Study Title	A Randomized, Double-Blinded, Placebo-Controlled Study for the Treatment of Ocular Chronic GVHD with Processed Amniotic Fluid (pAF) Drops (GVHD)
ClinicalTrials.gov ID (NCT Number)	NCT03298815
Principal Investigator (PI)	Catherine Lee, MD
Document (ICF, Protocol, SAP)	Protocol
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A Randomized, Double-Blinded, Placebo-Controlled Study for the Treatment of Ocular Chronic GVHD with Processed Amniotic Fluid (pAF) Drops (GVHD)
Protocol Number 7.0

Amniotic Fluid Trials

Protocol Version
Version Date: January 14, 2022
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This protocol is Protocol Number 7.0, and has been authored by Catherine Lee, M.D., University of Utah, for implementation with the investigators.

PROTOCOL TITLE:

A Randomized, Double-Blinded, Placebo-Controlled Study for the Treatment of Ocular Chronic GVHD with Processed Amniotic Fluid (pAF) Drops

Short Title: GVHD
Protocol Number: 7.0

Lead Investigator and Author:
Catherine Lee, M.D.
University of Utah

Protocol Version:
Version Date: January 14, 2022

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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Contents

List of Tables

1 Synopsis

Title	A Randomized, Double-blinded, Placebo-Controlled Study for the Treatment of Ocular Chronic GVHD with Processed Amniotic Fluid (pAF) Drops
Short Title	pAF for the treatment of Ocular GVHD
IRB Number	00103515
IND	18489
Phase	**I/II**
Design	This is a randomized, double-blinded, placebo-controlled study of the efficacy of pAF in patients with hematologic malignancies who have undergone Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) and are diagnosed with chronic GVHD of the eye.
Study Duration	Two years
Study Center(s)	University of Utah
Objectives	Primary Objectives: • To determine the safety of pAF in patients with chronic ocular GVHD. • To determine the clinical effects of pAF in patients with chronic ocular graft versus host disease. Secondary Objectives: • To determine the change in National Institutes of Health (NIH) Consensus Criteria (CC) ocular score of chronic GVHD at 30±3 days, 60±3 days, and 100±3 days from baseline related to the administration of pAF. • To determine change in Functional Assessment of Cancer Therapy-General (FACT G)(Quality of Life (QOL)) at 30±3 days, 60±3 days, and 100±3 days related to the administration of pAF. • To determine changes in visual acuity related to the administration of pAF. • To determine the effects of pAF on the corneal surface. • To determine the changes in dry eye symptoms using the grading provided by the International Dry Eye Workshop (DEWS) 2007 report.[1] • To determine the changes in patient reported pain level related to the administration of pAF using the 0–10 pain rating scale during the first 60 days of the study.

Continued on next page

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Number of Subjects	15–20 patients
Diagnosis and Main Eligibility Criteria	**Inclusion**: 1. Patients diagnosed within 5 years after hematopoietic stem cell transplant for any disease, with any graft and any conditioning regimen with at least one of the following: • Dry eye symptoms partially affecting (requiring lubricant drops > 3 × per day, punctal plugs, or thermally cauterized puncta) or significantly affecting (special eyewear to relieve pain) activities of daily living (ADL). • Unable to work because of ocular symptoms. • Loss of vision due to keratoconjunctivitis sicca (KCS). Patients may be using bilateral scleral lenses and/or bilateral punctal plugs at the time of accrual. 2. Patients who are 18 years of age or older. 3. Willing and able to provide informed consent. **Exclusion**: 1. Patients who have any other reversible cause for dry eye at the time of accrual. 2. More than 3 lines of therapy beyond corticosteroids with or without calcineurin inhibitors or sirolimus. 3. Relapsed malignancy at time of accrual after the most recent transplantation. 4. A difference in dryness between both eyes of more than 2 points of the grading provided by the International Dry Eye Workshop (DEWS) 2007 report.[1] 5. Patients who are pregnant or plan to become pregnant while participating in the study. 6. Patients who are not willing to discontinue the use of any eye drops, with the exception of non-medicated lubricant eye drops (artificial tears). All eye drops (excluding non-medicated lubricant eye drops) must be stopped at least seven days before treatment with pAF. 7. Inability to comply with the investigational plan and visit schedule for any reason, in the judgement of the investigator.

