Study Title	A Phase I/II Randomized, Double-Masked Placebo-Controlled Study for Determining the Safety of Processed Amniotic Fluid (PAF) Drops after Photorefractive Keratectomy
ClinicalTrials.gov ID (NCT Number)	NCT04281004
Principal Investigator (PI)	Mark Mifflin, MD
Document (ICF, Protocol, SAP)	Protocol
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A Randomized, Double-Masked, Placebo-Controlled Study For Determining The Safety Of Processed Amniotic Fluid (pAF) Drops After Photorefractive Keratectomy. (PRK)

Amniotic Fluid Trial Network
Cell Therapy & Regenerative Medicine

Protocol Version 1.05
Version Date: December 01, 2021
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This protocol is PRK Protocol Number 1.05, and has been authored by Mark Mifflin, M.D., University of Utah, for implementation with the PRK investigators.

PROTOCOL TITLE:

A Randomized, Double-Masked, Placebo-Controlled Study For Determining The Safety Of Processed Amniotic Fluid (pAF) Drops After Photorefractive Keratectomy.

Short Title: PRK
PRK Protocol Number: 1.05

Lead Investigator and Author:
Mark Mifflin, M.D.
University of Utah

Protocol Version: 1.05
Version Date: December 01, 2021

I confirm that I have read this protocol, I understand it, and I will conduct the study according to the protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and will adhere to the Ethical and Regulatory Considerations as stated. I confirm that if I or any of my staff are members of the Institutional Review Board, we will abstain from voting on this protocol, its future renewals, and its future amendments.

Principal Investigator Name: _____

Principal Investigator Signature: _____

Date: _____

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1 Synopsis

Title	A Randomized, Double-Masked, Placebo-Controlled Study for determining the safety of processed Amniotic Fluid (pAF) Drops after Photorefractive Keratectomy.
Short Title	pAF for the treatment of PRK
IRB Number	00099569
IND	19025
Phase	I/II
Design	This is a randomized, double-masked, placebo-controlled study to determine the safety of pAF in patients who undergo PRK.
Study Duration	Two years
Study Center(s)	Single Center-John A. Moran Eye Center
Objectives	Primary Objectives: • To determine the safety of using processed Amniotic Fluid (pAF) in patients following PRK. Secondary Objectives: • To determine if pAF hastens re-epithelialization following PRK compared to placebo. • To determine if pAF reduces post-operative pain following PRK compared to placebo. • To determine if pAF affects visual outcome following PRK compared to placebo. • To determine if pAF affects ocular surface staining and corneal regularity following PRK compared to placebo.

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Number of Subjects	63 patients
Diagnosis and Main Eligibility Criteria	**Inclusion**: 1. Patients aged 21 years and older. 2. Patients undergoing PRK for visual correction in both eyes. 3. Willing and able to give consent for study participation and comply with study procedures, including follow-up visits. **Exclusion**: 1. Patients with any active eye disease, including keratoconus or any other ectactic disorders. 2. Patients with documented uncontrolled diabetes. 3. Patients with severe dry eye as measured by corneal staining. 4. Patients with calculated PRK treatment resulting in residual stromal bed <300 um. 5. Patients who have had previous eye surgery or refractive laser procedures. 6. Patients with any active collagen vascular disease. 7. Patients who do not have potential of 20/20 or better best-corrected vision in each eye.
Study Product, Dose, Route, Regimen	Individual patients will be randomized to one of two post-operative drop regimens, control eyes that will receive placebo saline solution (NaCl 0.9%, Baxter Medical), and study eyes that will receive pAF four times daily for seven days. Both eyes of all patients will otherwise be treated with the standard post-operative drop regimen for our center. This includes moxifloxacin 0.3%, prednisolone acetate 1%, and ketorolac 0.5% on a tapering schedule. A bandage contact lens will also be placed as per standard post-PRK care. All patients will be instructed to use supplemental preservative free artificial tears from individual vials (any brand) no less than 3 but up to 8 times a day for both eyes for the first 7 days.

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Statistical Methodology	Standard statistics will be calculated and used to describe the two treatment groups in terms of all study variables. Statistics will be compiled for each variable at the study time points listed in the data collection protocol.

2 Objectives

Primary Objectives:

a. To determine the safety of using pAF to improve ocular surface healing in PRK patients compared to use of placebo drops.

Secondary Objectives:

a. To determine if pAF hastens re-epithelialization in patients following PRK compared to placebo.

b. To determine if pAF reduces post-operative pain following PRK compared to placebo.

c. To determine if pAF affects visual outcome following PRK compared to placebo.

d. To determine if pAF affects ocular surface staining and corneal regularity following PRK compared to placebo.

3 Rationale and Background

3.1 Photorefractive Keratectomy (PRK)

Photorefractive Keratectomy (PRK) is a common elective surgery used to correct refractive errors (nearsightedness, farsightedness, and astigmatism). An excimer laser is used to remove stromal corneal tissue to achieve the desired refractive outcome. The corneal epithelium is removed prior to excimer laser treatment, creating a very well controlled and reproducible surgical injury to the ocular surface. Post-operative healing is easily monitored and quite predictable, but does cause potential discomfort and visual blurring for the patient, hence any supportive measures or medication that hastens epithelial healing and recovery is desirable to allow patients to regain visual function more quickly.[1, 2] In rare circumstances, the creation of these epithelial defects may also be associated with other side effects or complications including delayed epithelial healing, recurrent erosions, or dry eye. We propose that amniotic fluid

drops may be beneficial in promoting ocular surface healing following PRK. Purified amniotic fluid (pAF) contains anti-inflammatory, anti-microbial and regenerative factors similar to solid amniotic membrane.[3] Amniotic membrane has growth factors and natural anti-inflammatory factors and has demonstrated therapeutic effect following a variety of ocular surface injuries or surgery including: Stevens Johnson Syndrome and pterygium removal.[4, 5] Anecdotal experience has indicated that amniotic fluid eye drops may be beneficial in graft-versus-host disease, but controlled studies are needed to determine if this has clear beneficial effects.

