Study Title: *A Prospective, Randomized, Controlled Pilot Clinical Study to Evaluate the Use of ProKera Plus® in the Management of Bacterial Corneal Ulcers*

Principal Investigator: *David B Warner M.D.*
University of Arkansas for Medical Sciences
4301 W. Markham Street
Little Rock, AR 72205
Telephone: *501.686.5822*
Email: *dbwarner@uams.edu*

Co-Investigators: *Heather V Broyles D.O.*
University of Arkansas for Medical Sciences
4301 W. Markham Street
Little Rock, AR 72205
Telephone: *501.686.5822*
Email: *hvbroyles@uams.edu*

William Henry, M.D.
University of Arkansas for Medical Sciences
4301 W. Markham Street
Little Rock, AR 72205
Telephone: *501.686.5822*
Email: *wahenry@uams.edu*

Study Coordinators: *Jeremy Lemmons*
University of Arkansas for Medical Sciences
4301 W. Markham Street
Little Rock, AR 72205
Telephone: *501-296-1156*
Email: JDLemmons2@uams.edu

Study location: *University of Arkansas for Medical Sciences*
4301 W. Markham Street
Little Rock, AR 72205

Background and Rationale

Bacterial keratitis is a serious bacterial infection of the cornea, usually caused by a persistent epithelial defect or ulcer that can lead to permanent vision loss from corneal scarring, perforation or endophthalmitis. An infectious corneal ulcer requires immediate treatment with intensive topical fortified broad-spectrum antibiotics to try to eliminate the pathogen. Corneal tissue destruction can be caused directly by infectious agents, the associated inflammatory response, or by ocular toxicity from frequent dosing of fortified antibiotics.[1] Sutured amniotic membrane transplantation (AMT) has been shown to reduce pain and promote healing in human bacterial keratitis.[2] ProKera® is a sutureless form of CryoTek amniotic membrane that is clipped into a dual polycarbonate ring system with the epithelial side up when in contact with the ocular surface. ProKera Plus® contains a double layer of CryoTek amniotic membrane tissue to provide extra therapeutic benefit. ProKera Plus® has several advantages over sutured AMT including ease of administration in a clinic setting and reduced overall procedural cost.

Several retrospective case reports have shown that ProKera® can facilitate resolution of pain and inflammation and promote rapid epithelization.[3] However, the role of ProKera® in the treatment algorithm of corneal ulcers has yet to be fully clarified. There are currently no prospective case studies comparing the use of ProKera® to standard of care conventional treatments in corneal ulcers. The utility of this device would provide valuable information in the treatment of bacterial corneal ulcers. **Our Main goal is to estimate standard deviation of our outcomes in order to design a larger clinical trial to more accurately address these objectives**

Objectives

1. To determine if ProKera Plus® can lead to better visual recovery when used with bacterial corneal ulcers compared to conventional treatment
2. To determine if ProKera Plus® can actively modify corneal wound healing during the course of managing bacterial corneal ulcers and decrease the overall time to re-epithelialization
3. To determine if ProKera Plus® can decrease pain associated with bacterial corneal ulcers compared to conventional treatment
4. To determine if ProKera Plus® can decrease the amount of corneal opacity and corneal thinning associated with bacterial corneal ulcers compared to conventional treatment
5. To determine if ProKera Plus® can decrease the need for further interventions or surgeries related to complications from bacterial corneal ulcers

Study Design and Procedures

This is a pilot study meant to gather information needed to design a larger, prospective, randomized, controlled clinical trial. A total of N=24 patients (12 per treatment arm) will be required for this pilot study.

There may be up to 30 patients consented to reach this total. The pilot study design is a prospective, randomized, controlled interventional study to compare the outcome of ProKera Plus® with conventional treatment in patients with vision-threatening bacterial corneal ulcers. The study will be conducted at the University of Arkansas Medical Sciences (UAMS) in two phases to answer the objectives stated above. Patients who present to an Ophthalmology clinic or Emergency Department at UAMS with a severe corneal ulcer based on the inclusion criteria will be evaluated and initially treated in the conventional, standard of care methods as follows:

- The patient's name, date of birth and medical record number will be accessed to enter the standard consult note in the EMR chart.

