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	Title: CLINICAL PROTOCOL: Safety of Topical Mesenchymal Stromal Cell Derived Secretome for Ocular Surface Disease
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Safety of Topical Mesenchymal Stromal Cell Secretome for Ocular Surface Disease

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonization Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

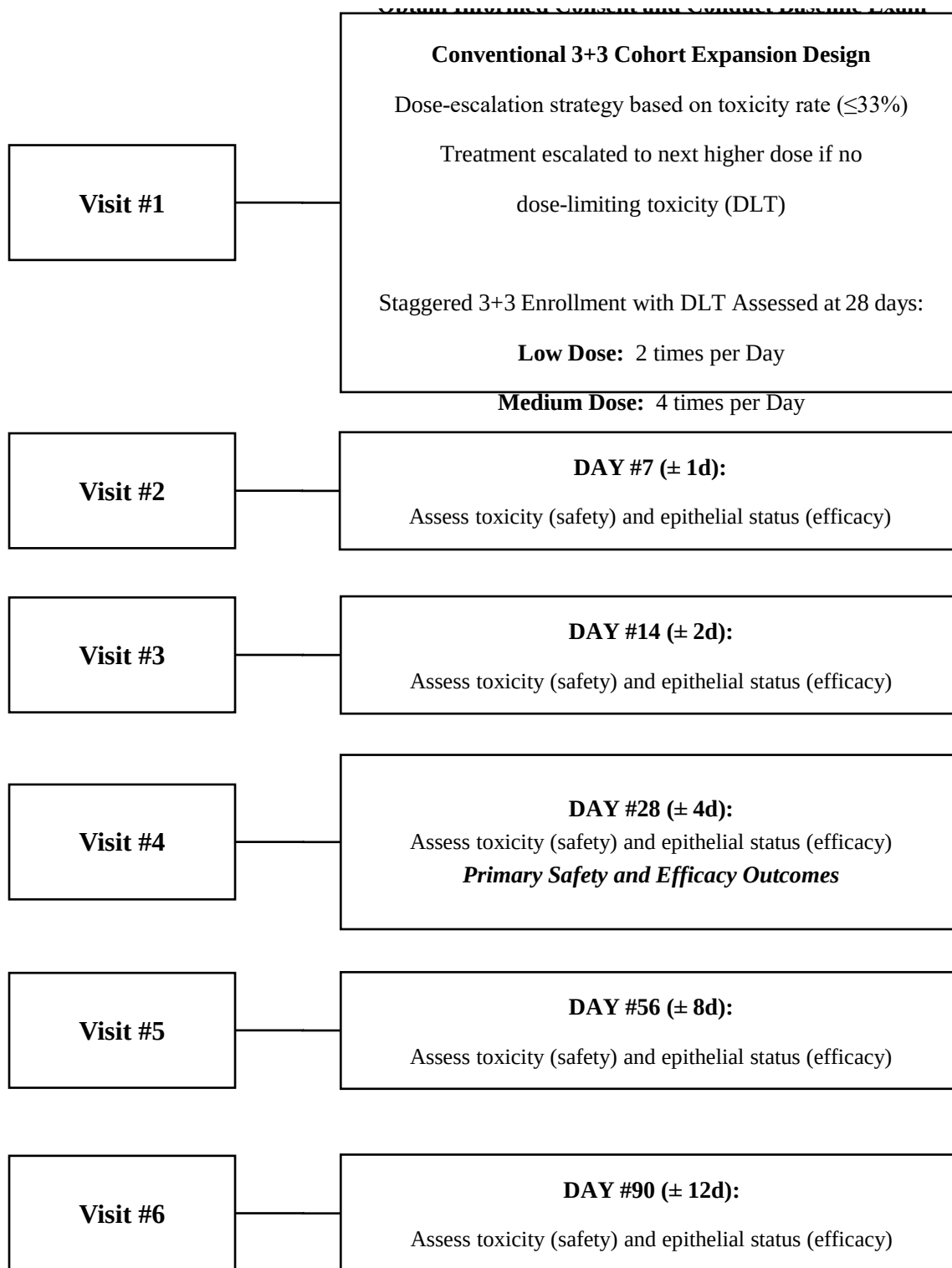
Investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of this trial will be required to complete Human Participants Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the University of Illinois at Chicago (UIC) Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is screened or enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent using a previously approved consent form.

1. Protocol Summary

1.1. Synopsis

Title:	Safety of Topical Mesenchymal Stromal Cell (MSC) Derived Secretome for Ocular Surface Disease
Study Description:	To evaluate the safety and maximal tolerated dose (MTD) of topical MSC Secretome (eye drops in patients with chronic ocular surface disease with neurotrophic keratitis through a dose-escalation study under a 28-day topical application protocol. <u>The primary purpose of this research is to determine safety and the maximum tolerated dose (MTD); efficacy is not the primary aim.</u>
Objectives:	Determine the dose of MSC Secretome through a first-in-human study through a dose-escalation strategy targeting a toxicity rate of 33% or less.
Phase:	1
Study Design:	Phase 1, 3 + 3 Conventional Cohort Expansion Study
Endpoints:	<u>Primary Safety:</u> Incidence of treatment emergent adverse events (TEAE) assessed at 28 days following treatment initiation (participants will receive continued monitoring through 90 days). <u>Primary Efficacy:</u> Proportion of patients with improved corneal epithelial barrier at 28 days compared to baseline - defined as $\geq 50\%$ reduction in corneal fluorescein staining score using National Eye Institute (NEI) grading scale as measured by the investigator via slit lamp examination. <u>Secondary Endpoints:</u> Other associated measures to assess treatment effect relative to baseline such as visual acuity, corneal epithelial thickness and corneal stromal haze as measured by anterior segment Optical Coherence Tomography (OCT)/Scheimpflug Imaging, and corneal sensation as measured by esthesiometry; and treatment tolerability measured using a visual analogue scale (VAS).
Study Population:	Participants with chronic stable ocular surface disease unresponsive to standard medical therapies with evidence of impaired corneal epithelial barrier (fluorescein staining score ≥ 6 by NEI grading scale) AND neurotrophic keratitis as evident by reduced corneal sensation (≤ 4 cm measured by Cochet Bonnet esthesiometry in at least one corneal quadrant).
Facility Enrolling Participants:	Department of Ophthalmology and Visual Sciences at the University of Illinois at Chicago, Chicago, IL.
Description of Study Intervention:	Bone marrow derived MSCs isolated from a healthy donor will be used to produce MSC Secretome under GMP conditions at the University of Illinois Hospital Clinical Stem Cell Laboratory. Frozen vials of MSC Secretome will be thawed and dispensed in eye drop bottles by the University of Illinois Eye and Ear Infirmary Pharmacy for topical application by the participant. Escalating doses of MSC secretome will be assigned (e.g., 2 times a day, 4 times a day, 6 times a day) under a 28-day topical application protocol.
Study Duration	90 days

1.1. Schema of Phase I Study: Conventional 3+3 Cohort Expansion Study**Figure 1. Overview of Phase I Protocol**

1.2. Schedule of Activities

Table 1 displays scheduled Phase I measurements per study visit. Outcome measures include assessment of treatment emergent adverse events (TEAE) via slit lamp exam (Primary Safety Outcome; DAY #28), and assessment of corneal epithelial barrier function/integrity as determined by fluorescein staining (Primary Efficacy Outcome; DAY #28). The primary study objective is to assess safety via maximal tolerated dose (MTD).

Table 1. Schedule of Measurements by Study Visit.

Phase I	Baseline Enrollment	DAY #7 (± 1d)	DAY #14 (± 2d)	DAY #28 (± 4d)	DAY #56 (± 8d)	DAY #90 (± 12d)
Assessment of eligibility and Informed consent	X					
Clinical Exam	X	X	X	X	X	X
Medical and ocular history	X					
Ocular discomfort VAS	X	X	X	X	X	X
High contrast visual acuity	X	X	X	X	X	X
Slit lamp exam	X	X	X	X*	X	X
Corneal epithelial fluorescein staining assessment	X	X	X	X†	X	X
Conjunctival lissamine green staining	X	X	X	X	X	X
Corneal sensation measurement	X			X	X	X
Intraocular Pressure Measurement	X		X	X	X	X
Digital Slit Lamp Photography	X		X	X	X	X
Anterior Segment OCT/Scheimpflug Imaging (epithelial thickness, stromal haze)	X		X	X	X	X
Vial Disbursement Form	X	X	X	X		
Subject Adherence Survey		X	X	X		
VAS = visual analogue scale	X = measured *PRIMARY SAFETY OUTCOME MEASURE, 28 days †PRIMARY EFFICACY OUTCOME MEASURE, 28 days					

2. Introduction

2.1. Background

2.1.1. Significant Unmet Need in Patients with Corneal Epithelial Disease

Corneal epithelial impairment in the setting of severe ocular surface disease represents an unmet clinical need. These include conditions where the corneal epithelium is unable to heal and/or is chronically impaired. This can occur in many settings such as chronic neurotrophic states; severe inflammatory surface diseases such as Sjogren's syndrome and graft versus host disease; and corneas with insufficient limbal epithelial stem cells (e.g. severe chemical injuries, or autoimmune diseases). Neurotrophic keratopathy (reduced corneal sensory nerves) is a common feature of many of these ocular surface diseases and can occur in many settings such as after herpetic/microbial keratitis, limbal stem cell deficiency, chemical injuries, and post-surgical (e.g. keratoplasty, LASIK).