3.2 Amniotic fluid (AF):

Early after conception and until the mothers water breaks for the delivery of their infant, the fetus is bathed in amniotic fluid. AF functions as a supportive cushion to the fetus and provides a protective environment. AF is a rich source of nutrients, cytokines and growth factors that are required for fetal development and maturation.[6] AF also contains stem cells with the potential to differentiate along multiple cell lineages.[7, 8] The protective and regenerative properties of AF are achieved via the exchange of water and solutes with surrounding tissues. This is accomplished via the utilization of different pathways during the course of a pregnancy that likely contribute to changes in the composition of the AF with gestational age.[6] A report that concentrates of AF inhibited the development of peritonitis was among some of the first evidence that AF had protective biological properties.[9] This was followed by a publication by Shimberg and co-workers that AF accelerates defense-repair mechanisms within damaged joints.[9, 10] Since these early publications, more sophisticated evaluations have revealed the presence of antimicrobial, immunomodulatory, and growth-promoting activities in AF.[6] Reports about antimicrobial activity in AF differs[11] among investigators. Some studies show that AF is inhibitory, while others show no effect against the same microorganisms. Yet, other reports provide evidence that AF with low antimicrobial activity is associated with a high incidence of an infectious syndrome in pregnant women.[12] Components with antimicrobial, antiviral and antifungal activity that are present in AF include lysozyme, peroxidase, transferrin, -lysin, immunoglobulins and zinc-peptide complexes.[11] Immunomodulatory properties of AF are evident from studies showing that enteral feeding of AF suppresses the pro-inflammatory responses in preterm pigs with necrotizing enterocolitis.[13] While growth promoting activities of AF are supported by animal studies as well as by in vitro studies showing that AF can enhance neochondrogenesis,[14] regenerate peripheral nerves[15] and bone,[16] accelerate re-epithelialization in corneas,[17] and promote healing of human skin wounds.[18] Some of the factors that are found in AF that may contribute to these activities include inflammatory mediators that include, but are not limited to TNF-a, IL-6, IL8, and IL-10,[19] trophic factors that include EGF, IGF-1, FGF, HGF and TGF-a,[20–24] and HA, an important factor in promoting re- epithelialization in human skin wounds.[18]

Based on the hypothesis that nutrients, cytokines and growth factors contained in the non-cellular fraction of AF are useful for reparative and regenerative treatments in patients, Pierce et al conducted a study at the University of Utah to address three issues. The first was to determine the feasibility of consenting and screening volunteer donors for the routine collection of AF from full-term pregnant women scheduled for caesarean- section (C-sections) and then processing the AF for clinical applications. The second aim was to develop a processing method that resulted in a cell-free AF preparation suitable for clinical applications. The third goal was to gain a better understanding about components of AF procured from full-term pregnancies.

With the above 3 goals in mind, human AF was collected by the staff of the Obstetrical and Gynecological department at the University of Utah hospital and was processed by technical staff of the Cell Therapy and Regenerative Medicine (CTRM) facility at the University of Utah. Physician executed abdominal incisions were performed through the abdominal and uterine muscles without cutting into the amnion membrane. Using a sterile soft suction catheter connected to a sterile MediVac Suction Container (Cardinal Health, Waukegan, IL), a blunt end insertion with a catheter was made into the amnion membrane and the AF was aseptically suctioned into a MediVac Container. The container was labelled, wrapped in frozen Insul-ice mats (Fisher Scientific, Hanover Park, IL) and placed in a temperature-monitored shipper that was validated for transport between 2 and 8 °C. The AF was transported to the CTRM facility at the University of Utah. Upon arrival at the CTRM facility, the product was immediately placed into a refrigerator at 2-8 °C until processing occurred. At the time of processing, the MediVac container with AF was aseptically placed in a biological safety cabinet and the AF was transferred via aseptic techniques into sterile centrifuge tubes. The total volume and gross appearance of the AF were recorded and samples were removed for sterility testing, cell counts and other relevant testing. The AF was centrifuged at 1400Xg for 20 min at 4 °C. Once centrifugation was complete, the supernatant was expressed into a new transfer pack and the remaining cell pellet was characterized and cultured as described below. The supernatant from the AF was processed using a proprietary filtration technology to sterilize and eliminate cellular debris from the final product. AF collections and final products were evaluated for total volume, fluid chemistries, total protein, and hyaluronic acid (HA) levels. Final products of processed AF (pAF) were also assessed for their cellular content and for their protein profiles using quantitative antibody arrays.

To validate the above described approach for collecting and processing AF, 36 pregnant women consented and passed the donor screening criteria. AF was successfully collected from 17 individuals. Median AF volumes were 70 mL (range 10-815 mL; n = 17). Fluid chemistries were similar, but some differences were noted in HA levels and cytokine profiles. Cytokine arrays revealed that an average of 304 ± 20 (mean±SD; n=3) of 400 proteins tested were present in AF with a majority of cytokines associated with host defense. Among the 300 proteins present in pAF were two proteins with functions known to be associated with ocular

development and corneal stroma maintenance.

3.3 Processed Amniotic Fluid (pAF): Preliminary Clinical Data

The presence of peptides in processed amniotic fluid (pAF) that have roles in ocular development and corneal stroma maintenance, along with the understanding that one of the in utero beneficial functions of AF is to bathe the fetuses eyes, led us to hypothesize that pAF may be a valuable treatment regimen to improve corneal re-epithelialization following PRK.

Our pAF has been clinically used in over 2000 applications for over 100 different conditions. A majority of treatments have been for wounds and burns with 3 patients receiving pAF for the treatment of ocular GVHD. No adverse events have been directly associated with the injection of pAF or when pAF has been topically applied for the treatment of ocular GVHD.

For the 3 patients treated for ocular GVHD at the University of Utah, each patient received a dose of 0.5 ml per eye daily in 2 divided doses, and no toxicities were observed. One patient with severe dry eye had a good partial response with a decrease in the need for artificial tears and the use of PROSE lenses. A second patient had stabilization of the disease after discontinuation of all immunosuppression. The third patient reported no change.