- The patient's past medical, ocular, surgical and family history will be obtained including the following historical information: medications patients takes, any medications allergies, if the patient smokes, drinks alcohol or uses recreational drugs, if the patient has ever been diagnosed with any eye condition or disease, any history of eye infections or eye surgeries including glaucoma surgery, and history of eye trauma or chemicals in the eye, if patient has ever worn contact lenses and if so how often they are worn/cleaned/changed/where they are bought, and recent exposure to pools, lakes or outside matter and any high risk sexual behavior (this information will help determine initial eligibility as well as subsequent data analyses.

Visual acuity, pupil check, extraocular motility, and intraocular pressure will be recorded. An anterior segment exam will be recorded using a slit-lamp. Dilating eye drops will be administered and dilated fundus exam will be obtained. If the ulcer is impeding view of the fundus, an ocular ultrasound will be performed to evaluate the posterior segment. All of this information will be documented in the consult note.

- Corneal ulcer scraping sent for microbial culture

- Initiation of fortified vancomycin 25mg/mL every 1 hour alternating with fortified tobramycin 15mg/mL every 1 hour, preservative free artificial tears every 2 hours, and doxycycline 100mg twice daily, with outpatient follow-up every 1-3 days at the UAMS Ophthalmology clinic or in the hospital if admitted for the above regimen based on clinical severity.

Phase 1: This phase of the pilot study will establish baseline characteristics for each patient that is randomized into the treatment arm for the phase 2 part of the study.

- After at least 48 hours of conventional treatment as described above, consent will be obtained regarding the use of experimental treatment with ProKera Plus® versus continuing conventional method of treatment. This will be randomized in a 1:1 ratio (n=12/group) by a block-randomization schedule to assign subjects to each of the two treatment groups. The master randomization schedule will be generated from sealed envelope.com prior to the start of the study, and the master randomization list will be kept in the Trial Master File. A set of tamper-evident envelopes will be created and each have the trial identification and a sequential number on it. Inside is the specific treatment allocation as determined by the Master Randomization List. The envelopes are to be kept private, and to only reveal a treatment allocation after receiving information demonstrating that the patient is eligible and has consented to the trial. After assessing subject's eligibility and consent, the next envelope in sequence is opened.

- At this time, each patient will undergo baseline slit-lamp photography with and without fluorescein staining, as well as anterior segment optical coherence tomography (ASOCT). These will be recorded in the patient's existing medical chart for the hospital encounter.
- Each patient will be in the study for 6 months. It will include continued outpatient follow-up in clinic for approximately 5-7 days or continued hospitalization for approximately 5-7 days if necessary, and 2 mandatory clinic visits that will happen on approximately day 16 and day 30 of the study. Additional clinic visits will be scheduled for approximately 3 months and 6 months for visual acuity and Slit Lamp exam, and a survey will be sent instead if patient is unable to physically come to clinic.

Phase 2: This phase of the pilot study will test the intervention of using ProKera Plus® versus continuing conventional treatment for the corneal ulcer as follows:

- Experimental Treatment Arm (determined at random as mentioned above) → ProKera Plus® will be placed in the eye with the corneal ulcer. Eye drops will then be adjusted based on culture speciation and sensitivities, and decreased to 4 times daily. If patient is being followed on an outpatient basis, they will be evaluated every 1-3 days for 5-7 days then be seen in clinic for repeat slit-lamp photography with and without fluorescein staining. If the patient is still hospitalized during this time, the patient will be evaluated every 1-3 days and brought to clinic prior to discharge. At this last visit before discharge, pain level will be assessed; in addition we will repeat slit-lamp photography with and without fluorescein staining The ProKera Plus® will be exchanged or replaced if it becomes cloudy or melts