The current standard of care for these patients with chronic epithelial disease include: lubrication, anti-inflammatories, contact lenses, autologous serum/plasma drops, amniotic membrane, and tarsorrhaphy.(1) (**Figure 2**). Yet there is still significant unmet need for more effective therapies to prevent pathologic responses including ulcerations, scarring, and neovascularization.

The proposed Phase 1 study involves the use of bone marrow derived Mesenchymal Stromal Cell (MSC) secretome. Specifically, the secreted factors from MSCs will be used as topical eye drop. As described later in the application, we plan to test the safety of MSC secretome in patients with chronic corneal epithelial disease.

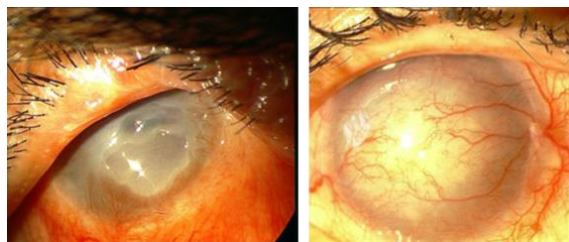


Figure 2. Right: Ulceration due to non-healing wound after severe chemical injury. Left: Significant scarring and neovascularization after severe chemical injury.

2.1.2. Rationale for using MSC Based Therapy

MSCs are found in most adult tissues, including bone marrow, fat and cornea/limbus, and play an important role in tissue repair and maintenance. Over the past decade, they have been increasingly investigated for their therapeutic potential in a wide range of human diseases given their significant regenerative, immunomodulatory and anti-scarring effects.¹⁻³ Based on our preclinical models and extensive published studies,²⁻¹¹ application of MSCs or their secreted factors can significantly promote corneal epithelial health and repair.

MSCs have a great safety track record. They have been, or are, under investigation in multiple clinical trials for promoting tissue repair and modulating immune responses (>100 clinical trials registered in the U.S.).³ To date none of the trials have reported any adverse effects related to the MSCs following systemic or local administration. Our group has recently begun a Phase 1 study to assess the safety of cryopreserved allogeneic bone marrow derived MSCs administered by subconjunctival injection (IND 18400).

Numerous studies have shown that the therapeutic effects of MSCs are mainly mediated through their secreted factors¹²⁻¹⁶. Our laboratory studies on wound healing have enabled us to optimize a treatment strategy based on MSC secreted factors (aka secretome) to promote epithelial repair, limit excessive inflammation and prevent secondary scarring in pre-clinical models.^{17 12,18}. We have specifically showed that exosomes derived from corneal MSCs are an important mediator of the secretome and can promote

corneal wound healing¹⁹. This proposed clinical trial will address the gap between these extensive laboratory studies with promising results and the clinical application of MSC secreted factors.

Secreted factors present considerable advantages over cells regarding manufacturing, storage, handling, product shelf life and their potential as a ready-to-go biologic product²⁰. Moreover, considering the fact that there are no live cells involved in this approach, there are no concerns about immunologic responses (e.g. rejection) and possible abnormal proliferation / neoplastic transformation.

We propose a Phase 1 study to evaluate the safety, efficacy and tolerability of topical allogeneic bone marrow-derived MSC Secretome in patients with corneal epithelial disease.

2.1.3. MSC Tissue Source

MSCs have been isolated from a number of tissues, all of which possess similar (not identical) immune modulating and tissue-repair-promoting effects. We propose to use bone marrow-derived MSCs isolated from healthy donors who were screened prior to donation for any and all reasonable medical diseases,²¹ which are considered a reliable source for scale-up of MSCs. Such cells have been used in numerous studies and through various routes (intravenous to intramyocardial, as examples) and have the longest and best safety/efficacy track record in U.S. clinical trials.

2.1.4. Production of MSC Secretome

The source of MSCs, the reagents and the facility is the same as described in our ongoing MSC study (IND 18400). Briefly, the Master Cell Bank and Working Cell Bank of human bone marrow derived allogeneic MSCs that will be used for the production of secretome are from Rooster Bio, Inc (DMF 18458). Expansion of the MSCs will be carried out in the cleanroom facility at the University of Illinois Hospital Clinical Stem Cell Laboratory under the direction of Dr. Nadim Mahmud exactly as described in the CMC section for IND 18400 from our group. The laboratory is an integral component of the University of Illinois Blood & Marrow Stem Cell Transplant Program. The Stem Cell Laboratory has been accredited by the Foundation for the Accreditation of Cellular Therapy (FACT) continuously since 2001 and is also registered with the FDA as a good tissue practice (GTP) facility. The laboratory and the Cell Processing Laboratory is located within the hospital which consists of approximately 1,775 sq ft total area including a classified clean room (Class 10,000 / ISO 7), a main graft processing laboratory area (Class 100,000 / ISO 8) and office spaces. Further detail is provided in the CMC section of IND 18400.

Following the expansion of MSCs, the cell culture media will be switched to a basal medium. After 72 hours the media will be collected as the MSC secretome product. The product will be aliquoted in sterile vials and stored at < -80 °C. The product will undergo extensive lot release testing before it is ready for clinical use.

2.1.5. Dispensing and Application of the MSC Secretome Product

Once a patient is enrolled in the study, a frozen vial of MSC Secretome Product will be thawed by University of Illinois Eye and Ear Infirmary (EEI) Pharmacy which has extensive experience compounding investigational eye medications. The MSC Secretome will be transferred to an eye dropper bottle which will be dispensed to the patient on ice packs (Following an SOP for dispensing compounded medications). The patient will take the bottle home and refrigerate after each application. The bottle is discarded at maximum 1 week and a new bottle is started.

2.2. Risk/Benefit Assessment

2.2.1. Known Potential Risks

Use of any human tissue in the production process represents a small risk of infectious transmission. To mitigate this risk, the bone marrow-derived MSCs have been harvested from a healthy donor who has been extensively screened and tested for infectious, malignant, neurological and auto-immune diseases, as well other pertinent exposures, and social habits. Further, MSCs will be produced at the University of Illinois Hospital clean room facility under direction of Co-Investigator Dr. Nadim Mahmood. The cells and the secretome will undergo extensive sterility testing as outlined in the CMC. To date, there have been no reported serious adverse event (AEs) related to the use of MSCs or secretome.

An additional known risk is related to the contamination of the product after dispensing. Specifically, since the product is preservative free and contains growth media, there is potential for contamination by microbial agents if not handled properly. To mitigate this risk, the MSC secretome product will be kept refrigerated at all times (except during application) and will be discarded after 7 days. This is the same protocol used for preservative free autologous serum eye drops which are dispensed by the University of Illinois EEI pharmacy on a regular basis. To date, the EEI pharmacy has dispensed autologous serum eye drops several thousand times and there have been no reports of corneal infections that have been traced to contamination of the compounded serum eye drops.

A potential risk is an ocular surface inflammatory/allergic reaction to the MSC Secretome drops with secondary worsening of the corneal disease; however, the risk is expected to be very low given the safety data from preclinical studies. These and other unknown potential adverse reactions are described in more detail below under the section on Adverse Events.

Participants may experience no improvement in their corneal epithelial disease as a result of this research. Impaired corneal epithelium over time can lead to corneal scarring, additional adverse corneal sequelae (e.g., ulcers, infection), and decreased vision.

2.2.2. Potential Benefits

On an individual level, taking part in this study may improve the participant's epithelial disease by improving the epithelial barrier, integrity, and/or phenotype. This could limit destructive visual sequelae including both relatively common and less severe negative outcomes (e.g., increased infection risk, scarring, decreased visual acuity), as well as less frequent but severe outcomes (e.g., profound loss of vision or eye).

At the societal level, information from this study may help investigators learn more about chronic epithelial disease including non-healing epithelial defects, as well as their treatment. It may also aid in identifying which types of surface diseases are more likely to benefit from use of MSC secretome.

2.2.3. Assessment of Potential Risks and Benefits

The associated risks are essentially limited to a risk of transmission of infectious agents and risk of contamination of the secretome product after dispensing. The risks during the production process are minimized by strict adherence to cGMP (current Good Manufacturing Practice) guidelines and quality control testing of the final product prior to release. Furthermore, participants included in this clinical trial will be screening on the basis of their ability to strictly adhere to the study visits and proper handling of the MSC secretome product. Patients with active corneal infections or severe corneal melts

(at high risk for further melting and perforation) will be excluded during this phase of the study. Medical follow-up required by this protocol is in line with current best practice and standard-of-care supportive therapies.