4 Eligibility Criteria

Inclusion Criteria:

1. Patients aged 21 years and older.
2. Patients undergoing PRK for visual correction in both eyes.
3. Willing and able to give consent for study participation and comply with study procedures, including follow-up visits.

Exclusion Criteria:

1. Patients with any active eye disease, including keratoconus or any other ectactic disorders.
2. Patients with documented uncontrolled diabetes.
3. Patients with severe dry eye as measured by corneal staining.
4. Patients with calculated PRK treatment resulting in residual stromal bed <300 um.
5. Patients who have had previous eye surgery or refractive laser procedures.

6. Patients with any active collagen vascular disease.

7. Patients who do not have potential of 20/20 or better best-corrected vision in each eye.

5 Study Design and Procedures

This is a randomized, double-masked, placebo-controlled study to evaluate the safety of using pAF in patients following PRK.

Pre-operatively each patient will be randomized to one of two post-operative drop regimens. Neither the patient nor their doctors will know if they are receiving pAF or placebo. The vials containing pAF and placebo (NaCl 0.9%, Baxter Medical) will be packaged the same by the Cell Therapy and Regenerative Medicine (CTRM). The assigned drop will be administered in both eyes throughout the study.

Regimen 1 will serve as the placebo control. For the first week only, patients in this regimen will receive placebo saline solution (NaCl 0.9%, Baxter Medical) for use four times daily. Patients will otherwise be treated with our standard post-operative drop regimen, which includes prednisolone acetate 1% suspension (4x/day for 7 days, then 2x/day for 2 additional weeks), moxifloxacin 0.3% (4x/day for 7 days), and ketorolac 0.5% (2x/day for 3 days only). The prednisolone acetate will be tapered to fluorometholone 0.1% suspension (3x/daily on days 22-60 then 2x/daily on days 61-90). A bandage contact lens will also be placed following surgery as is standard care for PRK patients.

Regimen 2 will have a bandage contact lens placed post-PRK and have an identical drop regimen with the exception of replacing the placebo saline with pAF four times daily for one week.

All patients will be instructed to use supplemental preservative free artificial tears from individual vials (any brand) no less than 3 but up to 8 times a day in both eyes the first week. After the first week, they will continue to be allowed use of preservative free tears as needed per the standard post PRK regimen in our center.

Patients are instructed to use oral medications as needed post-surgery. Ibuprofen or naproxen may be helpful in preventing pain for the first 3 days following the procedure. Patients will also be prescribed hydrocodone-acetaminophen 5-325 mg every four hours as needed for breakthrough pain. Patients will record how many pain pills are taken each day on a worksheet provided by the research team after the surgery.

The patients will have a pain rating sheet and will be asked to grade their peak subjective pain on a scale of 0 to 10 (with 10 being the most painful) for the first 7 days. They will grade each eye separately.

The day of surgery will be designated day 1, as surgery occurs in the early morning. The size of the epithelial defect created by PRK will be measured at the time of surgery completion.

Surgeons will administer the first dose of masked study drug (placebo or pAF fluid) directly after surgery, the morning of day 1, and three more times that day. Patients will then administer drops four times a day total for the next 6 days, for a total of 7 days of study drug administration. The patients will begin filling out the pain survey the night of day 1.

At a designated time on day 1 (2^{nd} visit post-surgery), 3, 4, 5, 6, 7, and 8 each patient will be examined by one of the study investigators who will be masked to the drop regimen. The investigator will grade the size of the epithelial defect on day 1(AM), 3, 4, 5, 6, 7, and 8 by measuring the largest horizontal and vertical meridians using slit lamp biomicroscopy. If a patient has re-epithelialized by an earlier exam day (i.e., day 3) they will be able to skip all subsequent in person examinations except for day 8 (i.e., skip days 4, 5, 6, and 7). All patients will still need to record their pain rating scale at the end of each day and return for the day 8 visit.

The study investigators will be masked to which drop is being used for each patient. Patients will be required to store all drops in the freezer and will be masked as to which type of drop is being used.

Patients will also be seen at post-operative months 1, 3, 6 and 12. At each of these visits visual acuity and manifest refraction will be checked, along with a measure of ocular surface staining and corneal regularity. These are all standard procedures performed for post-operative PRK patients regardless of study participation.

5.1 Schedule of Events

	Baseline	**Day of Surgery (Friday)**		**Days after Surgery**									
	Pre-Op Screening[1]	Day 1 (AM)	Day 1 (2^{nd} visit post-surgery)	Day 3	Day 4*	Day 5*	Day 6*	Day 7*	Day 8 Friday AM (in-person visit)*	Month 1 (Day 30±10)*	Month 3 (Day 90±14)*	Month 6 (Day 180±30)*	Month 12 (Day 365±30)*
Informed Consent	X												
Eligibility	X												
Medical History	X												
Concomitant medications[2]	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization[3]		X											
PRK Surgery		X											
Dispense Masked Study Drug[4]		X											
Study Drug Compliance[5]			X	X	X	X	X	X					
Pain Assessment[6]			X	X	X	X	X	X					
Size of Epithelial Defect[7]		X		X	X	X	X	X	X				
Uncorrected VA[8]	X								X	X	X	X	X
Manifest Refraction	X									X	X	X	X
Target Post-OP Refraction	X												
BCVA[8]	X									X	X	X	X
Ocular Surface Staining	X									X	X	X	X
Corneal Topography	X									X	X	X	X
Ocular Adverse Events			X	X	X	X	X	X	X	X	X	X	X

1. The pre-op screening/baseline visit may be performed in 2 separate visits.

2. Record systemic medications at baseline and any subsequent changes; Record start and stop dates of ocular medications prescribed as standard care post-PRK surgery, note any deviations from prescribed regimens.