1. Active Comparator Control Arm → Standard of care eye drops will be adjusted based on culture speciation and sensitivities. If culture speciation is not available at 48 hours, eye drops will decrease to every 2 hours. If speciation has resulted, eye drop regimen will be adjusted to either every 2 hours or 4 times daily depending on the severity. If the patient is being followed on an outpatient basis, they will be seen in clinic every 1-3 days for 5-7 days and at one visit we will repeat slit-lamp photography with and without fluorescein staining. If the patient is still hospitalized, they will continue to be evaluated every 1-3 days for approximately 5-7 days, and brought to clinic prior to discharge. At this last visit before discharge, pain level will be assessed and we will repeat slit-lamp photography with and without fluorescein staining.
2. For the patients that are hospitalized, they will be discharged from the hospital approximately 5 (+/- 3) days from the start of the study based on demonstrated improvement, and seen in the clinic prior to discharge. This will be given there are no other conditions keeping patient hospitalized at this time.
3. The following will be obtained at visit 3(refer to protocol table): subjective data including pain level and subjective visual improvement, and objective data including visual acuity, slit-lamp photography with and without fluorescein, and ASOCT.
4. The following will be obtained at visit 4(refer to protocol table): subjective data including pain level, subjective visual improvement, and objective data including visual acuity, slit-lamp photography with and without fluorescein. Additional visits may be scheduled if wound healing is delayed and patient

requires closer follow-up.

5. Visits 5 & 6(refer to protocol table) are the only visits that are solely for research purposes. If the patient is unable to come to an in-person clinic visit at this time, a patient questionnaire will be mailed instead which evaluates pain and visual acuity on a subjective level. If the patient is able to come to clinic, the questionnaire will be done in person, objective visual acuity will be obtained as well as a slit lamp exam and pain assessment.

Primary Outcome Measures:

1. Visual Recovery [Data points: Day of presentation, visits 1, 2, 3, and 4; visits 5 and 6 only if patient is able to make it to these visits in person]

- Measured by the Traditional Snellen Eye Chart or Revised 2000 Series ETDRS charts (Precision Vision, La Salle, Illinois, USA) recorded values of anywhere from 20/20 to 20/400 or count fingers vs hand motion vision in front of face. These values will be converted to logarithm of the minimum angle of resolution (logMar) for statistical analysis

Secondary Outcome Measures:

2. Corneal Re-epithelialization [Data points: Visits 1, 2, 3, and 4]

- Measured by fluorescein staining size on examination using slit lamp photography

3. Corneal Opacity size and Thinning [Data points: Visits 1 and 3]

- Measured by ASOCT

4. Pain [Data points: At day of presentation and at visits 1, 2, 3, and 4; visits 5 and 6 only if patient is able to make it to these visits in person]

- Assessed subjectively using the Visual Analog Scale (VAS) ranging from 0 (none) to 10 (worst possible pain)

5. Complications/Adverse Events [Time Frame: up to 3 months after initial treatment]

- Quantified by need for additional procedures such as corneal gluing or corneal transplantation due to corneal perforation, need for vitreous tap/inject for development of endophthalmitis, or any additional surgical procedure necessitated by infection. In addition, there will be documentation and quantification of any adverse event from the insertion of the ProKera Plus® including but not limited to cloudy vision while inserted, allergic reaction, inability to completely blink or close the eyelid with possible need for tape to close eyelids, or any new microbial infection or transmission of viral disease.

6. A questionnaire, constructed by the study team, will be given to participants at visits 5 & 6.(see attached)

Study Population

The study population will consist of all patients ages 18 or older who present to the ophthalmology consulting service or ophthalmology eye clinic for eye care at University of Arkansas for the Medical Sciences.

Inclusion Criteria

- Subjects 18 years of age or older, all sexes and races
- Willing to sign a written informed consent to participate
- Corneal ulcer criteria: at least 3mm in diameter, opacification located within 3mm of visual axis, infiltrate occupying at least 50% of the corneal thickness, moderate AC cell reaction, and clinical picture consistent with bacterial infection.

Exclusion Criteria

- History of Immunodeficiency
- History of connective tissue disorders or severe atopic disease
- History of chemical eye injuries
- History of known limbal stem cell deficiency
- History of neurotrophic keratopathy
- History of recent eye surgery, or glaucoma surgery with bleb or drainage tube
- History of drug reactions to Ciprofloxacin or Amphotericin B
- Risk factors and clinical appearance consistent with fungal keratitis
- Visual acuity that is greater than 20/40
- Visual acuity that is light perception or worse

Risks and Benefits

A risk to study participants is the potential for loss of confidentiality of study data. Measures to protect the confidentiality of study data will be implemented as described in the Data Handling and Recordkeeping section below.