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Study Product, Dose, Route, Regimen	pAF will be given as one drop in 1 eye and saline (placebo) will be given in the other eye. pAF and placebo will be used in each eye twice daily, for a total of 30 days. In patients wearing bilateral scleral lenses, the drops will be applied prior to lens insertion in the morning and immediately after removal at night
Statistical Methodology	This is randomized, double-blinded, placebo-controlled study. The clinical effects of pAF will be analyzed using descriptive statistics, Wilcoxon rank sum, and Fisher's exact test where applicable. Test for a significant response will be carried out using McNemar's test. The proportion of subjects who experience adverse events and serious adverse events will be summarized descriptively; no inferential tests will be performed.

2 Objectives

Primary Objectives:

1. To determine the safety of pAF in patients with chronic ocular GVHD.
2. To determine the clinical effects of pAF in patients with chronic ocular GVHD.

Primary Endpoint:

1. Overall response (Complete Response & Partial Response) as defined in section 5.

Secondary Objectives and Endpoints:

1. To determine the change in National Institutes of Health (NIH) Consensus Criteria (CC) ocular score of chronic GVHD at 30±3 days, 60±3 days, and 100±3 days from baseline related to the administration of pAF
2. To determine change in FACT G (QOL) at 30±3 days, 60±3 days, and 100±3 days related to the administration of pAF.
3. To determine changes in visual acuity related to the administration of pAF.
4. To determine the effects of pAF on the corneal surface.
5. To determine the changes in dry eye symptoms related to the administration of pAF using the grading provided by the International Dry Eye Workshop (DEWS) 2007 report.[1]
6. To determine the changes in patient reported pain level related to the administration of pAF using the 0–10 pain rating scale during the first 60±3 days of the study.

3 Rationale and Background

3.1 Chronic GVHD

Allogeneic (Allo) Hematopoietic Stem Cell Transplantation (HSCT) is a potentially curative treatment modality for patients with hematologic malignancies who would otherwise have a poor outcome with other conventional treatment approaches alone. Allo HSCT causes donor-derived immune responses that can result in the desired graft-versus-tumor effect as well as the undesired complication, Graft versus Host Disease (GVHD). Chronic GVHD is the main complication for long-term survivors of a successful AlloHSCT. Chronic GVHD occurs in more than 50% of all patients who undergo an allo HSCT and the majority of patients who develop acute GVHD as a complication of their allo HSCT. Chronic GVHD can involve multiple organs and require prolonged immunosuppressive therapy. Advanced chronic GVHD is typically manifested by significant fibrous tissue deposition in different organs, leading to disabling symptoms, most commonly because of sicca syndrome and sclerodermatous forms of the disease. Sicca syndrome can lead to severe pain, visual impairment, and mucositis leading to malnutrition. Sclerodermatous chronic GVHD can cause dramatic limitations in the range of motion, with different degrees of immobility, and occasionally restrictive pulmonary disease. At this stage, current treatment strategies are almost always ineffective, at least in part due to the irreversibility of manifestations related to fibrosis. The mainstay of first-line immunosuppressive therapy in patients with chronic GVHD is systemic glucocorticoids and there are no standard second-line therapies. Systemic glucocorticoids have limited efficacy and significant long-term complications. Despite the many alternative immunosuppressive agents to systemic glucocorticoids, no single class of immunosuppressive agents has persistently produced a steroid-sparing effect in patients with chronic GVHD.[2–8] In conclusion, chronic GVHD and its current standard therapy have a major negative impact on the quality of life (QOL) and survival in patients in whom allo HSCT was able to achieve a cure from their original hematologic malignancy. Therefore, there is a desperate need for more effective agents in treating chronic GVHD.