3. Randomization to study drug assignment will occur when patient presents day of surgery.

4. First dose of study drug applied to each eye at completion of surgery. Three additional doses administered by patient on day of surgery, then 4x per day through Day 7.

5. Patient will document each use of study drug and oral pain medication in diary.

6. Patient will assess highest level of daily pain/discomfort at the end of each day.

7. Grade defect dimensions until healed.

8. Visual acuity will be measured on Snellen charts. Conversion to logMAR equivalents will be completed on the back end by the Biostatisticians.

* Study procedures may be performed via telehealth or by phone at the discretion of the treating provider.

Table 1: Schedule of Events

5.2 Dosage

Based on randomization to receive the test article or the placebo, the patient will receive Comar Eye Dropper Bottles that contain pAF or control saline solution (NaCl 0.9% Baxter Medical). The patient will apply one drop 1-2 drops (one drop is approximately 50μL) in each eye four times daily for a total of 7 days. The patient will apply 1-2 drops (0.25mL) in each eye four times daily for a total of 7 days. Patients will be instructed to treat their eyes at regular intervals during the day, approximately at breakfast, lunch, dinner, bedtime, or the equivalent. Surgeons will administer the first dose of pAF or placebo to patients the morning of surgery, Day 1.

The study treatment assignment for each patient will be randomly selected. Neither the patient nor their doctors will know if they are receiving pAF or placebo. The eyedropper bottles containing pAF and the placebo will be packaged identically.

Patients will be instructed to use preservative free artificial tears from individual vials (any brand) no less than 3 but up to 8 times a day in both eyes during the first 7 days of the study.

5.3 Drug Dispensation and Randomization

Upon acceptance into the study, and prior to surgery, the patient will be randomized as to whether they will receive the pAF or the placebo. The study coordinator will notify CTRM who will then select, package and release Comar Eye Dropper Bottles containing pAF or the placebo saline solution (NaCl 0.9% Baxter Medical) to the clinical site. A small box of eight (8) eyedropper bottles containing 3.0 mL of either pAF or placebo will be provided in a Thermosafe box with dry ice to the patient for 7 days of treatment.

The small boxes will be packaged on dry ice and transported to the patient. The patient will be provided with the instructions on how to store the eye drops at home, how to use the eye drops and how to handle the dry ice. The patient will transport the eye drops to their home and immediately transfer them to their kitchen refrigerator freezer. For each day of use the patient will dispense the eye drops as follows:

1. The patient will remove the box containing the bottles from the freezer and remove a single bottle from the small box. They will verify that the lot number on the bottles match the labels on the small box. If the lot numbers on the bottle do not match the lot numbers on the small box, the patient will be instructed to notify the study coordinator. The study coordinator will notify CTRM, and CTRM will replace the bottle with the correct lot number. The patient will have to come to the Moran to trade out the bottle(s). **On Day 1 of surgery, surgeons will administer the first dose prior to patients leaving the surgery center).**

2. They will thaw the frozen fluid in the bottle by placing the bottle in their hand or by placing it at room temperature until the entire volume is completely thawed.

3. For the 1st daily application (e.g. morning), they will remove the cap from the bottle. They will invert the bottle over the right eye and squeeze to dispense 1-2 drops into the right eye. They will then invert the bottle over the left eye and squeeze to dispense 1-2 drops into the left eye. They will then tightly cap the bottle.

4. They will then place the thawed bottle into refrigerate at a temperature between 1°C and 10°C (e.g. refrigerator). They will not re-freeze the thawed bottle.

5. For each subsequent application of the day, they will remove the same thawed bottle that they used for the 1st application from the refrigerator and repeat steps 3 and 4.

6. After the final application of the day, they will place the bottle into a box provided to them for the placement of used bottles.

7. The patient will repeat these steps for each daily application.

8. After the 7th day of treatment, the patient will return all used and unused bottles to the clinic. This will allow us to account for drug dispensed and monitor compliance.

9. The patients will also voluntarily record if they missed any doses of their medication.

5.4 Assessment of Primary Endpoint(Safety)

Safety and tolerability will be evaluated by the PI from the findings of scheduled eye examinations at days 1 (day of surgery) through 8 (in-person follow-up visit) and at months 1, 3, 6 and 12 after initiation of pAF therapy. More frequent safety evaluations may be performed if clinically indicated or at the discretion of the investigator. Information from the eye examination at these additional safety evaluations will be collected and entered into the EDC as adverse events as appropriate.

All ocular adverse events will be recorded from randomization through Month 12. Any events that occur from time of consent to randomization will be recorded as medical history.

6 Safety Monitoring

6.1 Adverse Event Reporting

The site investigator is responsible for evaluating all adverse events at their Clinical Center and ensuring that they are properly reported. Only ocular adverse events (as specified in 6.1.2) that occur during this study will be recorded. The nature of each experience, date and time

(where appropriate) of onset, outcome, course, and relationship to treatment will be documented.

6.1.1 Definition of Adverse Event and Serious Adverse Event

Adverse Event (AE) means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). An event constitutes a disease, a set of related signs or symptoms, or a single sign or symptom.

Serious Adverse Event (SAE): A serious adverse event (SAE) for this population is an adverse event that:

1. results in death; or
2. is life-threatening (the patient was, in the view of the site investigator, in immediate danger of death from the event as it occurred); or
3. requires inpatient hospitalization or prolongs an existing hospitalization; or
4. results in persistent or significant disability or incapacity; or
5. results in congenital anomaly/birth defect; or
6. any other event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

6.1.2 Classification of an Adverse Event (Relatedness, Severity, and Expectedness

Relatedness: The suspected relationship between study interventions and any adverse event will be determined by the site investigator using the criteria below. Relatedness must be assessed by an investigator and may not be assessed by a research coordinator.

- Not Related: The event is clearly related to other factors, such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.
- Possibly Related: The event follows compatible temporal sequence from the time of beginning the assigned study intervention, but could have been produced by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

- Probably Related: The event follows a reasonable temporal sequence from the time of beginning the assigned study intervention, and cannot be reasonably explained by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Severity:
The severity, which is a measure of intensity, of clinical adverse events and laboratory abnormalities will be recorded by the site investigator and categorized. The following guidelines will be used to describe severity.