Potential risks include possible discomfort with insertion of ProKera Plus® and cloudy vision while inserted, allergic reaction if drug allergies to Ciprofloxacin or Amphotericin B, inability to completely blink or close the eyelid with possible need for tape to close eyelids, and rare possibility of microbial infection and transmission of viral disease through the donor tissue although unlikely

Potential benefits include decreased pain, ability to decrease eye drop frequency, decreased corneal scarring and therefore possible increased chance of visual recovery.

Knowledge gained from the study could potentially benefit patients in the future and determine the utility of ProKera Plus® in treating bacterial corneal ulcers as part of standard-of-care treatment for severe ulcers.

Data Handling and Recordkeeping

The Principal Investigator will carefully monitor study procedures to protect the safety of research subjects, the quality of the data and the integrity of the study. All study subject material will be assigned a unique identifying number. The key to the code will kept in a locked file in the Principal Investigator's office. Only the Principal Investigator Dr. Warner, Dr. Broyles and Dr. Henry will have access to the code.

Data safety monitoring will involve the following: any feedback, complaint, error or accidents that may possibly occur during the trial will be reported to UAMS and Tissue Tech company. In addition any adverse event that may occur during the trial will be properly monitored by the following: if potentially attributable to the ProKera Plus®, a description of the adverse event, the date if occurred, and the serial number and expiration date of the device will be recorded and mailed to Tissue Tech.

During the study, the information collected will be obtained from EMR on UAMS servers and de-identified and stored on research service drive (encrypted database/spreadsheet) on UAMS servers. Dr. David Warner, Dr. Heather Broyles, and Dr. Will Henry will have access to the research service drive. In addition, the de-identified data including all measurements and data obtained from the primary and secondary outcomes will be shared with Tissue Tech.

At the conclusion of the study, the information will be permanently de-identified. The information will be kept on site for a minimum of 7 years according to UAMS policy. The records will be destroyed according to current HIPAA regulations and institution policy.

Specimen Handling and Storage

The specimens obtained from the patient include corneal ulcer wound scrapings done at bedside and plated Blood Agar Plate, Chocolate Agar and Potato Dextrose Agar Plates. This is part of the normal clinical standard-of-care that will occur regardless of study participation. These specimens will be walked over to the clinical microbiology lab at UAMS as part of routine care

The ProKera Plus® will be obtained from Tissue Tech Inc. It will be stored in the tissue freezer at -80ºC to 4ºC. After being thawed at room temperature for five minutes, the ProKera Plus® will be rinsed with saline solution and inserted at the bedside after topical application of 0.5% proparacaine hydrochloride.

The ProKera Plus® will be removed when measuring visual acuity and for photographic documentation and reinserted afterwards. It will be exchanged or replaced when it becomes torn, cloudy, or melts.

Statistical Considerations:

Sample Size: A total of N=24 patients (12 per treatment arm) will be required for this pilot study. With this sample size, we can get robust variance estimates for the primary and secondary outcomes of interest.

We intend to use a one-sided 80% upper confidence limit (UCL) of the sample standard deviation (***s***) estimated from this pilot study to calculate the power and sample size requirements for larger clinical trial. With N=24, the 80% UCL of **s** is 1.16***s**.[4] This estimate is relatively precise, resulting in only a 16% increase in s, and practical in that the required sample can be obtained from a single site within two years.

Summaries of demographic and medical characteristics of the patients who agree to participate will be calculated for each treatment group. Continuous variables will be summarized using means, standard deviations, medians and ranges. Counts and percentages will be used to summarize categorical measures.