3. Outcomes and Endpoints

3.1. Study Primary Objective

To evaluate the safety and MTD of MSC Secretome in chronic ocular surface disease through a dose-escalation study (Phase I Study).

Table 2A describes Phase I Outcomes and Endpoints, with justification for each endpoint. The MTD will be assessed, defined as the highest dose at which zero to one of six patients experienced a dose-limiting toxicity (DLT).

DLT will be defined according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE v5) adverse event reference. Any ocular or systemic treatment emergent adverse event (TEAE), with a Grade 2 toxicity without resolution after 14 days or a Grade 3 or higher toxicity will be considered a DLT, unless it can clearly be attributed to another cause.

If a DLT occurs in two patients in a dosing cohort, then the MTD is considered exceeded and the dose will not be escalated.

DLT Escalation Rules are described in detail in **Section 4.3 Phase I Dose-Limiting Toxicity Escalation Rules (also see Table 3)**.

Table 2A. Phase I Outcomes and Endpoints

OUTCOMES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary Safety		
Ocular Safety	The MTD will be assessed, defined as the highest dose at which 0 to 1 of 6 patients experienced a DLT.	Clinically meaningful morbidities associated with non-healing corneal wounds may be lessened with MSC Secretome
General Safety	DLT will be defined according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE v5) adverse event reference. Any non-corneal, ocular or systemic treatment emergent adverse event (TEAE), with a Grade 2 toxicity without resolution after 14 days or a Grade 3 or higher toxicity will be considered a DLT, unless it can clearly be attributed to another cause. If a DLT occurs in 2 patients in a dosing cohort, then the MTD is considered exceeded, and the dose will not be escalated. Participants in a cohort will be required to complete DAY 28 assessment before dose escalation (i.e., staggered enrollment). A staggering interval of two days between each participant will be included within a cohort to avoid simultaneous exposure to unknown risks.	Occurrence of Grade 2 or higher TEAE is an accepted endpoint for safety assessment
Primary Efficacy		
Corneal Epithelial Barrier Function and Integrity	Change in corneal fluorescein staining score from baseline to DAY 28 as determined by the investigator on slit lamp examination. Presence/absence of an epithelial defect at DAY 28 will also be recorded.	Epithelial integrity and barrier function is a measure of epithelial health and is necessary for good visual acuity; therefore, it is a clinically meaningful outcome
Secondary Endpoints		
Visual Acuity	Change in best corrected distance visual acuity from baseline to DAY 28	Visual acuity is a clinical measure of corneal health
Corneal Epithelial Thickness	Percent change in corneal epithelial thickness from baseline to baseline on DAY 28 as measured by anterior segment OCT (AS-OCT)	Corneal epithelial thickness provides an objective measure of corneal epithelial health.
Corneal Stromal Haze	Percent change in corneal stromal haze from baseline to DAY 28 as imaged by AS-OCT / Scheimpflug Imaging. OCT images analyzed by Image-J/Metamorph software	Corneal stromal haze evaluation provides an objective measure of stromal clarity/scarring
Tolerability	Drop discomfort measured using a VAS scale 0 – 100, where 0 is no discomfort and 100 the worst discomfort. Measured on DAYS 7, 14, and 28.	Provides a measure of tolerability

DLT Dose-limiting toxicity; MTD Maximal tolerated dose; TEAE Treatment Emergent Adverse Event.

4. Study Design

4.1. Overview

The primary goal of the ***Safety Study of Topical MSC Secretome for Corneal Epithelial Disease***, otherwise known as the “***MSC Secretome Study***,” is to assess the safety of topical application of bone marrow-derived MSC Secretome and to assess potential efficacy in promoting corneal epithelial barrier function/integrity; with the objective of improving clinical outcomes in patients with corneal epithelial disease.

4.2. Phase I Study Design

The MSC Secretome Study is a 28-day Phase 1, 3 + 3 Conventional Cohort Expansion Study dose-escalation study designed to evaluate the safety and MTD as per FDA guidelines²² with MSC Secretome in chronic ocular surface disease. The overarching MSC Secretome Study Design parallels important FDA guidelines “Consideration for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products, Guidance for the Industry,”²² with dose-exploration studies identifying the MTD early in development. Participants will receive the MSC secretome treatment for 28 days, and will be followed for a total of 90 days.

4.3. Phase I Dose-Limiting Toxicity Escalation Rules

DLT Escalation Rules for a Phase I Cohort Expansion Study adapted from Ivy et al²³ guide the DLT Escalation Rules that will be employed in this study (**Table 3**). DLT will be defined according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE v5) adverse event reference. Any ocular or systemic treatment emergent adverse event (TEAE), with a Grade 2 toxicity without resolution after 14 days or a Grade 3 or higher toxicity will be considered a DLT, unless it can clearly be attributed to another cause. Table 2B provides a list of possible toxicities for grade 2-4 (other toxicities not listed will be evaluated and graded based on the effect on visual acuity and need for intervention)

Final DLT outcomes will be assessed at DAY #28 (if not having previously met the 14-day rule), and all participants will be followed for a total of 90 days.

If a DLT occurs in 2 patients in a dosing cohort, then the MTD is considered to have been exceeded and the dose will not be escalated. DLT will be assessed at DAY #28, and cohort enrollment of participants at the next dose level will be staggered pending DLT assessment at each given dose. A staggering interval of two days between each participant will be included within a cohort to avoid simultaneous exposure to unknown risks. Descriptive statistics will assess safety to determine the MTD and the Recommended Phase 2 Dose (RP2D; toxicity <33%). The MTD will be defined as the highest dose at which 0 to 1 of 6 patients experienced a DLT.

Table 2B: Definitions and Examples of Toxicity Grades 2-4 for DLT Determination

Grade 2: vision loss of 3 lines or less or not requiring invasive intervention
- worsening epithelial disease: increased staining by >50%, epithelial defect increase by > 4.0 mm, relative to baseline - new or worsening inflammation: conjunctivitis, scleritis, or uveitis 1-2+ cells in anterior chamber (regardless of visual acuity) - new or worsening corneal stromal disease: increased haze > 50% from baseline, stromal loss 10-30% from baseline
Grade 3: loss of vision by more than 3 lines up to 20/200 or requiring invasive intervention
- new mild to moderate infectious/inflammatory keratitis with vision loss up to 20/200 - new corneal ulceration with > 30% stromal loss from baseline - new uveitis with 3-4+ cells in anterior chamber
Grade 4: loss of vision by > 3 lines worse than 20/200 or requiring emergent invasive intervention
- new severe infectious/inflammatory keratitis - corneal perforation - Anaphylactic reaction, New Stevens-Johnson syndrome

Table 3. Phase I Cohort Expansion Study: Dose-Limiting Toxicity (DLT) Escalation Rules. *

Number of Participants with DLT at a Given Dose	Conventional 3+3 Cohort Expansion Design Study Escalation Decision Rule
0 of 3 participants	Enter 3 participants at the next dose level
1 of 3 participants	Enter at least 3 more participants at this dose level • If 0 of 3 experience DLT, proceed to next dose level • If ≥ 1 experience DLT, then dose escalation is stopped, and this dose is declared MTD • 3 additional participants will be entered at the next lowest dose level if only 3 participants were treated at that dose
≤ 1 of 6 participants at highest dose level below the maximally administered dose	Considered the maximum tolerated dose (MTD) and generally RP2D (toxicity <33%) At least 6 participants must be entered at the RP2D

*Adapted from Ivy et al²³

DLT Dose-limiting toxicity; MTD Maximal tolerated dose; RP2D Recommended Phase 2 Dose

4.4. Justification for Phase I Sample Size

A 3 + 3 Conventional Cohort Expansion Study dose-escalation study design²³ will be used in this Phase I study. As such, a minimum of 3 and a maximum of 18 participants (pending DLT results and Escalation Rules) will be enrolled to determine the MTD. Three doses will be evaluated, including a low-dose treatment (topical application two times per day); medium-dose treatment (topical application four times per day); and high-dose treatment (topical application six times per day).

4.5. Phase I Treatment Description

The MSC Secretome eye drop will be dispensed by the pharmacy in one-week supply bottles without preservatives, and as such need to be kept refrigerated between applications. Standard-of-care supportive therapy will be continued in all participants (e.g., topical antibiotics, lubrication, steroids). Treatment dosages will be assigned as outlined according to dose-escalation rules.

4.6. Justification for Phase I Treatment Dose

The MSC Secretome dose frequencies selected were determined based on preclinical rodent and rabbit models, in which a dose-response effect was seen where increasing doses produced an increased treatment response. No toxicity was evident in preclinical models, suggesting that traditional starting doses appropriate in oncology clinical trials (e.g., one tenth lethal dose for mice²⁴) may not be appropriate, especially as manufacturing capabilities limit dosing to such high potential levels.