Ocular GVHD in particular affects more than half of the patients within 2 years of the diagnosis of chronic GVHD.[9, 10] The fibroproliferative nature of ocular chronic GVHD makes it irreversible in its advanced stages, and at this point systemic therapies are ineffective. Thus, the management is supportive, through control of surface inflammation, lubrication, control of drainage and evaporation.[11] Despite some advances in the area of supportive care, such as scleral lenses that effectively control evaporation, the problem of ocular chronic GVHD is far from resolved and it is still associated with worse overall health-related quality of life.[10]

3.2 Amniotic fluid (AF): Preclinical Data

Early after conception and until the mother's 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.[12] AF also contains stem cells with the potential to differentiate along multiple cell lineages.[13, 14] 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.[12] A report that concentrates of AF inhibited the development of peritonitis was among some of the first evidence that AF had protective biological properties.[15] This was followed by a publication by Shimberg and co-workers that AF accelerates defense-repair mechanisms within damaged joints.[15, 16] Since these early publications, more sophisticated evaluations have revealed the presence of antimicrobial, immunomodulatory, and growth-

promoting activities in AF.[12] Reports about antimicrobial activity in AF differs[17] 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.[18] Components with antimicrobial, antiviral and antifungal activity that are present in AF include lysozyme, peroxidase, transferrin, -lysin, immunoglobulins and zinc-peptide complexes.[17] 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.[19] While growth promoting activities of AF are supported by animal studies as well as by in vitro studies showing that AF can enhance neochondrogenesis,[20] regenerate peripheral nerves[21] and bone,[22] accelerate re-epithelialization in corneas,[23] and promote healing of human skin wounds.[24] 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,[25] trophic factors that include EGF, IGF-1, FGF, HGF and TGF-a,[26–30] and HA, an important factor in promoting re-epithelialization in human skin wounds.[24]

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 1400×g 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. Some of the peptides encountered and classified according to their function are found on Table 1.

Pro-inflammatory	OPN, PAI-I, CD163, RAGE, IL17, IL1R3
Host defense	IL-27, LAG-3, GITR, PD1
Innate Immunity	hCGb, Galectin-3, TLR-2, Osteoactivin
Antimicrobial	TSP-1, lactoferrin, CXCL14, Trappin-2, CCL-28, MIG
Anti-inflammatory	IL1-ra, MBL
Embryonic development	DKK1, DKK3
Angiogenesis	VEGF R1, Transferring, TIMP-2
Wound healing	OPN, PAPP-A, FAP

Table 1: List of Peptides according to their function

3.3 Processed Amniotic Fluid (pAF): Preliminary Clinical Data

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 NIH CC eye score and decrease in the need for artificial tears and the use of scleral lenses. A second patient had stabilization of the disease after discontinuation of all immunosuppression. The third patient reported no change.

4 Eligibility Criteria

4.1 Inclusion Criteria:

1. Patients diagnosed within 5 years after hematopoietic stem cell transplant for any disease, with any graft and any conditioning regimen with at least one of the following:

   - Dry eye symptoms partially affecting (requiring lubricant drops > 3 × per day, punctal plugs, or thermally cauterized puncta) or significantly affecting (special eyewear to relieve pain) activities of daily living (ADL).
   - Unable to work because of ocular symptoms.
   - Loss of vision due to keratoconjunctivitis sicca (KCS).

2. Patients who are 18 years of age or older.

3. Willing and able to provide informed consent.

4.2 Exclusion Criteria:

1. Patients who have any other reversible cause for dry eye at the time of accrual.

2. More than 3 lines of therapy beyond corticosteroids with or without calcineurin inhibitors or sirolimus.

3. Relapsed malignancy at time of accrual after the most recent transplantation.

4. A difference in dryness between both eyes of more than 2 points of the grading provided by the International Dry Eye Workshop (DEWS) 2007 report.[1]

5. Patients who are pregnant or plan to become pregnant while participating in the study.

6. Patients who are not willing to discontinue the use of any eye drops, with the exception of non-medicated lubricant eye drops (artificial tears). All eye drops (excluding non-medicated lubricant eye drops) must be stopped at least seven days before treatment with pAF.