- Mild: The event requires minimal or no treatment and does not interfere with the participants daily activities.
- Moderate: The event results in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe: The event interrupts a participants usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".

Expectedness of the Event:
All adverse events, including serious adverse events, will be evaluated as to whether their occurrence was expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information described for the study intervention. The following events will be reported as AEs::

- Infection
- Delayed healing of the epithelial defect (after the 7-day post-surgery visit)
- Scarring or haze
- Severe irritation of conjunctiva or periocular skin in the first week
- Poor refractive outcome (worse than 20/40 at 3 months or beyond)
- Pain or severe irritation at 3 months or beyond
- Corneal erosion
- Corneal haze
- Need for touch up surgery

There is a theoretical risk of infection with amniotic fluid drops. A patient could have an immune reaction to the study drops.

6.1.3 Treatment or Action Taken:

For each adverse event, the site investigator will record whether an intervention was required:

- Medical or surgical procedure
- Concomitant medication: started, changed, or discontinued
- Other, specify
- No action taken

6.1.4 Outcome of Event:

Finally, the site investigator will record the clinical outcome of each adverse event as follows:

- Death
- Recovered and the patient returned to baseline status
- Recovered with permanent sequelae
- Symptoms persist

6.1.5 Time Period for Adverse Events

For purposes of this study, events that occur following randomization through the last study visit will be reported as adverse events. Serious adverse events, unexpected medically attended events, and new onset chronic illnesses will be recorded from randomization through twelve months after the last study dose. Specifically, events that occur following consent to participate in the study, but prior to actual randomization, are not adverse events. These should be recorded as baseline conditions.

6.1.6 Data Collection Procedures for Adverse Events

After patient randomization all adverse events (including serious adverse events) will be recorded according to relatedness, severity, and expectedness, as well as their duration and any treatment prescribed. Any medical condition present at the time of randomization, recorded in the patient's baseline history at study entry, which remains unchanged or improves (unless the clinician feels it is clinically relevant), will not be recorded as an adverse event at subsequent evaluations. However, worsening of a medical condition that was present at the time of randomization will be considered a new adverse event and reported.

Adverse events will be coded using the MedDRA coding vocabulary. Coding will be done centrally at the Data Coordinating Center as this requires specific training.

6.1.7 Unanticipated Problems (UP)

Unanticipated problems (UP) are defined as incidents, experiences, or outcomes that are unexpected, related or possibly to participation in the study, and suggest that the research places subjects at a greater risk of harm than was previously known or recognized. The site investigator will report unanticipated problems to the Data Coordinating Center within 24 hours of becoming aware of the event. A detailed completed report will be required to be sent to the Data Coordinating Center within 3 working days of the event. After receipt of the complete report, the Data Coordinating Center will report these unanticipated problems to the CTRM in an expedited manner (as close to 24 hours as possible).

In accordance with local IRB requirements, the site investigator may be required to report such unanticipated problems to the IRB in addition to notifying the Data Coordinating Center. In the event that the medical monitor believes that such an event warrants emergent suspension of enrollment in the trial, and funding staff cannot be reached expeditiously, the Data Coordinating Center will notify the study investigator and all site investigators to cease enrollment in the trial.

6.1.8 Monitoring Serious Adverse Events

A qualified physician will be designated to fulfill the function of the medical monitor for this study. Site investigators and/or research coordinators will report serious adverse events to the Data Coordinating Center within 24 hours of becoming aware of the event. A detailed completed report will be required to be sent to the Data Coordinating Center within 3 working days of the event, and the medical monitor will assess all serious adverse events reported from site investigators.

For each of these serious adverse events, the site investigator will provide sufficient medical history and clinical details for a safety assessment to be made with regard to continuation of the trial. The medical monitor will sign off on each SAE report after review. All SAE reports will be retained at the Data Coordinating Center, and all SAE reports will be available for review by funding staff. The SAE reporting process may be incorporated into the Electronic Data Capture (EDC) System in use for the study.

In the unlikely event that the medical monitor believes an unexpected and study-related SAE warrants emergent cessation of enrollment in the trial, funding staff will be immediately consulted. If these individuals concur with the judgment of the medical monitor, or if the funding staff cannot be reached expeditiously, the Data Coordinating Center will notify the study investigator and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the funding staff.

In accordance with local IRB requirements, the site investigator may be required to report such events to the IRB in addition to notifying the Data Coordinating Center.

6.1.9 Follow-up of Serious, Unexpected and Related Adverse Events

All serious, unexpected and related adverse events, that are unresolved at the time of the patient's termination from the study, will be followed by the site investigators until the events are resolved, subject is lost to follow-up, the adverse event is otherwise explained or has stabilized, or 12 months have passed from the time of last study dose.

7 Assessment of secondary endpoints

1. Re-epithelialization will be graded by trained, masked examiners on days 1, 3, 4, 5, 6, 7, and 8 unless patient re-epithelializes sooner in which case they can skip visits until day 8. Dimensions of the epithelial defects will be directly measured using slit lamp bio-microscopy. The baseline epithelial defect will be measured by surgeons on Day 1 at the time of surgery. Examiners will be masked as to which patients are using pAF vs. placebo. A vertical and horizontal measure of defect in mm will be performed, and defect area calculated. A complete re-epithelization (absence of a defect) is defined when the measures equal zero.

2. Pain will be measured through a 0-10 scale on days 1, 2, 3, 4, 5, 6, and 7. Participants will rate their peak pain at the end of each day and directly return the forms at their in-person visit on day 8 to the examiners. They will complete a separate survey for each eye.

3. Uncorrected visual acuity will be measured at post-operative day 8, months 1, 3, 6, and 12 by trained physicians.

4. Manifest refraction and best-corrected visual acuity will be measured at months 1, 3, 6, and 12 by trained physicians.