Clinical treatment dose escalation follows an intermediate between a true and modified Fibonacci dose-escalation scheme given 1) the lack of toxicity seen in preclinical models; 2) likely biological (e.g., paracrine) versus toxicologic treatment effects;²⁴ and, 3) safety of MSCs in clinical models for other applications (e.g., intravenous injection). This allows balance between safety and the proportion of participants treated at a sub-therapeutic dose, especially as toxicity is not expected to be substantial in the predicted therapeutic range.²² As the upper limits of treatment dosing described in this clinical trial are limited by manufacturing capabilities and practicalities of participant administration, we expect a primary goal of this trial is identification of MTD.

The treatment duration of 4 weeks was selected as it is expected to provide safety data as well as some indications of efficacy. This will provide guidance on the duration of therapy for a future phase 1b study.

4.7. Phase I Recruitment Goals and Dose-Escalation Assignment

Approximately 9-18 participants will be recruited from UIC and assigned according to a conventional 3+3 cohort expansion study with escalating doses. Treatment will be escalated to the next higher dose administration if no dose limiting toxicity exists (i.e., 1st 3 participants assigned to topical administration at two times per day, next 3 at four times per day if no toxicity; final 3 at six times per day if no toxicity). DLT Escalation Rules adapted from Ivy et al²³ will be employed, as described in **Section 4.3 Phase I Dose-Limiting Toxicity Escalation Rules (Table 3)**.

Dose assignment as described above will be maintained and provided by Dr. Joslin at UIC.

4.8. End of Study Definition

A participant is considered to have completed the study if he or she has completed all visits including the End of Study visit (DAY #90).

4.9 Stopping rules

Grade 2 toxicities listed in Table 3 (DLT definition) that persist beyond 7 days will prompt a stop in dosing of that subject but not a halt in the study enrollment. Grade 3 or 4 toxicities listed will prompt an immediate

halt in the study dosing for that subject as well as halt in study enrollment until the toxicity data can be further studied.

5. Study Population

5.1. Inclusion Criteria

Approximately 3-18 male and female participants of all racial, ethnic, and religious backgrounds will be recruited from UIC without bias. Consecutive patients meeting eligibility criteria will be approached for participation. **Tables 4 and 5** provide specific details regarding Phase I Inclusion and Exclusion Criteria, respectively.

Here, we elect to recruit cases with stable ocular surface disease of moderate severity given that safety/toxicity can be assessed more precisely compared to patients with more severe and unstable disease. Likewise, we have chosen to exclude patients with milder disease since the risks associated with the study cannot be justified in that patient population.²²

Table 4. Phase I Inclusion Criteria.

Age	Patients 18 years of age or older
Ocular Health	- Chronic corneal epithelial disease with fluorescein staining score ≥ 6 by NEI grading scale - Reduced corneal sensation (≤ 4 cm measured by Cochet Bonnet esthesiometry) in at least one corneal quadrant - A stable ocular surface with no objective clinical evidence of significant ($> 50\%$) improvement/worsening of the epithelial disease in the last 30 days - Epithelial disease refractory to conventional non-surgical treatments (e.g., preservative-free artificial tears, gels or ointments; discontinuation of preserved topical drops; anti-inflammatory therapy) - If both eyes of subject meet the inclusion criteria, the eye with the higher fluorescein staining score will be enrolled to the study.
Study Procedures	Only patients who satisfy all Informed Consent requirements may be included in the study. The patient and/or his/her legal representative must read, sign and date the Informed Consent document before any study-related procedures are performed. The Informed Consent form signed by patients and/or legal representative must have been approved by the UIC IRB for the current study. Patients must have the ability and willingness to comply with study procedures.

5.2. Exclusion Criteria

Table 5. Phase I Exclusion Criteria

Ocular Health	- Any active or suspected ocular infection (bacterial, viral, fungal or protozoal). - Evidence of corneal ulceration with stromal loss $> 10\%$, - Presence of an epithelial defect ≥ 1.0 mm in the largest diameter in the study eye - Presence of any size epithelial defect that has been persistent for more than 30 days - Patients unable to discontinue or intermittently remove therapeutic contact lens in the study eye (to apply drops) during the 4-week study period - History of any ocular surgery (including laser or refractive surgical procedures) in the study eye within the 3 months prior to study enrollment.
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	History of chemical injury within the last 6 months prior to study enrollment
Study Procedures	- Known hypersensitivity to one of the components of the study or procedural medications (e.g., fluorescein). - History of drug, medication or alcohol abuse or addiction. - Use of any investigational agent within 4 weeks of screening visit. - History of previous enrollment in the MSC Secretome Study at a lower dose - Participation in another clinical study at the same time as the present study. - Participants who are pregnant at the time of study enrollment.

5.3. Screen Failures

Demographic data for potential participants assessed for eligibility but excluded from the study will be collected in addition to reasons for study exclusion (e.g., did not meet inclusion criteria, declined participation) in order to meet CONSORT (Consolidated Standards of Reporting Trials) reporting guidelines.

5.4. Strategies for Recruitment and Retention

Participants will be recruited from the Cornea Clinic at the UIC Department of Ophthalmology. Eligibility based on severity of corneal injury and other eligibility criteria as described above will be assessed by Dr. Djalilian in the Cornea Service. Participants will receive a \$25 stipend per visit (up to \$175 stipend for participation in all study visits). Research staff and Doctors will use flyers for recruitment of patients. Initial recruitment of patients will be discussed via phone or in person with a detailed study script. In order to make the research questions comprehensible to the study population, a participant flash card will be utilized to aid with the REDCAP questionnaire.

6. Study Intervention Discontinuation and Participant Discontinuation/Withdrawal

6.1. Investigator Termination of Study Participation

An individual's study participation may be terminated by the investigator from the study at any time. Reasons for participant discontinuation from study treatment may include:

- An AE (including illness)
- Protocol violation
- Use of a prohibited concomitant therapy

The Investigator may withdraw a participant from the trial at any time if he/she deems it medically necessary. Participants who discontinue the study will complete the Treatment Discontinuation Visit. Participants who discontinue study treatment without withdrawing their consent will remain in the study to complete the remaining visits.

6.2. Participant Discontinuation/Withdrawal from Study

All participants may withdraw from the trial at any time. REDCap documentation should include the reason for discontinuation. In event of participant withdrawal, the investigator will make every effort to complete the final study visit procedures. All withdrawn participants will be followed until resolution of any AEs or until the unresolved AEs are judged by the investigator to have stabilized.

Reasons for participant withdrawal may include:

- Participant withdrawal of consent
- The Investigator terminates the trial
- Lost to follow-up
- An AE (including illness)
- Protocol violation

All participants with unresolved AEs should be contacted one month after their final visit to determine if more information on the event is available. Even if participants have been removed from the trial or if an AE remains ongoing, Investigators should follow up with participants for 90 days.

6.3. Lost to Follow-Up

If a participant is lost to follow-up, a minimum of three contacts including one certified letter will be attempted and documented in participant records.

7. Study Assessments and Procedures

7.1. Summary Schedule of Study Procedures by Visit and Outcome Measures

Phase I study procedures are presented in **Table 6**. Outcome measures will include slit lamp exam to assess safety via toxicity (primary outcome) with assessment of epithelial staining/integrity (primary efficacy), and anterior segment OCT for change in corneal haze and epithelial thickness (secondary efficacy). Phase I planned descriptive statistics will assess safety and MTD at DAY #28 in addition to any AEs (AEs monitoring continued through DAY #90). The MTD will be assessed, defined as the highest dose at which zero to one of six patients experienced a DLT. DLT will be defined according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE v5) adverse event reference. Any non-corneal, ocular or systemic treatment emergent adverse event (TEAE), with a Grade 2 toxicity without resolution after 14 days or a Grade 3 toxicity will be considered a DLT, unless it can clearly be attributed to another cause.

Table 6. Schedule of Measurements by Study Visit (duplicate of Table 1).

Phase I	Baseline Enrollment	DAY #7 (± 1d)	DAY #14 (± 2d)	DAY #28 (± 4d)	DAY #56 (± 8d)	DAY #90 (± 12d)
Assessment of eligibility and Informed consent	X					
Clinical Exam	X	X	X	X	X	X
Medical and ocular history	X					
Ocular discomfort VAS	X	X	X	X	X	X
High contrast visual acuity	X	X	X	X	X	X
Slit lamp exam	X	X	X	X*	X	X
Corneal epithelial fluorescein staining assessment	X	X	X	X†	X	X
Conjunctival lissamine green staining	X	X	X	X	X	X
Corneal sensation measurement	X			X	X	X
Intraocular pressure measurement	X		X	X	X	X
Digital Slit Lamp Photography	X		X	X	X	X
Anterior Segment OCT/Scheimpflug Imaging (epithelial thickness, stromal haze)	X		X	X	X	X
Vial Disbursement Form	X	X	X	X		
Subject Adherence Survey		X	X	X		
VAS = visual analogue scale	X = measured					

*PRIMARY SAFETY OUTCOME MEASURE, 28 days †PRIMARY EFFICACY OUTCOME MEASURE, 28 days
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7.2. Detailed Study Activities by Visit

7.2.1. Visit #1: Screening, Enrollment and Baseline Exam

Purpose: Patients will be screened for eligibility by assessing all inclusion and exclusion criteria based on existing medical and clinical information, and interview with the patient prior to consenting and enrollment. Eligible patients will be consented and enrolled and the study team will perform baseline assessments.