7. Inability to comply with the investigational plan and visit schedule for any reason, in the judgement of the investigator.

5 Study Design and Procedures

This is a randomized, double-blinded, placebo-controlled study of the efficacy of pAF in patients with hematologic malignancies who have undergone Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) and are diagnosed with chronic GVHD of the eye. Patients will be allowed to continue the use of non-medicated lubricant eye drops (artificial tears) for ocular GVHD treatment prior to participating in this study. Patients are allowed to resume the use of eye drops after the 30 day treatment period at the discretion of the treating physician.

5.1 Dosage

pAF will be given as one drop (50μL) in one eye twice daily and saline (placebo) will be given as one drop in the second eye twice daily for a total of 30 days. Patients will be instructed to treat their eyes once in the morning and once in the evening. In patients wearing bilateral scleral lenses, the drops will be applied prior to lens insertion in the morning and immediately after removal at night.

The assignment of which eye gets which drop will be randomly selected. Neither the patient nor their doctors will know which eye is receiving AF and which eye is receiving placebo. The eyedropper bottles containing pAF and placebo (i.e. physiological saline) will be packaged the same.

5.2 Patient Study Calendar

	Screening/ Baseline	Day 30±3 days[6]	Day 60±3 days[6]	Day 100±3 days[6]	Early Discontinuation of Study Drug
Informed Consent	×				
Eligibility	×				
Medical History	×				
Concomitant meds	×	×	×	×	×
Randomization	×				
Dispense Drug	×				
Physical Exam	×	×	×	×	×
Drug Compliance		×			
FACT G (QOL)	×	×	×	×	×
DEWS assessment	×	×	×	×	×
NIH CC Response Assessment for Chronic GVHD[1,3]	×	×	×	×	×
Complete Eye Exam[2,3]	×				
Urine Pregnancy Test[4]	×				
FU Eye Exam[2]		×	×	×	×
Adverse Events		×	×	×	×
Review Study Diary[5]		×	×		×

[1] Health Care Provider Global Rating, Eye Score, Patient Global Rating

[2] Including DEWS assessment by ophthalmologic assessment and visual acuity

[3] The NIH CC Response Assessment for Chronic GVHD and the Complete Eye exam can be performed within seven days of each other.

[4] Pregnancy test is required if participant is of childbearing potential.

[5] The study team may call the participant before the 30-day visit to address diary questions and completion.

[6] Follow-up visits may be conducted via phone call if in-person visits cannot be completed.

Table 2: Schedule of Events

5.3 Drug Dispensation and Randomization

Upon acceptance into the study, the CTRM facility will be notified and the patient will be randomized as to which eye will receive the drug and which will receive the placebo. Randomization will be done using the MS Excel randomization method. Based on the results of the randomization selection, CTRM will then select appropriate coded boxes. Four boxes containing 8-eyedropper bottles per box will be labeled with a yellow dot and stamped with an “R” for right eye. Each eyedropper bottle is labeled with a yellow dot and stamped with an “R”. Four boxes containing 8-eyedropper bottles per box will be labeled with a blue dot and stamped with an “L” for left eye. Each eyedropper bottle is labeled with a blue dot and stamped with an “L”. The boxes will be labeled as Amniotic Fluid Eye Drops, each set (right and left) will contain a total of 32 eyedropper bottles of pAF and 32 eyedropper bottles of placebo.

The 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 a single right eye and left eye, eyedropper bottle from the freezer. They will verify that the lot number on the eyedropper bottles match the labels on the box it was removed from.