5. Ocular surface staining will be measured at post-operative months 1, 3, 6, and 12 using an area density index.[25] This is a published and validated index used to measure corneal staining. Corneal regularity will also be measured at months 1, 3, 6, and 12 with the Surface Regularity Index (SRI) obtained via Zeiss Atlas 9000 Corneal Topography™.

8 Other Medications

- Oral pain medications usage will be tracked for the first 7 days.

9 Patient Withdrawal From Study

- A subject may withdraw their consent at any time without affect to their care.

10 Discontinuation of Study Drug

The study drug will be discontinued if any of the following occur:

- Development of severe ocular infection.
- Any other condition that in the opinion of the treating physician and ophthalmologist are not compatible with participation in this study.

11 Discontinuation of Study

- Any ocular serious, related and unexpected adverse effect that occurs in 1/3 of the patients (e.g. 1 out of 3, 2 out 6, 3 out of 9, etc.)
- The determination of the number of patients having serious, related and unexpected ocular adverse events in the study group will be reviewed by the DSMB, who may be unblinded to the study drug. Thus, the blind will not be broken throughout the study.
- The purpose of the DSMB is to advise the study investigators regarding the continuing safety of study subjects and the continuing validity and scientific merit of the study.
- The DSMB Chairperson will prepare a summary of each DSMB meeting that conveys the public conclusions of the DSMB, with respect to protocol alterations and recommendations concerning continuation of the study.
- In the unlikely event that the medical monitor believes an unexpected and study-related SAE warrants emergent cessation of enrollment in the trial, staff and the DSMB chairperson will be immediately consulted. If these individuals concur with the judgment of the medical monitor, or if the staff and the DSMB chairperson cannot be reached expeditiously, the DCC will notify the study investigator and all site investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the staff after discussion with the DSMB.

12 Statistical Considerations

12.1 General Statistical Methods:

Simple descriptive statistics of patient characteristics, operation parameters, and vision/ocular measurements will be calculated (overall and by pAF eye drop group vs. controls) at baseline, post-op and each follow-up visit. Outcomes will be described using appropriate summary measures and graphical approaches.

Between-group comparisons will be performed on:

- Time to complete re-epithelization (primary efficacy outcome).
- Visual Acuity (best-corrected and uncorrected) (this measure at 30 days is the key secondary efficacy outcome).
- Dimensions of the epithelial defects directly measured using slit lamp biomicroscopy (exploratory efficacy outcome).
- Pain in each eye using a 0-10 point VAS scale on days 1, 2, 3, 4, 5, 6, and 7. (exploratory efficacy outcome).
- Timing and frequency of use of rescue pain medication.
- Corneal staining and surface irregularity.
- Adverse event rate and complication rate.

For the primary efficacy outcome, a mixed effects model with time to healing as outcome and assigned treatment arm as a predictor, including a random effect for each patient to model correlation between eyes in the same participant, will be fit to carry out the primary analysis. A two-sided probability value with an alpha level of 0.05 will be used to test the null hypothesis that time to complete re-epithelialization is not different between the two treatment arms. The primary analysis will be performed on an intention to treat basis.

12.2 Analyses for Other Outcomes:

The key secondary efficacy outcome at 30 days will be analyzed (on a logarithmic scale) using a mixed effects ANOVA model as for the primary aim. Exploratory outcomes will be analyzed in appropriate fashion; for example, each patients pain level (worst in either eye) will be examined on each evaluation day and compared between treatment groups for each day, while each patients peak pain level at any time, and number of days until the patient is pain-free, will be compared

between treatment arms. A Statistical Analysis Plan will fully delineate all analyses to be performed, including additional analyses to assess robustness of the primary and secondary efficacy analyses.

12.3 Statistical Power and Sample Size: Time to complete re-epithelization

Time to complete re-epithelization will be measured from the end of surgery until the date of the exam where complete re-epithelization is observed. While evaluation times will be collected, since the time of surgery and the time of the follow-up exam are dependent upon scheduling, time to complete re-epithelialization will be analyzed as an integer, being simply the number of calendar days from the day of surgery to the date of the first examination demonstrating complete re-epithelization.

12.4 Sample Size calculations for pAF PRK study

A mixed effects ANOVA model for difference in time to complete re-epithelization was used to determine sample size. A within-subject correlation in healing times of r = 0.5 was assumed. Calculations were performed to achieve a power of 80% and 90% and a level of significance of 5% (two sided).

12.5 Sample size calculations:

Eliacik et al.,[26] found a mean time to re-epithelization of 3.1 ± 0.6 days for the Comfilcon Group and 3.6 ± 0.5 days for the Lotrafilcon Group. We used this 0.5-day difference as an estimate of treatment effect, and considered within-group standard deviations in the range of 0.5 to 0.7 days. Another key parameter in the estimation of statistical power is the intraclass correlation between eyes in the same patient. This correlation was estimated to be in the range of 0.5 to 0.75, as within-patient healing times have been reported to be nonuniform to a modest extent in the study PIs experience. The table below shows true treatment differences detectable with 80% and 90% power under different estimates of the variability of re-epithelization time and intraclass correlation, **assuming that 56 participants (28 participants and therefore 56 eyes in each treatment arm) complete the trial.** These values were calculated using PASS [PASS 15 Power Analysis and Sample Size Software (2017). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass.], treating the study as a cluster-randomized trial with clusters (each participants eyes) of size two.

Assumed Within-Group Standard Deviation of Primary Outcome	Assumed ICC between a Participants Eyes	Treatment Difference Detectable with 80% Power	Treatment Difference Detectable with 90% Power
0.5 days	0.5	0.33 days	0.38 days
0.5 days	0.6	0.34 days	0.39 days
0.5 days	0.75	0.35 days	0.41 days
0.6 days	0.5	0.39 days	0.45 days
0.6 days	0.6	0.41 days	0.47 days
0.6 days	0.75	0.42 days	0.49 days
0.7 days	0.5	0.46 days	0.53 days
0.7 days	0.6	0.47 days	0.55 days
0.7 days	0.75	0.49 days	0.57 days

Based on numerous power estimates (the above and others), a sample size of 56 patients completing the trial, yields acceptable power to find a significant treatment difference, if the true treatment difference is at least 0.5 days for the time to complete re-epithelialization outcome. In order to collect additional safety data and allow for a small number of patients being lost to follow-up, the study will enroll a total of 63 participants.