Primary Analysis: The primary outcome of the study is visual recovery as measured by change in logMAR scores from baseline to approximately16-days post-treatment. As stated above, the primary goal of this pilot study is to estimate the sample variance of the temporal changes in these logMAR scores; however, we also intend to perform statistical testing. A two-sample t-test will be used to compare the treatment arms with respect to this outcome and a two-tailed 5% α-level will be used to determine statistical significance. Prior to the analysis, the assumption of the t-test procedure will be assessed. If violated, alternative nonparametric testing procedures, such as a Wilcoxon rank sum test, will be employed. With N=24, the t-test described above will have 80% power to detect standardized differences in group means of 1.20 standard deviations or larger, or 90% to detect differences of 1.39 standard deviations or larger

Secondary Analyses: As above, two-sample t-tests will be used to compare the treatment arms with respect to ASOCT measures and VAS pain scores. Log-likelihood ratio tests will be used to compare the groups with respect to dichotomous outcomes, such as re-epithelialization at approximately16-days or the need for further interventions or surgeries. A 1% α-level will be used to determine statistical significance for all secondary analyses.

Ethical Considerations

This pilot study will be conducted in accordance with all applicable government regulations and University of Arkansas for Medical Sciences research policies and procedures. This protocol and any amendments will be submitted and approved by the UAMS Institutional Review Board (IRB) to conduct the study.

The formal consent of each subject, using the IRB-approved consent form, will be obtained before that subject is submitted to any study procedure. All subjects for this study will be provided a consent form describing this study and providing sufficient information in language suitable for subjects to make an informed decision about their participation in this study. The person obtaining consent will thoroughly explain each element of the document and outline the risks and benefits, alternate treatment(s), and requirements of the study. The consent process will take place in a quiet and private room, and subjects may take as much time as needed to make a decision about their participation. Participation privacy will be maintained and questions regarding participation will be answered. No coercion or undue influence will be used in the consent process. This consent form must be signed by the subject or legally authorized representative, and the person obtaining the consent. A copy of the signed consent will be given to the participant, and the informed consent process will be documented in the research record.

Dissemination of Data

The study will be listed on clinicaltrials.gov in accordance with FDA requirements. Results of this study may be used for presentations, posters, or publications. The publications will not contain any identifiable information that could be linked to a participant.

References

1. Gangopadhyay N, Daniell M, Weih L, et al. Fluoroquinolone and fortified antibiotics for treating bacterial corneal ulcers. Br J Ophthalmol. 2000; 84:378-384.
2. Sheha, H., Liang, L., Li, J., & Tseng, S. C. (2009). Sutureless amniotic membrane transplantation for severe bacterial keratitis. *Cornea*, *28*(10), 1118–1123.
3. Sheha H, Liang L, Li J, Tseng SC. Sutureless amniotic membrane transplantation for severe bacterial keratitis. *Cornea*. 2009;28(10):1118-1123.
4. Whitehead, A. L., Julious, S. A., Cooper, C. L., & Campbell, M. J. (2016). Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable. *Statistical methods in medical research*, *25*(3), 1057–1073.

Appendices

Supplemental Eye Symptom Questionnaire

Please circle below which eye was treated:

RIGHT EYE LEFT EYE

1. In general, would you say your overall health is:

- o Excellent
- o Very Good

- Good
- Fair
- Poor

2. At the present time, would you say your eyesight using the **affected eye** (with glasses or contact lenses, if you wear them) is:

- Excellent
- Good
- Fair
- Poor
- Very poor

3. In the last month, how much of your eyesight in the **affected eye** has improved if at all?

- None
- Very little
- A noticeable but not significant amount
- A lot

4. In the last month, how much pain or discomfort have you had in & around the **affected eye** (ex: burning, itching, or aching)?

- None
- Mild
- Moderate
- Severe
- Very Severe

5. If you answered at least **Mild** in the previous question, how often did you have the pain/discomfort?

- Not Applicable
- Rarely
- Sometimes
- Often

6. In the last month, how much of a problem did you have with burning or stinging in the **affected eye**?

- No problem at all
- A little bit of a problem

- Somewhat of a problem
- Very much of a problem

7. In the last month, how much of a problem did you have with the **affected eye** feeling gritty?

- No problem at all
- A little bit of a problem
- Somewhat of a problem
- Very much of a problem

7. In the last month, how much of a problem did you have with the eye being sensitive to light?

- No problem at all
- A little bit of a problem
- Somewhat of a problem
- Very much of a problem

8. In general, do you currently use contact lenses in either eye?

- No
- Yes

9. Do you have any other concerns regarding your affected eye? Please fill in the blank space below:

_____

_____

_____

Thank you for taking this survey!