2. They will thaw the frozen fluid in the eyedropper bottles 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 eye dropper bottle cap from the eyedropper bottle labeled right. Invert bottle over the right eye and squeeze the bottle to dispense 1 drop into the right eye. They will then tightly cap the eyedropper bottle.

4. They will then remove the eye dropper bottle cap from the eyedropper bottle labeled left and invert the bottle over the left eye and squeeze the bottle to dispense 1 drop into the left eye. They will then tightly cap the eyedropper bottle.

5. They will refrigerate both bottles containing remaining pAF at temperatures between 1 °C and 10 °C (e.g., refrigerator). They will not re-freeze the fluid in the bottles

6. For the 2nd daily application, they will remove the same eyedropper bottles that they used in the morning from the refrigerator and repeat steps 7 & 8.

7. After the second application, they will place both eyedropper bottle into a box that was provided to them for the placement of bottles that had been used.

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

9. After the 30 day treatment the patient will return all used and unused bottles to the clinic.

The patients will be instructed to return all used and unused vials along with their diary. This will allow us to account for drug dispensed and monitor compliance.

5.4 Concomitant Medications and Procedures

Only medications with ocular effects will be recorded at screening and at subsequent study visits. These medications will be recorded in the medical record and appropriate CRF.

Patients may use non-medicated saline eye drops (artificial tears), provided they are applied in both eyes greater than 30 minutes following the application of the study eye drops.

All other eye drops, other than non-medicated saline eye drops, are prohibited while the patient is on study. If the patient is on medicated eye drops, a seven day washout is required before starting treatment. Patients can enroll if they have bilateral punctal plugs or if puncta were previously thermally cauterized, but the insertion of punctal plugs or cauterization is not allowed while the subject is on study.

5.5 Assessment of Response:

Assessment of response will be performed at 30±3, 60±3 and 100±3 days after initiation of therapy with pAF. The response is a combination of the NIH CC assessments and the ophthalmologic assessment (DEWS evaluation) as follows:

- NIH Consensus Conference (CC) for assessment of response in chronic GVHD
- Ophthalmologic assessment: Dry eye assessment will be performed over the course of four eye exams, including a baseline comprehensive eye exam (including a dilated fundus examination), and three follow-up exams (without dilation) on Day 30±3, Day 60±3 and Day 100±3. Signs and symptoms of dry eye will be assessed at each exam using the grading scale provided by the International Dry Eye Workshop (DEWS) 2007 report.[1] DEWS assessment will include visual symptoms, severity and frequency of discomfort, conjunctival injection, conjunctival staining (using NaFl), corneal staining (using NaFl), corneal/tear signs (including filamentary keratitis and debris), the eyelids and meibomian glands, and fluorescein tear break-up time (TFBUT). Each sign/symptom will be graded on a scale of 1 to 4, with 4 being most severe. A total score will be taken at each exam, and a cumulative decrease in overall score >2 will constitute improvement with treatment. Note: Schirmer testing will not be performed or graded, as it has no proven value in ocular chronic GVHD.

Responders would be defined as a one point improvement in the dry eye grading scale (DEWS), without worsening in the eye score (NIH Consensus Criteria Ocular Assessment).

5.6 Assessment of Secondary Endpoints:

This includes the measurement of QOL with the FACT-G tool, patient reported pain assessment and measurement of visual acuity by ophthalmology.

5.7 Assessment of Safety:

Safety and tolerability will be evaluated by the investigator from the results of reported signs and symptoms, scheduled physical examinations, vital sign measurements, and clinical laboratory test results done as standard of care at day 30±3, day 60±3 and day 100±3 after initiation of pAF therapy. More frequent safety evaluations may be performed if clinically indicated or at the discretion of the investigator. SAEs will be reported within 24 hours of discovery to the central Data Coordinating Center.

Toxicity will be reported using Common Terminology Criteria for Adverse Events (CTCAE) version 4. Only ocular AEs will be collected.