Each patient who enters study screening will be assigned a participant ID number for traceability. The participant ID will consist of a 2-digit site number and a 3-digit participant number (i.e. 01-001, etc.; as only UIC is participating, all participants will receive the same site number).

Any individual who fails to meet the eligibility criteria is considered a Screen Failure. Screen Failure will have their demographic information and reason for screen failure captured in REDCap. If failure is due to underlying etiology, there will be the opportunity for one re-screen at subsequent patient visits.

All patients will be screened for trial eligibility by completing the following procedures:

- Obtain written informed consent and HIPAA authorization
- Assess all inclusion/exclusion criteria
- Obtain Demographics
- Review Medical and Ocular History
- Assess concomitant medication and therapy
- Record medications currently being taken by the participant
- Determine best uncorrected and corrected visual acuity
- Perform screening slit lamp exam

Among Eligible Participants, the following baseline assessment will be completed:

- All female participants screened for pregnancy as part of REDCap self-reported demographics questionnaire
- Review Concomitant Medications
- Perform additional clinical measurements: corneal sensation measurement, tonometry, digital corneal photography, anterior segment OCT imaging
- Perform slit lamp exam with fluorescein and lissamine green staining
- Assign participant to treatment groups based on dose-escalation treatment assignment
- Provide participant instructions on continued supportive care
- Dispense the Study medication (MSC secretome)
- Vial Disbursement Form

7.2.2. Visit #2: “DAY #7”

Purpose: To assess safety via toxicity and evaluate status of epithelium

- Assess concomitant medication and therapy
- Assess Drop-related discomfort using a visual analogue scale (VAS)
- Visual acuity and perform slit lamp exam, record fluorescein and lissamine green staining
- Assess adverse events
- Provide participant instructions on continued supportive care
- Visit to fall within 6-8 days of baseline/enrollment visit
- Dispense the Study medication (MSC secretome)
- Vial Disbursement Form
- Subject Adherence Survey

7.2.3. Visit #3: “DAY #14”

Purpose: To assess safety via toxicity and evaluate status of epithelium

- Assess concomitant medication and therapy
- Assess Drop-related discomfort using VAS
- Visual acuity and perform slit lamp exam, record fluorescein and lissamine green staining
- Perform additional clinical measurements: corneal sensation measurement, pneumotonometry, digital corneal photography, anterior segment OCT imaging
- Assess adverse events
- Provide participant instructions on continued supportive care
- Visit to fall within 12-16 days of baseline/enrollment visit
- Dispense the Study medication (MSC secretome)
- Vial Disbursement Form
- Subject Adherence Survey

7.2.4. Visit #4: “DAY #28”

Purpose: To assess safety via toxicity and evaluate status of the epithelium

- Assess concomitant medication and therapy
- Assess Drop-related discomfort using VAS
- Visual acuity and perform slit lamp exam, record fluorescein and lissamine green staining
- Perform additional clinical measurements: corneal sensation measurement, tonometry, digital corneal photography, anterior segment OCT imaging
- Assess adverse events
- Provide participant instructions on continued supportive care
- Visit to fall within 24-32 days of baseline/enrollment visit
- Vial Disbursement Form
- Subject Adherence Survey

7.2.5. Visit #5: “DAY #56”

Purpose: To assess safety via toxicity and evaluate status of the epithelium

- Assess concomitant medication and therapy
- Assess discomfort using VAS
- Visual acuity and perform slit lamp exam, record fluorescein and lissamine green staining

- Perform additional clinical measurements: corneal sensation measurement, tonometry, digital corneal photography, anterior segment OCT imaging
- Assess adverse events
- Provide participant instructions on continued supportive care
- Visit to fall within 48-64 days of baseline/enrollment visit

7.2.6. Visit #6: “DAY #90” (final study visit)

Purpose: To assess safety via toxicity and evaluate status of the epithelium

- Assess concomitant medication and therapy
- Assess discomfort using VAS
- Visual acuity and perform slit lamp exam, record fluorescein and lissamine green staining
- Perform additional clinical measurements: corneal sensation measurement, tonometry, digital corneal photography, anterior segment OCT imaging
- Assess adverse events
- Provide participant instructions on continued supportive care
- Visit to fall within 78-102 days of baseline/enrollment visit

7.3. Safety and Other Assessments

7.3.1. Safety and Maximal Tolerated Dose

Safety will be determined by assessing the MTD, defined as the highest dose at which zero to one of six patients experienced a DLT. DLT will be defined according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE v5) adverse event reference. Any non-corneal, ocular or systemic treatment emergent adverse event (TEAE), with a Grade 2 toxicity without resolution after 14 days or a Grade 3 toxicity will be considered a DLT, unless it can clearly be attributed to another cause. Final DLT outcomes will be assessed at DAY #28 (if not having previously met the 14-day rule), and all participants will be followed for a total of 90 days. All participants in a cohort of a given dose will be required to complete their DAY #28 assessment before future participants with a dose escalation will be enrolled. DLT Escalation Rules described in detail in **Section 4.3 Phase I Dose-Limiting Toxicity Escalation Rules (Table 3)** will be used in determining treatment dose. If a DLT occurs in 2 patients in a dosing cohort, then the MTD is considered exceeded and the dose will not be escalated.

7.3.2. Safety and Adverse Event Monitoring

Safety monitoring will continue by collection of all AEs following treatment up until DAY #90. Adverse events collected at each study visit will be recorded for duration, severity, causality, and need for treatment in general and for eye-associated AEs in particular. All AEs will be reviewed regularly and coded for preferred terminologies using Medical Dictionary for Regulatory Activities (MedDRA) for analysis. More detail and definitions follow in **Section 7.5 Adverse Events and Serious Adverse Events**.

7.4. Efficacy Assessments

The primary purpose of this research is to determine safety of the treatment via the MTD; efficacy is not a primary aim. However, the effectiveness of the treatment will be assessed as detailed below to determine if study treatment has a positive effect, a negative effect or no effect.

7.4.1. Epithelial Status Assessment (primary efficacy)

The primary outcome measure is improved corneal epithelial barrier function at DAY #28 assessed by viewing the cornea under slit lamp examination after instillation of sodium fluorescein, assessing the change from baseline in corneal fluorescein staining score. The presence/absence of an epithelial defect on DAY #28 will also be recorded.

7.4.2. Visual Acuity (secondary efficacy)

Best-corrected distance visual acuity will be measured using standard ETDRS protocols.

7.4.3. Corneal Epithelial thickness (secondary efficacy)

Corneal epithelial thickness measured by anterior segment OCT imaging (Cirrus 6000, Pachymetry, a-scans and b-scans) will assess the treatment effect on thickness on DAY #28 and compared relative to baseline.

7.4.4. Corneal Scarring/Haze (secondary efficacy)

Corneal scarring/haze will be assessed via anterior segment OCT imaging (Cirrus 6000; HD cornea, a-scans) to assess the treatment effect on DAY #28 relative to baseline (Images analysis done by Image-J/Metamorph)

7.4.5. Ocular discomfort and Tolerability of MSC secretome drops (secondary efficacy)

Drop discomfort will be measured at DAYS #7, #14, and #28 using a visual analogue scale (VAS; range 0 – 100), where 0 represents no discomfort and 100 the worst discomfort. Ocular discomfort will be similarly assessed on days 56 and 90.

7.4.6. Durability of Corneal Epithelial Status Improvement (secondary efficacy)

Durability of the improved corneal epithelial barrier function/integrity will be assessed an outcome measure at DAY #56 and DAY #90. Participants with improved corneal epithelial barrier function/integrity to DAY #28 will be assessed at each subsequent follow-up visits (e.g., DAYS #56, #90) to assess persistence of the healing response. Epithelial barrier function/integrity status will be assessed as described in **Section 7.4.1**.

7.4.7. Time to Improvement of Corneal Epithelial Barrier (secondary efficacy)

The time required for an improved epithelial barrier function will be assessed at each visit throughout the trial as described in **Section 7.4.1**

7.5. Adverse Events and Serious Adverse Events

7.5.1. Definition of Adverse Events

An AE is any untoward medical occurrence temporally associated with the use of an investigational medicinal product, whether or not considered causally related to that product. An AE can be any unfavorable and unintended sign (for example, an abnormal laboratory finding), symptom, or disease. Investigators, or his/her designee, will document AEs. All AEs must be recorded on an AE Form of REDCap. Adverse events will be coded using the MedDRA AEs dictionary. All AEs will be reported in the participant's source documents regardless of relationship to the study product.

All AEs will be followed during the trial period. The Investigator will use his/her discretion in ordering additional tests as necessary to monitor the resolution of such events. Event outcome at resolution or at time of last follow up will be recorded as event resolved, resolved with sequelae, ongoing at discontinuation, or death. Any events ongoing after the participant’s last visit will be followed by attempting to contact the participant at a minimum for up to 28 days after the visit to determine status. Beyond that timeframe, Investigators should continue to follow up events of concern outside of the trial per their standard of care. The trial database will be finalized in these cases with events noted as ongoing.

