LV Prasad Eye Institute

CLINICAL STUDY PROTOCOL

STUDY TITLE:
A proof of concept study to evaluate the clinical safety and efficacy of *Ex-vivo* Cultivated Allogenic Limbal Stem Cell Transplantation for Treatment of Superficial Corneal Pathologies

PROTOCOL NUMBER- HERF-SSC-SCP-01

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CO-ORDINATING CLINICAL CENTRE	Hyderabad Eye Research Foundation L V Prasad Eye Institute Kallam Anji Reddy Campus Road No. 2, Banjara Hills Hyderabad - 500034, Telangana India	
FUNDING SOURCE/ RESEARCH GRANT	Hyderabad Eye Research Foundation	
INVESTIGATIONAL AGENT	Ex-vivo Cultivated Allogenic Limbal Stem Cell	
INDICATION	Superficial Corneal Pathologies	
CLINICAL PHASE	Proof of Concept (PILOT), Investigator Initiated Study	
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PROTOCOL APPROVAL PAGE

PROTOCOL APPROVAL PAGE

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INVESTIGATORS' STATEMENT FOR AGREEMENT TO PROTOCOL

Protocol Number	HERF-SSC-SCP-01

Protocol Title: A proof of concept study to evaluate the clinical safety and efficacy of Ex-vivo Cultivated AllogenicLimbal Stem Cell Transplantation for Treatment of Superficial Corneal Pathologies.

I have read and understood this trial protocol and attached annexes and any amendment and/or supplements thereto. Having fully considered all the information availiable, I consider that it is ethically justificable to perform the study according the above protocol.

In addition, I agree to conduct this protocol and any amendment and/or supplements according to applicable national laws and regulations relevant to perform this study, Schedule Y of Drugs and Cosmetics Act along with all relevant amendments, National Guideleines for Stem Cell Research issued jointky by ICMR and DBT and current ICH GCP (E6) Guidelines and in a manner consistent with the Declaration of Helsinki

Furthermore, I agree to make no additions and/or changes except when necessary to protect the safety of the subjects.

Dr. Sayan Basu MS (Ophthalmology) Consultant Corneal Surgeon and Scientist LV Prasad Eye Institute, Hyderabad Signature Date 2/4/19	**Dr. Vivek Singh PhD** Scientist, Prof. Brien Holden Eye Research Center, Champalimaud Translational Centre for Eye Research. LV Prasad Eye Institute, Hyderabad Signature Date 02/04/19

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REVISION LOG

Protocol Version	Section	Amendment	Reason for Changes	Date
2.0	4.4	Release Criteria	Phenotypic markers study by Immunohistochemistry for purity check excluded from the final product specification because of no availability of cells for the purity study.	12.11. 2018
2.0	21.1	Flow Chart for Production of Ex vivo Allogenic Limbal Stromal Stem Cells	Sampling and testing details update as per intermediate and final product specifications.	12.11.2018

LIST OF ABBREVIATIONS

AE	Adverse Experience
ANOVA	Analysis of Variance
ASOCT	Anterior Segment Optical Coherence Tomography
BCL	Bandage Contact Lens
BCVA	Best Correct Visual Acuity
cDNA	Complimentary Deoxyribonucleic Acid
CDSCO	Central Drugs Standard Control Organization
CI	Confidence Interval
CRF	Case Report Form
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events (CTCAE
CTRI	Clinical Trial Registry India
DCGI	Drugs Controller General India
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ECG	Electrocardiogram
ECM	Extra Cellular Matrix
ETDRS	Early Treatment Diabetic Retinopathy Study
EU/mL	Endotoxin Unit/millilitre
FBS	Fetal bovine serum
FDA	Food and Drugs Administration
GCP	Good Clinical Practices
GMP	Good Manufacturing Practices
HERF	Hyderabad Eye Research Foundation
HLA-DR	Human Leukocyte Antigen – antigen D Related
hLMSC	Human Limbus-Derived Stromal/ Mesenchymal Stem Cells
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonization
ICMR	Indian Council of Medical Research
IC-SCR	Institute Committee on Stem Cell Research
IEC	Independent Ethics Committee
IIS	Investigator Initiated Study
IOP	Intraocular Pressure
IRB	Institutional Review Board
LBSCs	limbal biopsy–derived stromal stem cells
LVPEI	L V Prasad Eye Institute
MI	Myocardial Infarction
MSC	Mesenchymal Stem Cells
NYHA	New York Heart Association

PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
RA	Regulatory Authorities
RNA	Ribonucleic acid
SAEs	Serious Adverse Events
SSC	Stromal Stem Cells
SCP	Superficial Corneal Pathologies
UPIN	Unique Patient Identification Number
VAS	Visual Analogue Scale
WHO	World Health Organization
WHOART	World Health Organization Adverse Reaction Terminology

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PROTOCOL SYNOPSIS

Study Title:

A proof of concept study to evaluate the clinical safety and efficacy of Ex-vivo cultivated allogenic Limbal Stem Cell Transplantation for Treatment of Superficial Corneal Pathologies.

Study Number: HERF-SSC-SCP-01

Investigational Agent: Ex-vivo Cultivated Limbal Stem Cells.

Study Phase: Proof of Concept (Pilot), Investigator Initiated Study.

Study Purpose: To evaluate the safety and efficacy of ex-vivo cultivated allogenic limbal stromal stem cells for the treatment of visually significant superficial corneal stromal scarring and other pathologies.

This study proposes to investigate the transplantation of ex-vivo cultivated allogenic limbal stromal cells for the treatment of the corneal pathologies. The limbus is an ideal source as the stem cells are numerous and located very superficially in the tissue [17]. Pre-clinical work suggests human corneal stromal stem cells can be isolated from the cadaveric tissues, cultivated in conditions suitable for cell based therapy and used to prevent fibrosis in a murine model of corneal stromal scarring. Further, these cells are able to successfully engraft, differentiate, and mediate wound healing in the corneal stroma such that the tissue remains healthy, free of fibrotic tissue, and optically transparent. The clinical implications of these findings are substantial in that it represents the potential to lessen the burden on donor tissue necessary for corneal allografts by using cultured cells to regenerate tissue. We foresee the ability of a clinician to and grow and expand the cells in number and after surgically removing the scar tissue from the wounded eye, apply the cultured limbal stem cells to regenerate healthy, transparent tissue.

Study Population: This study will include 20 (male and female) participants, aged between 18-60 years, who have unilateral blindness due to various corneal pathologies like superficial (defined as involving the anterior 200μM of the corneal stroma on ASOCT imaging) corneal ulcers, burns, scars. All the patients who will be included in this study would need to undergo stem cell transplantation and who would not require any second intervention.

Patient excluded from the study would be those with bilateral corneal disease, corneal scars with limbal dysfunction (clinically defined as absent limbal palisades or conjunctivalization of the cornea) or ocular surface disease including dry eye disease (defined as a Schirmer's test of less than 10mm at 5 minutes), Unknown etiology, post-herpetic eye disease or eyes with active intra-ocular inflammation, Children (<18 years of age), Less than 3 months after documented clinical resolution of acute disease and Inability/refusal to give written informed consent or to undergo any of the anterior segment imaging tests. Patient should have not participated in another clinical study within 30 days of their enrolment on this study.

Those who sign informed consent and meet all the entry criteria will be enrolled to the study. Participants will be screened for eligibility for study entry based on their ophthalmic presentation. Thorough ophthalmic screening will be done prior to enrollment in the study which will include a detailed clinical examination to ascertain the ocular disease status and other underlying causes for reduced vision. Screening for systemic conditions and health of the participant will be done prior to the

surgery as per Institute practice. All the screening procedure should be accomplished within 14 ±7 days from the date the participant signs the informed consent.

Study Objectives:

Primary Objective:

To evaluate the safety of ex-vivo cultivated allogenic limbal stromal stem cells for the treatment of visually significant superficial corneal stromal scarring and other pathologies including any ocular or systemic adverse effects at the various post-operative time points.

Secondary objective (s):

The secondary outcome measures are:

1. Visual improvement using ETDRS Vision Charts method.
2. Change in the corneal haze after treatment using clinical photography and scheimpflug imaging.