12.6 Analysis of Safety Data

The primary safety analyses will be based on the as-treated population, defined as subjects who are randomized and receive at least one dose of study agent. Safety endpoints will include ocular adverse events, ophthalmic exam findings, and use of rescue treatments. The occurrence of ocular adverse events will be recorded from the time of randomization through the last study visit. AEs will be categorized by System Organ Class (SOC) Preferred Term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA) and by severity using the Common Terminology Criteria for Adverse Events (CTCAE) dictionary.

In general, all AE tables will be presented by treatment group, causality and severity. Events in the right and left eyes will be considered as separate events. The following will be summarized and presented overall and by treatment group:

- The number and percentage of subjects experiencing an SAE.
- The number and percentage of subjects experiencing an SAE by preferred term and by severity of event.
- The number and percentage of subjects experiencing an SAE by preferred term and by relationship to study drug.

- The number and percentage of subjects experiencing an AE.
- The number and percentage of subjects experiencing an AE by preferred term and by severity of event.
- The number and percentage of subjects experiencing an AE by preferred term and by relationship to study drug.
- The number and percentage of AEs that cause a subject to discontinue study drug.
- The number and percentage of AEs leading to death.

13 Data Management

13.1 Clinical Site Data Management

The Data Coordinating Center (DCC) will develop an electronic data capture system for this trial. Currently the DCC uses multiple applications, such as OpenClinica or REDCap, and will elect to use the most appropriate application at the time of implementation of the study.

The DCC will use an electronic discrepancy management system to notify sites of inconsistent or erroneous data entry, which will be corrected by the clinical site. The discrepancy/management system maintains an audit trail of all discrepancy resolution.

13.2 Study Monitoring

The investigators recognize the importance of ensuring data of excellent quality. Study monitoring is critical to this process. Monitoring has been a very effective tool for maintaining data quality in previous network studies, and we will utilize this process to ensure excellent quality data in the proposed study. The DCC utilizes risk-based methodology to identify and correct problems that may arise at sites. The risk-based approach to monitoring focuses on oversight activities and preventing or mitigating key risks to data quality, as well as to processes critical to human subject protection and integrity of the trial or study.

Study monitors must be provided with appropriate access to study materials and the medical records for study subjects. If the medical records are in electronic form, the clinical investigator or an authorized individual must provide any assistance necessary to facilitate the study monitor's review of data in the electronic medical record.

13.2.1 Site Monitoring Plan

A supplemental study-specific risk-based monitoring plan, separate from the protocol will be completed which outlines specific criteria for monitoring. This plan may include the number of planned site visits, criteria for focused visits, additional visits or remote monitoring, a plan for chart review and a follow up plan for non-compliance. The monitoring plan also describes the type of monitoring that will take place (e.g., sample of all subjects within a site; key data or all data), the schedule of visits, how they are reported and a time frame to resolve any issues found.

13.2.2 Clinical Site Monitoring

Site monitoring visits will be performed by a trained site monitor during the study period to ensure regulatory compliance, patient safety, and to monitor the quality of data collected. Essential document binders, regulatory documents and data collection forms may be reviewed. Interim visits will take place depending on grant budget, site enrollment, and compliance issues identified. The site monitor will provide each site with a written report, and sites will be required to follow up on any deficiencies. It is anticipated that the study monitoring visits for this protocol will consist of a site initiation visit (prior to patient enrollment), interim visits, and a close out visit. The site initiation may take place as group training made up of site investigators and research assistants.

13.2.3 Remote Monitoring

The Data Coordinating Center may supplement on-site monitoring with remote monitoring activities. Remote monitoring involves detailed review of the data entered by the site and consultations with the site investigator and/or research coordinator to review safety and data quality. This may require uploading copies of medical records, patient study files, regulatory documentation, or other source documents for the monitor to review. Alternatively, other methods, such as remotely viewing source documentation, may be utilized. This helps assure protocol compliance and accurate data collection. The Data Coordinating Center may conduct more remote monitoring activities early in the trial to assure protocol compliance and identify any training issues that may exist. Remote monitoring documents will be retained in accordance with federal requirements.

14 Protection of Human Subjects

14.1 Institutional Review Board Approval

The Data Coordinating Center and clinical center PI must obtain approval from the IRB prior to participating in the study. The Data Coordinating Center will track IRB approval status and will not permit subject enrollment without documentation of initial IRB approval and maintenance of that approval throughout subsequent years of the project.

14.2 Informed Consent

If a subject is greater than 18 years of age or attains the age of 18 years during the study period, informed consent is required. Subjects who are capable of giving consent and who are alert and competent, will be asked, following an appropriate discussion of risks and benefits, to give consent to the study.

14.3 Potential Risks

Loss of confidentiality of the subject is a potential risk of the study; however, safeguards are in place to protect against this. There are no known or anticipated additional medical risks from participating in the trial.

14.4 Protection Against Potential Risks

Regarding loss/breach of privacy and confidentiality, all applicable parties (e.g. clinical sites, DCC) will be responsible for ensuring that appropriate data security procedures are in place.

14.5 Potential Benefits

There may be a potential benefit in helping decrease pain and healing time with pAF treatment.

15 Regulatory Considerations

15.1 Food and Drug Administration

This trial is being conducted under an Investigational New Drug application. The clinical investigator at each participating site will complete a Form FDA 1572, “Statement of Investigator.”

An annual progress report will be submitted to the FDA within 60 days of the anniversary of the date that the IND went into effect (21 CFR 312.33).