The Investigator should consider AEs both as they relate to the MSC Secretome and as they relate to the procedures involved in the trial. The Investigator is responsible for determining initial relationship and severity of AEs.

7.5.2. Definition of Serious Adverse Events (SAE)

A serious adverse event (SAE) is defined by federal regulation as any AE occurring at any dose that results in any of the following outcomes: death, life-threatening AE, requires hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in participant hospitalization, or the development of drug dependency or drug abuse.

7.5.3. Classification of an Adverse Events

7.5.3.1. Severity of Event

The severity of all AEs will be assessed by the Investigator and graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE v5) adverse event grading system. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE. **Table 7**, which has been adopted from CTCAE v5, provides a list of Eye disorders that are potentially relevant to the MSC Secretome Study. The table is followed by examples of possible AEs for each severity grade.

Table 7. Adverse and Serious Adverse Events Description, Definitions and Grading, as defined by the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE v5) adverse event grading system

COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS (CTCAE)	GRADE 1	GRADE 2	GRADE 3	GRADE 4
BLURRED VISION	Intervention not indicated	Symptomatic; moderate decrease in visual acuity (best corrected	Symptomatic with marked decrease in visual acuity (best	Best corrected visual acuity of 20/200 or

		visual acuity 20/40 and better or 3 lines or less decreased vision from known baseline); limiting instrumental ADL.	corrected visual acuity worse than 20/40 or more than 3 lines of decreased vision from known baseline, up to 20/200); limiting self care ADL	worse in the study eye
CORNEAL ULCER	-	-	Corneal ulcer without perforation in the study eye	Perforation in the study eye
DRY EYE	Asymptomatic; clinical or diagnostic observations only; symptoms relieved by lubricants	Symptomatic; moderate decrease in visual acuity (best corrected visual acuity 20/40 and better or 3 lines or less decreased vision from known baseline)	Symptomatic with marked decrease in visual acuity (best corrected visual acuity worse than 20/40 or more than 3 lines of decreased vision from known baseline, up to 20/200); limiting self care	-
EYE PAIN	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	-
EYELID FUNCTION DISORDER	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; nonoperative intervention indicated; limiting instrumental ADL	Limiting self care ADL; operative intervention indicated	-
GLAUCOMA	Less than 8 mmHg of elevated intraocular pressure (EIOP); no visual field deficit	EIOP which can be reduced to 21 mmHg or under with topical medication and on visual field deficit	EIOP causing visual field deficits	Visual field deficit within the central 10 degrees of the visual field in the study eye
KERATITIS	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; moderated decrease in visual acuity (best corrected visual acuity 20/40 and better or 3 lines or less decreased vision from known baseline)	Symptomatic with marked decrease in visual acuity (best corrected visual acuity worse than 20/40 or more than 3 lines of decreased vision from known baseline, up to 20/200); corneal ulcer; limiting self care ADL	Perforation; best corrected visual acuity of 20/200 or worse in the study eye
NIGHT BLINDNESS	Symptomatic but not limiting ADL	Symptomatic; moderate decrease in visual acuity (best corrected visual acuity 20/40 and better or 3 lines or less decreased vision from known baseline); limiting instrumental ADL.	Symptomatic with marked decrease in visual acuity (best corrected visual acuity worse than 20/40 or more than 3 lines of decreased vision from known baseline, up to 20/200); limiting self care ADL	Best corrected visual acuity of 20/200 or worse in the study eye
PERIORBITAL EDEMA	Soft or non-pitting	Indurated or pitting edema; topical intervention indicated	Edema associated with visual disturbance; increased intraocular pressure, glaucoma or retinal hemorrhage;	-

			optic neuritis; diuretics indicated; operative intervention indicated	
PHOTOPHOBIA	Symptomatic but not limiting ADL	Limiting instrumental ADL	Limiting self care ADL	-
UVEITIS	Anterior uveitis with trace cells	Anterior uveitis with 1+ or 2+ cells	Anterior uveitis with 3+ or greater cells; intermediate posterior or pan-uveitis	Best corrected visual acuity of 20/200 or worse in the study eye
EYE DISORDERS-OTHERS, SPECIFY	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated; no change in vision	Moderate; minimal, local or noninvasive intervention indicated; limiting instrumental ADL; best corrected visual acuity 20/40 and better or 3 lines or less decreased vision from known baseline	Severe or medically significant but not immediately sight-threatening; limiting self care ADL; decrease in visual acuity (best corrected visual acuity worse than 20/40 or more than 3 lines of decreased vision from known baseline, up to 20/200)	Sight-threatening consequences; urgent intervention indicated; best corrected visual acuity of 20/200 or worse in the study eye

**** A single dash (-) indicated Definition of AE is not available.**

***** Grade 5- Death related to Adverse Event.**

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. Examples of possible ocular surface Grade 1 AEs

- transient symptoms after instillation of drops such as stinging, itching, mild blurriness, mild eye pain/headache.
- transient signs lasting less than 24 hours after instillation of drops such as conjunctival injection, tearing, frequent blinking, and mild eyelid puffiness.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*. Examples of ocular surface Grade 2 AEs

- Worsening epithelial disease: increased staining by >50% above baseline,
- New epithelial defect > 4.0 mm or increase in epithelial defect size by more than 4.0 mm
- Persistent conjunctivitis, episcleritis,
- Increase corneal haze/scar
- Stromal disease: stromal loss 10-30%

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**. Examples of ocular surface Grade 3 AEs

- Clinical picture consistent with infectious keratitis
- Corneal ulceration with > 30% stromal loss

Grade 4 Life-threatening consequences; urgent intervention indicated. Examples of possible ocular surface Grade 4 AEs

- Anaphylactic reaction, Stevens-Johnson syndrome
- Corneal perforation

Grade 5 Death related to AE.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc. **Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

7.5.3.2. Relationship to Study Intervention

All AEs must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal or rechallenge. Rechallenge information is not required to fulfill this definition.
- **Not Related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments). Or, the AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

7.5.3.3. Expectedness

As with the use of any human tissue, the possibility of infectious agent transmission cannot be completely eliminated although all screening and microbial testing results were satisfactory for the tissue and tissue donor. Possible significant AEs include microbial infection and transmission of viral disease.

The MSC Secretome drops may cause temporary stinging and blurring of vision, which should resolve quickly.

Disease Related Events that may occur during the study include:

- Compromised vision
- Ocular discomfort
- Infection
- Scarring
- Stromal ulceration
- Melting
- Perforation

Any AE that involves ocular tissues besides eyelids/eyelid margin, cornea, conjunctiva and anterior chamber would be considered as unexpected.

7.5.4. Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by study monitor/auditors.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured in an AE Case Report Form in REDCap. Information to be collected includes event description, date of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and date of resolution or stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship.

Any medical condition that is present at the time that the participant is screened will be considered as baseline medical history and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

The Investigator will record all reportable events with start dates occurring any time after informed consent is obtained until the final study visit. At each study visit, the investigator will inquire about the occurrence of AEs/SAEs since the last visit. Event outcome at resolution or at time of last follow up will be recorded as "event resolved", "resolved with sequelae", "ongoing at discontinuation", or "death".

7.5.5. Adverse Event Reporting

Disease related events expected in this population may be reported by the Investigator if they differ in nature or frequency from what is expected, or if they appear to have a causal relationship to the test article. All AEs must be reported in the participant's source documents and recorded on an AE Case Report Form regardless of whether or not they are considered to be related to the test article.

7.5.6. Serious Adverse Event Reporting

In the event of any SAE reported or observed during the trial, whether or not attributable to the trial product, the Investigator, or designee, shall report the event to the Clinical Monitor within 5 days being made aware of the event. An initial report should be made with the understanding that it may be lacking in details.

A SAE Form must be completed for all SAEs and submitted within 5 days of the Investigator's knowledge of the event and to the Institutional Review Board following the reporting requirements. When new significant information is obtained as well as when the outcome of an event is known, the Investigator will provide this information to the Sponsor as soon as it becomes available. Depending on the nature of the SAE, copies of the participant's medical records as well as results of any relevant laboratory tests performed maybe required to be submitted. If the participant was hospitalized, a copy of the discharge summary should be forwarded as soon as it becomes available. In certain cases, a letter from the Investigator that summarizes the events related to the case may be required.

In addition, all Serious Adverse Events are reported to the DSMC within 7 days and if needed a phone call/ meeting is scheduled prior to reporting to the FDA (to determine relatedness - if indicated).

Likewise, the Investigators will notify the FDA of Adverse Events that meet the following three criteria set forth by the FDA: Serious (S), Unexpected (U), and Suspected Adverse Reactions (SAR) as soon as possible and no later than 15 calendar days after determining the information qualifies for reporting (life threatening or fatal reactions are reported no less than 7 days). All IND safety reports are submitted on Form 3500A and will be accompanied by Form 1571. The submission must be identified as: “IND safety report” for 15-day reports, or “7-day IND safety report” for unexpected fatal or life-threatening suspected adverse reaction reports, or “Follow-up IND safety report” for follow-up information. The report will be submitted by email (followed up with a telephone call to confirm receipt) to the review division’s Regulatory Project Manager that has the responsibility to review the IND application.

