graft per DCDI protocol
  - Hepatitis B
  - Hepatitis C
  - HIV
- Slit lamp exam
- BCVA
- IOP
- Fundus exam or B-scan
- AS-OCT
- IVCM
- Slit lamp photos
- Symptom assessment
- Conjunctival swab and culture
  - (if MRSA positive, the original protocol will be followed for vancomycin)
- Schirmer's test (optional)
- Punctal plus (optional)

The visit will not require punctal plugs, bandage contact lenses, prednisolone, vancomycin, preservative-free tears and autologous serum tears as participants will already be using them as treatments from initial CALEC. The preoperative antibiotic drop will be given prior to the biopsy and transplant in the respective operative eyes, as with the initial CALEC (section 6.2.1). The Schirmer test may not be needed as participants would have had it to receive the initial CALEC and would have had plugs if necessary. . The full set of labs will not be repeated as they would have been done to enter the study for the initial CALEC. The labs that are required for manufacturing of CALEC will be repeated as noted above, for participants pursuing second CALEC transplant.

**15.5. BIOPSY AND TRANSPLANT**

<u>For participants choosing to undergo CLAU</u>

- The pre-operative visit will occur within 60 days after baseline and within 1-5 days prior to transplant, and testing will include slit lamp exam, visual acuity, IOP.
- The biopsy and transplant procedures and subsequent medication regimens will follow standard care.

<u>For participants choosing to undergo CALEC</u>

- The post-biopsy and pre-operative visits and testing schedule, including all procedures and measurements, will be the same as the current protocol (APPENDIX VI).
- The CALEC manufacturing (section 6.1), biopsy and transplant procedures (section 6.2) and subsequent medication regimens (section 6.5) will follow the protocol for initial CALEC
- If a participant experiences failure of the biopsy to generate a viable cell sheet, the participant will not be eligible to rescreen for another biopsy attempt. The participant will be monitored by the study team for 30 days to check for any adverse events related to the biopsy procedures. If the donor eye has a complication or adverse event that does not resolve after 30 days, the study team will continue to monitor until it resolves. The participant will discontinue the study after the monitoring period.

## 15.6. STUDY PROCEDURES, MEASUREMENTS, AND VISIT SCHEDULE

For all participants choosing to undergo a second transplant (either CALEC or CLAU), the post-operative visit and testing schedule, including all procedures, measurements and data collection, will be the same as the current protocol (APPENDIX VI). The protocol schedule will be followed until a common study closeout date is reached.

## 15.7. ANALYSIS CONSIDERATIONS

### 15.7.1. SAFETY

- **Safety events of interest, as defined in section 7.1.1.:**
  - **Primary safety analysis for protocol objective**: Safety events will only be tabulated up to (not including) the date of the biopsy for the second CALEC or CLAU transplant.
  - **Additional analysis evaluating second CALEC transplant**: Safety events following the biopsy for the second CALEC transplant will be summarized separately.

### 15.7.2. FEASIBILITY

- **Feasibility measures, as defined in section 7.1.2.:**
  - **Primary feasibility analysis for protocol objective:** The biopsy attempts for only the initial CALEC transplants will be included in the primary feasibility outcome calculation of feasibility success
  - **Additional analysis evaluating second CALEC transplant:** The biopsy attempts for second CALEC transplant will be summarized separately

### 15.7.3. EFFICACY

- **Efficacy outcome measures, as defined in section 7.2, will be evaluated the following ways:**
  - **Primary efficacy analysis for protocol objective**: Efficacy outcome data will only be included up to (not including) the date of biopsy for the second CALEC or CLAU transplant
  - **Additional analyses evaluating second CALEC transplant**:
    - Efficacy outcome data will be evaluated relative to original baseline through the latest protocol visit prior to the study closeout date.
    - Efficacy outcome data will be evaluated relative to second CALEC baseline through the latest protocol visit prior to the study closeout date.