An AE may consist of the following:

1. A new event which was not pre-existing at initial study drug administration.
2. A pre-existing event which recurs with increased intensity or increased frequency subsequent to study drug administration.

3. An event which is present at the time of study drug administration which is exacerbated following initial study drug administration.

A Serious Adverse Event (SAE) is defined by FDA and NCI as any adverse drug event (experience) occurring at any dose that in the opinion of either the investigator or sponsor results in any of the following outcomes:

1. Death
2. Life-threatening adverse drug experience
3. Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours)
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital anomaly/birth defect
6. Important Medical Event (IME) that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, it may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

IRB Notification: Events meeting the University of Utah IRB reporting requirements (http://www.research.utah.edu/irb/) will be submitted through the IRB's electronic reporting system within 10 working days.

Reporting will be performed according to FDA guidance (https://www.fda.gov/downloads/regulatoryinformation/guidances/ucm126572.pdf)

5.8 Discontinuation of Study Drug:

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

- Significant worsening of ocular chronic GVHD during the treatment period. This decision will be at the discretion of both the treating transplant physician and ophthalmologist.
- Study drug can be discontinued by the study participant prior to the Day 30±3 clinic visit, in which case the Early Discontinuation of Study Drug visit will occur.
- Discovery of new information which makes the subject ineligible to continue participation in the study.
- Any other condition that in the opinion of the treating physician and ophthalmologist are not compatible with participation in this study.
- A subject may withdraw their consent at any time without affect to their care.

5.9 Discontinuation of Study:

Significant worsening of ocular chronic GVHD in the eye treated with amniotic fluid over a period of 30 days, in >8 patients.

- The determination of the number of patients meeting this criteria will be performed by 1 designated person in CTRM, who will be the only one unblinded. Thus, the blind will not be broken throughout the study.

6 Statistical Considerations

Analysis of the primary endpoint will be coded as each having responded or not responded at the 30 day visit. Because a participant's eyes are more likely to be similar compared to eyes from two different participants we will conduct a paired analysis using McNemar's test. Assuming a significance level of 0.05, and a response rate in the control eye of 5%, 15 participants will provide 80% power to detect a difference if the response in the treated eye is 60%. With 19 participants we will have 80% power to detect a response rate of 50%. Due to the small sample size it is likely an exact p-value will be calculated.

The impact of different covariates on ORR will be analyzed using descriptive statistics, Wilcoxon rank sum test, and Fisher's exact test where applicable. Differences in NIH Eye and DEWS scores will be analyzed using Wilcoxon signed-rank test.

The proportion of subjects who experience adverse events and serious adverse events will be summarized descriptively; no inferential tests will be performed.

7 Data Management

7.1 Clinical Site Data Management

The Data Coordinating Center will create the electronic data capture (EDC) system that for data entry by clinical site research coordinators and investigators. Data will be entered via the web into the EDC. Worksheets and study documents will be maintained in locked filing cabinets in locked offices at the site.

7.2 Electronic Data Capture System

The Data Coordinating Center currently uses OpenClinica or REDCap as its data capture systems; this may be changed at any time without requiring a protocol amendment.

7.3 Study Monitoring

The investigators recognize the importance of ensuring data of excellent quality. Site monitoring is critical to this process. We will utilize this process to ensure excellent quality data in the proposed study. Our site

monitoring plan is designed to identify problems with sites and methods for handling problems that arise. Site monitors must be provided with full 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 site monitor's review of data in the electronic medical record.

7.3.1 Site Monitoring Plan

A supplemental study-specific monitoring plan, separate from the protocol will be completed which outlines specific criteria for monitoring. This plan will include the number of planned site visits, criteria for focused visits, or additional visits, a plan for chart review and a follow up plan for non-compliant sites. 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. Remote site monitoring schedules will be determined by the Data Coordinating Center in coordination with the study principal investigators.

7.3.2 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 interim visits.

8 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 Rittal's 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 center's 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 Dell's 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 week's 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.

8.1 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.

8.2 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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