Reporting Events to Participants

Any important risk that will change the risk-to-benefit ratio will be conveyed to study participants through updated consent forms.

Study results will be shared with participants.

7.5.7. Events of Special Interest

Not Applicable.

7.5.8. Reporting of Pregnancy

Pregnancy occurring during the study is not an AE, but it should be brought to the attention of the sponsor as soon as possible. Additional REDCap query will be included at each study visit to assess pregnancy status of study participants. The continuation of a participant who becomes pregnant will be determined on a case-by-case basis.

8. Statistical Considerations

8.1. Statistical Hypotheses

Specific Aim: Evaluate the safety and MTD of MSC Secretome in chronic ocular surface disease through a dose-escalation study (Phase I Study).

- Primary Safety Endpoint: *Compare incidence of TEAE related to ocular and systemic safety over the course of the study at 28 days, by dose-escalation group*
- Primary Efficacy Endpoint: *Evaluate whether any dose-escalation group is superior in corneal epithelial barrier/integrity (determined by fluorescein staining).*

8.2. Sample Size Determination

9-18 participants with chronic ocular surface disease will be assigned escalating doses of MSC Secretome, based on a traditional Phase I, 3 + 3 Conventional Cohort Expansion Study dose-escalation study designs. As reviewed in **Section 4.4 Justification for Phase I Sample Size**, study size is based on treatment-related DLT at each dose, and is designed to evaluate the safety and MTD as per FDA guidelines²² of MSC Secretome in chronic ocular surface disease.

8.3. Population for Analyses

- Safety Analysis Datasets will be used for all safety analyses (e.g., all participants).
- Intention-to-Treat (ITT) Analysis Dataset will be used for all efficacy analyses (e.g., all participants).

8.4. Statistical Analyses

8.4.1. General Approach

Phase I planned descriptive statistics will assess safety and MTD across three escalating dosages at DAY #28, in addition to monitoring participants through DAY #90 for AEs. In addition, all AEs will be analyzed using either a standard t-test (continuous outcomes) and Chi-square (categorical) analyses, as appropriate, as described below.

8.4.2. Baseline Descriptive Statistics

Baseline descriptive demographic data will be provided for all participants and by treatment group.

8.4.3. Analysis of the Safety Endpoint

MTD will be identified as described above in **Section 3.1 Study Primary Objective**, based on DLT Escalation Rules described in **Section 4.3 Phase I Dose-Limiting Toxicity Escalation Rules (also see Table 3)**. Briefly, the MTD will be assessed, defined as the highest dose at which 0 to 1 of 6 patients experienced a DLT. DLT will be defined according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE v5) adverse event reference. Any non-corneal, ocular or systemic treatment emergent adverse event (TEAE), with a Grade 2 toxicity without resolution after 14 days or a Grade 3 toxicity will be considered a DLT, unless it can clearly be attributed to another cause.

In addition, analysis of the Ocular and Systemic Safety Endpoints at DAY #28 will be conducted to compare the proportion with the Safety Endpoint across groups **Table 8**. A similar analysis will be completed for monitoring through DAY #90.

Table 8. Ocular and Safety Outcomes: Adverse Events

OUTCOMES	ENDPOINTS
• Ocular Safety • Systemic Safety	Incidence of TEAE over the course of the study at 28 days, by group

TEAE Treatment Emergent Adverse Event

Adverse Events will be coded (e.g., MedDRA), and counted only once for a given participant. Adverse Events will be counted only once for a given participant, and presented (e.g., severity, frequency, and relationship of AEs to study intervention) by System Organ Class. Information about each reported AE will include the following: start date, stop date, severity, relationship, expectedness, outcome, and duration. Adverse events leading to premature discontinuation from the study intervention and serious treatment-emergent AEs will be presented in a separate table.

8.4.4. Analysis of the Primary Efficacy Endpoint

Analysis of the Primary Efficacy Endpoint, defined as the proportion of participants with improved corneal epithelial barrier function/integrity at 28 days following treatment initiation as determined by fluorescein staining, will be conducted to compare the proportion with the Primary Efficacy Endpoint across groups. Standard t-tests will be used as appropriate.

8.4.5. Analysis of the Secondary Efficacy Endpoint

Analysis of the Secondary Efficacy Endpoints at 28 days, as defined in **Table 9**, will be conducted to compare the proportion with the Secondary Efficacy Endpoint across groups. Standard t-tests will be used as appropriate.

Table 9. Secondary Efficacy Outcomes and Definitions.

OUTCOMES	ENDPOINTS
- Visual acuity - Corneal Stromal Haze - Corneal Epithelial thickness - Tolerance of drops	- Best-corrected, high contrast, spectacle visual acuity (ETDRS) - Percent change from baseline in the corneal stromal haze relative to baseline as imaged by anterior segment OCT and analyzed by Image-J software - Percent change from baseline in the corneal epithelial thickness relative to baseline as measured by anterior segment OCT imaging - Measured by VAS (range, 0 to 100)

9. Supporting Documentation and Operational Considerations**9.1. Regulatory, Ethical and Study Oversight Considerations****9.1.1. Informed Consent Process****9.1.1.1. Consent and Other Informational Documents Provided to Participants**

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting any study procedure. IRB-approved consent forms will be provided to the FDA, and will contain at a minimum the following information:

- That the trial involves research.
- The purpose of the trial.
- The trial treatment(s) and the probability for assignment to each treatment.
- The trial procedures to be followed, including all invasive procedures.
- The participant's responsibilities.
- Those aspects of the trial that are experimental.
- The reasonably foreseeable risks or inconveniences to the participant.
- The reasonably expected benefits.
- The alternative procedure(s) or course(s) of treatment that may be available to the participant, and their important potential benefits and risks.
- The compensation and/or treatment available to the participant in the event of trial-related injury.
- The anticipated prorated payment, if any, to the participant for participating in the trial.
- The anticipated expenses, if any, to the participant for participating in the trial.

- m) That the participant's participation in the trial is voluntary and that the participant may refuse to participate or withdraw from the trial, at any time, without penalty or loss of benefits to which the participant is otherwise entitled.
- n) That the monitor(s), the auditor(s), the IRB/IEC, and the regulatory authority(ies) will be granted direct access to the participant's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the participant, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent form, the participant or the participant's legally acceptable representative is authorizing such access.
- o) That records identifying the participant will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the trial are published, the participant's identity will remain confidential.
- p) That the participant or the participant's legally acceptable representative will be informed in a timely manner if information becomes available that may be relevant to the participant's willingness to continue participation in the trial.
- q) The person(s) to contact for further information regarding the trial and the rights of trial participants, and whom to contact in the event of trial-related injury.
- r) The foreseeable circumstances and/or reasons under which the participant's participation in the trial may be terminated.
- s) The expected duration of the participant's participation in the trial.
- t) The approximate number of participants involved in the trial.

9.1.1.2. Consent Procedures and Documentation

Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants will have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. Participants will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A signed copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Only authorized trial staff should obtain consent and the most currently approved IRB consent form must be used.

9.1.2. Study Discontinuation and Closure

The trial may be terminated at any time by the Investigators if serious side effects occur, if the Investigators do not adhere to the protocol, or if, in the Investigator's judgment, there are no further benefits to be achieved from the trial. In the event that the clinical development of the investigational product is discontinued, the Investigator shall inform all regulatory authorities.

To ensure participant safety, the following approach is included to allow suspension or termination of the trial should a serious unforeseen risk arise: in the event that there are 2 reports of the same serious, unexpected and related AE, the Medical Monitor will within 5 calendar days of notification of the second event make a recommendation to Investigators whether to enhance surveillance, suspend enrollment, halt all further exposures, terminate the trial or continue without change.

If suspended, the trial may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the Investigators, IRB and/or FDA.

9.1.3. Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the Investigators and their staff. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval by the Investigators.

All research activities will be conducted in as private a setting as possible.

The study monitor, representatives of the IRB, or the FDA may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the UIC IRB, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored by the Investigators. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used at the clinical site will be secured and password protected.

9.1.4. Future Use of Stored Specimens and Data

This protocol does not include storage of any biological samples for future testing. All study data will remain de-identified and kept securely by the sponsor or by a qualified contracted vendor, for at least the period of time required by FDA.