15.2 Health Insurance Portability and Accountability Act

Data elements collected include the date of birth and date of admission. Prior to statistical analyses, dates will be used to calculate patient age at the time of the study events. The final data sets (used for study analyses and archived at the end of the study) will be de-identified, and will exclude these specific dates. Data elements for race, ethnicity, and gender are also being collected. These demographic data are required for Federal reporting purposes to delineate subject accrual by race, ethnicity, and gender.

15.3 Inclusion of Women and Minorities

There will be no exclusion of patients based on gender, race, or ethnicity.

15.4 Protocol Amendments

Any amendments or administrative changes to an IRB approved protocol will not be initiated without submission of an amendment for IRB review and approval. Any amendments to the protocol that significantly affect the safety of subjects, the scope of the investigation, or the scientific quality of the study are required to submit the amendment for FDA review.

16 Data Coordinating Center

The Data Coordinating Center (DCC) in the Department of Pediatrics at the University of Utah School of Medicine provides data coordination and management services for a variety of national research networks. Anchoring these services is a new state-of-the art, energy efficient data center completed in 2013. The data center facility supports more than 1400 users around the world and provides a secure, reliable, enterprise-wide infrastructure for delivering critical DCC systems and services. The new data center was built using high industry standards and energy efficient cooling solutions. The data center is cooled by Rittals LCP inline cooling technology, providing efficiency, redundancy and modularity. Cooling is based upon a hot/cold aisle design that allows for even air distribution with minimal hot spots. The data center electrical power system contains a redundant Mitsubishi uninterruptible power system (UPS) with a diesel backup generator. The data center is protected with a FM200 fire suppression system, early warning smoke detectors and a heat detection warning system to act as a secondary system to the smoke detectors. Security guards are on-site conducting access control and rounds. Entry

into the data center is restricted by card access and layered security measures and controls. The data center and external building access points are monitored with video surveillance.

- In 2011 the data center began a large scale VMware server virtualization deployment. Currently, the data center has virtualized about 99% of its environment. The virtual environment consists of more than 200 virtual servers. The data centers virtualization solution provides key advantages: high availability in the event of hardware failure, virtual servers automatically go back online in a seamless process.
- Flexible infrastructure disk storage, memory and processor capacity can be increased or reallocated at any time.
- Rapid deployment servers can be provisioned on-demand with minimal waiting on hardware of software.

The data center also enhanced its storage resources by implementing a networked storage system to support its virtualized environment. The data center currently manages over 50 terabytes of data. The storage solution consists of Dells EqualLogic PS Series Storage system for providing a virtualized storage area network (SAN). Some of the benefits that are realized through this technology are:

- Storage architecture is no longer be a bottleneck for IT services;
- Performance is better than with the previous architecture;
- Tiered storage is now possible;
- Provisioning and reclamation of SAN disk will be much easier; and most important;
- The new architecture includes a redesign of the SAN fabric to include complete redundancy.

Production servers running critical applications are clustered and configured for failover events. Servers are backed up with encryption through a dedicated backup server that connects across an internal 10 gigabit network to a tape drive. DCC storage area networking (SAN) applications, clusters, and switch-to-switch links are also on a 10 gigabit network. Incremental backups occur hourly Monday through Friday from 6 am to 6 pm. Incremental backups also are performed each night with full system backups occurring every Friday. Tapes are stored in a fireproof safe inside the data center facility, and full backups are taken off site on a weekly basis to an off-site commercial storage facility. In the event of catastrophic failure, such as a fire in

the server facility, daily backups would probably survive because of the fire suppression system and fireproof safe, but there would be obvious delay in re-establishing data center function because the servers will not survive such a disaster. Total destruction of the data center facility could cause the loss of up to one weeks data. In future investments, the data center is making co-location, disaster recovery and business continuity solutions a top priority. DCC information systems are available 24 hours a day, 7 days a week to all users unless a scheduled maintenance interruption is required. If this occurs, we notify all users of the relevant systems, and data entry can be deferred until after the interruption is over. Critical systems availability has exceeded 99.9% for the past two years, and there has been no unscheduled downtime in over five years.

16.1 Security and Confidentiality

The data center coordinates the network infrastructure and security with the Health Sciences Campus (HSC) information systems at the University of Utah. This provides us with effective firewall hardware, automatic network intrusion detection, and the expertise of dedicated security experts working at the University. Network equipment includes four high-speed switches. User authentication is centralized with two Windows 2012 domain servers. Communication over public networks is encrypted with virtual point-to-point sessions using transport layer security (TLS) or virtual private network (VPN) technologies, both of which provide at least 128 bit encryption. All of our Web-based systems use the TLS protocol to transmit data securely over the Internet. Direct access to data center machines is only available while physically located inside our offices, or via a VPN client.

All network traffic is monitored for intrusion attempts, security scans are regularly run against our servers, and our IT staff is notified of intrusion alerts. Security is maintained with Windows 2012 user/group domain-level security. Users are required to change their passwords every 90 days, and workstations time out after 5 minutes of inactivity. All files are protected at group and user levels; database security is handled in a similar manner with group-level access to databases, tables, and views in Microsoft SQL Server. Finally, all laptop computers in use in the DCC or in the Department of Pediatrics are whole-disk encrypted.

The data center uses control center tools to continuously monitor systems and failure alerts. Environmental and network systems are also monitored to ensure up time. Highly trained system administrators on staff are available to respond in high risk emergency events. All personnel involved with the DCC have signed confidentiality agreements concerning data encountered in the course of their daily work. All personnel (including administrative staff) have received Human Subjects Protection and Health Information Portability and Accountability Act (HIPAA) education. We require all users to sign specific agreements concerning security, confidentiality, and use of our information systems, before access is provided.

16.2 Record Access

The medical record and study files (including informed consent) must be made available to authorized representatives of the Data Coordinating Center, upon request, for source verification of study documentation. In addition, medical information and data generated by this study must be available for inspection upon request by representatives (when applicable) of the Food and Drug Administration (FDA), NIH, other Federal funders, and the Institutional Review Board (IRB) for each study site.

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