9.1.5. Key Roles and Study Governance

Co-Principal Investigator	Co-Principal Investigator
Ali R. Djalilian, MD	Charlotte E. Joslin, OD, PhD
Professor, UIC	Professor, UIC
1855 W. Taylor St. Chicago, IL 60612 312-996-8937	1855 W. Taylor St. Chicago, IL 60612 312-996-5410
adjalili@uic.edu	charjosl@uic.edu

9.1.6. Data and Safety Monitoring Committee (DSMC)

The Data and Safety Monitoring Committee (DSMC) will be charged in evaluating the safety and effectiveness of the treatment. DSMC members who have agreed to serve include the following:

Loretta Szczotka-Flynn, OD, PhD
Director, Contact Lens Service, UH Cleveland Medical Center
Director; Vision Research Coordinating Center, UH Cleveland Medical Center
Professor, Case Western Reserve University School of Medicine
Department of Ophthalmology & Visual Sciences
Department of Population & Quantitative Health Sciences

Ula Jurkunas, MD
Associate Professor of Ophthalmology
Harvard Medical School
Co-director, Cornea Center of Excellence
Massachusetts Eye and Ear Infirmary
Associate Scientist, Schepens Eye Research Institute

Jennifer Thorne, M.D., Ph.D
Cross Family Professor of Ophthalmology
Chief, Division of Ocular Immunology.
Wilmer Eye Institute
Johns Hopkins School of Medicine
Professor of Epidemiology
Johns Hopkins Bloomberg School of Public Health.

Kathryn Porter, JD, MPH
Research Scientist
Treuman Katz Center for Pediatric Bioethics
Seattle Children's Research Institute
Director, Research Bioethics Consultation Service
Institute of Translational Health Sciences

National Eye Institute Representative on DSMC:

Donald Everett, MA
Program Director for Collaborative Clinical Research
National Eye Institute, National Institutes of Health
6700 B. Rockledge Dr., Suite 3400
Bethesda, MD 20817

The DSMC will finalize the protocol. The DSMC will meet 2 times per year via tele/video conference throughout the course of the study with the second meeting to be scheduled after enrollment of the first 3 patients.

9.1.7. Clinical and Regulatory Monitoring

Clinical Monitoring

A Clinical Monitor, who is not an investigator in the study, will assure that the trial data are accurate, complete, and verifiable, and that all AEs have been appropriately identified and documented. The Clinical Monitor will be a clinician with expertise in corneal disease and will regularly inspect all the patient medical records, REDCap instruments, and imaging data. A report is generated for every DSMC meeting and anytime requested by the DSMC. The Clinical Monitor for the MSC Secretome Study will be:

Asim Farooq, MD
Assistant Professor of Ophthalmology and Visual Science
Department of Ophthalmology
University of Chicago
afarooq@bsd.uchicago.edu

Regulatory Monitoring

Regulatory Monitoring will assure that study is conducted according to the approved protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and all applicable regulatory requirement(s) by the IRB and FDA. The Regulatory Monitor will have specific expertise in clinical trial monitoring, including FDA requirements, documentation and reporting. They will review/audit the study data to assure that all protocol requirements, applicable FDA regulations, and Investigator's obligations are being fulfilled; and resolve any inconsistencies or deficiencies in the trial records. A report by the Regulatory Monitor is generated for every DSMC meeting and anytime requested by the DSMC.

9.1.8. Quality Assurance and Quality Control

Investigators shall implement and maintain quality control and quality assurance procedures with written standard operating procedures to ensure that the trial is conducted and data are generated, documented, and reported in compliance with the protocol, GCP, and applicable regulatory requirements. Specifically, this trial shall be conducted in accordance with the provisions of the Declaration of Helsinki.

9.1.9. Data Handling and Record Keeping

9.1.9.1. Data Collection and Management

REDCap, provided by UIC's CCTS and updated as systems evolve, will be used for electronic data capture, data entry and data management. REDCap is a secure, web-based application with Secure Socket Layer (SSL) authentication for collection and entry of research data as well as building and managing online databases, and allows enabling of range checks to query when entered values surpass a pre-programmed range. REDCap offers easy data manipulation with audit trails and ad hoc reporting functionality, and an automated export mechanism to common statistical packages (SAS, Stata, SPSS). Data will be retrieved and converted into SAS files regularly (e.g., bi-monthly), creating a Master Database with appropriate backups. Regular reports summarizing data acquisition and recruitment will be generated. Drs. Joslin and Djalilian will work closely with the study staff to ensure MSC Secretome Study quality control and data validation (e.g., REDCap's field validation including data quality module versus double data entry).

REDCap is 21 CFR Part 11 capable, supporting electronic signatures through a unique username and password combination. All study staff will require FDA's Good Clinical Practice (GCP) training and

certification, and all study-related procedures, data entry, and management will follow GCP standards. Only authorized users will have access to study-related systems through assigned. Standard UIC security measures will be implemented. Differential user levels commensurate with study tasks will be implemented to maximize study security.

Confidentiality procedures as specified in the MSC Secretome Study SOP will be implemented for all study-related procedures and utilize the MSC Participant ID Number. Data transfers between UIC and clinical sites will occur via a secure file transfer protocol (FTP) site. All data acquired at clinical sites will be entered real-time in REDCap (direct data entry) and stored at UIC as de-identified data.

During the trial, Investigators will maintain adequate records for the trial, including medical records, records detailing the progress of the trial for each patient, REDCap signed informed consent forms, investigational product disposition records, correspondence with the IRB, AE/SAE reports, and information regarding participant discontinuation and completion of the trial.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the Investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

Study source documentation will be the REDCap electronic record, identified through the MSC Participant ID Number. Printed PDF copies of entered data saved from the REDCap system for each visit will be maintained in participant binders that include signed consent forms. Participant binders will be stored in locked cabinets at clinic sites and assessed for study completeness by MSC Secretome Study PIs during annual site visits.

All documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Hardcopies of study visit worksheets may be provided for use as source document worksheets for recording data for each participant enrolled in the study. Clinical data (including AEs, concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered via REDCap, a 21 CFR Part 11-compliant data capture system. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly.

9.1.9.2. Study Records Retention

All records and documents pertaining to the trial including, but not limited to, data and source documents, will be maintained by the Investigator for a period of 10 years after completion of the trial or for a period of at least 2 years following the removal of an IND, whichever is longer. If the Investigator retires, relocates, or for any other reason withdraws from responsibility of keeping the study records, custody must be transferred to the Co-PI or to a person who will accept responsibility and is approved by the Co-PIs.

9.1.10. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol or GCP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of serious or repeated deviations, corrective and preventative actions are to be developed by the site and implemented promptly.

It is the responsibility of the Investigator to use continuous vigilance to identify and report deviations. The Investigator may not deviate from the protocol without a formal protocol amendment having been established and approved by the UIC IRB, except when necessary to eliminate imminent hazards to the participant or when the change(s) involve only logistical or administrative aspects of the trial. A deviation may result in the participant having to be withdrawn from the trial, rendering that participant non-evaluable.

9.1.11. Publication and Data Sharing Policy

At a minimum, the trial and its results will be posted on ClinicalTrials.gov

9.1.12. Conflict of Interest Policy

Each investigator will be asked to complete a financial disclosure prior to the initiation of this study, and one year following database lock.

9.2. Additional Considerations

None.

9.3. Abbreviations

AE	Adverse Event
CFR	Code of Federal Regulations
CGTP	Current Good Tissue Practice
CONSORT	Consolidated Standards of Reporting Trials
DLT	Dose-Limiting Toxicity
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
MedDRA	Medical Dictionary for Regulatory Activities
MSC	Mesenchymal stem cells
MSC Secretome	
Study	“Safety of Topical Mesenchymal Stromal Cell Derived Secretome for Ocular Surface Disease”
MTD	Maximal Tolerated Dose
TEAE	Treatment Emergent Adverse Event
UIC	University of Illinois at Chicago

9.4. Protocol Amendment History

Version	Date	Description of Change	Brief Rationale
1.0	February 9, 2021	Original issue	Original issue
1.1	April 27, 2021	Change to anterior segment OCT for all anterior segment imaging	Provides superior anterior segment imaging with epithelial thickness map
1.2	May 18, 2021	Inclusion and Exclusion Criteria Revised Clinical Monitoring Revised Adverse Events section revised	Per DSMC recommendation the study population and inclusion/exclusion criteria have been refined to achieve a balance between severity and stability Per DSMC recommendation a specific Clinical Monitor has been designated for the study. Per DSMC recommendation examples of ocular AEs of different severity levels are provided. Also the timing for reporting of serious AEs to the DSMC and the details for FDA reporting has been included.
1.3	May 20, 2021	Editing to Document	Minor Adjustments to Table of Contents and Document
1.4	June 10, 2021	Regulatory Monitoring added to the protocol	Per DSMC recommendation external Regulatory Monitoring has been added.
1.41	June 22, 2021	Added back Scheimpflug Imaging to complement OCT imaging	Scheimpflug Imaging provides more standard quantitative measure of corneal haze
1.5	June 28, 2021	Added specific examples of Grade 2-4 toxicities included in DLT definition	Per FDA recommendation
1.6	August 11, 2021	Editing to Document	Per UIC IRB recommendation
1.7	October 4, 2021	Editing to Document	Per UIC IRB recommendation
1.8	November 11, 2021	Editing to Document	Minor edition
1.9	December 6, 2022	Editing to Document	Adding patient survey and disbursement forms
2.0	December 8, 2023	Clarification	Clarification

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