Study Design:

This would be a single-center prospective, open labeled, non-randomized interventional study. This study is an Investigator Initiated Study (IIS). The Ethics Committee of the LV Prasad Eye Institute, Hyderabad, would prospectively approve this study. This study would be conducted in strict adherence to the tenets of the Declaration of Helsinki, Indian GCP Guidelines and Schedule Y of Drugs and Cosmetics Act and associated amendments and Ethical Guidelines for Biomedical Research on Human Participants and current National Stem Cell Research Guidelines.

Once the participants are found to be suitable for limbal transplant surgery, the patients will be administered written informed consent and audio /visual consent as per regulations. Detailed ophthalmic examination will be done to ensure that the patient is eligible for the trial.

All the screening procedures will be accomplished within 14± 7days. On Day 0 that is the date of surgery a Unique Participant Identification Number (UPIN) will be assigned to each patient and it would be in addition to hospital medical record number. The surgery will be done under local or general anesthesia (depending on age and patient preference).

In this prospective interventional study patients with unilateral superficial corneal scars will undergo a surgical procedure. Limbal ring from a cadaveric donor tissue, which is therapeutically accepted and serologically tested, is collected. This tissue will then be cultivated in the stem cell biology laboratory using standardized culture technique. Briefly the limbal tissue will be cut up into small pieces and digested overnight using an enzyme (Collagenase L). The cells obtained from the digest will be cultured on a petri-dish using 2% serum and growth factors. The cultured cells will be passaged three times to remove all epithelial cells from the culture.

In the second procedure, the eligible patients will under corneal transplant surgery, when the central corneal epithelium will be removed using a surgical sponge. 0.1ml of stromal cells in a concentration of $5x10^3$ cells/µl diluted in the thrombin component of fibrin glue (TISEEL, Baxter) will be applied to the debrided corneal stroma. A soft bandage contact lens will be placed over the cornea at the end of the procedure. The patient will receive topical antibiotic and steroid eye drops in the post-operative period. Periodic comprehensive ophthalmic evaluation along with anterior segment optical coherence tomography (ASOCT) scanning and slit-lamp photography will be done at Day 1, Day 7, Day 30, Day 90, Day 180 and Day 360 and 720 Days post-surgery. The primary outcome measure of this study is to

note any ocular or systemic adverse effects of this intervention at the various post-operative time points. The secondary outcome measures are visual improvement and change in the density and appearance of the corneal scarring and other pathologies after treatment.

Planned Number of Participants: 20 adult male and female participants will be enrolled from a single trial site.

About Stromal Cells Cultured from Limbal Rims

Therapeutically accepted and serologically tested cadaveric corneas, within four days of collection, are obtained from Ramayamma International Eye Bank (LVPEI, Hyderabad, http://eyebank.lvpei.org). These corneas are washed with 1.25mM penicillin-streptomycin with Phosphate Buffer saline, pH (7.4), for 3mins followed by another wash with PBS. Iris and endothelial layer were scrapped for visibility. Complete 360° limbal rims are isolated using a surgical blade in buffer saline and chopped for using a small, curved corneal scissors, in incomplete media (plain DMEM media, Sigma-Aldrich, D0567). The tiny limbal tissues pieces are be subjected to collagenization by adding 20µl of reconstituted Collagenase-IV (17104019, Thermofischer, at the rate of 10IU/µl of Collagenase-IV; per every 1 ml of incomplete media) to the incomplete media. Incubation was carried out for16-hours at 37°C in 5% CO_2 chamber.

Post 16-hour incubation, the enzymatic digestion is stopped by adding 2ml of complete media (with 2% FBS). The collagenised tissue fragments would then be spun down three times at 1000rpm for 3minutes, at room temperature, in saline. 3ml complete media (plain DMEM media, Sigma-Aldrich, with 2% fetal bovine serum, with added epidermal growth factor and insulin) is added to the pellet and kept for culture with frequent media replacement every 2days. P_0 cells (as shown inFig1) are purely limbal epithelial cells. No stromal cells grow in passage P_0. Stromal cells initiate at P_1 generation and pure culture of stromal cells is obtained from P_2 onwards (as shown in Fig2).

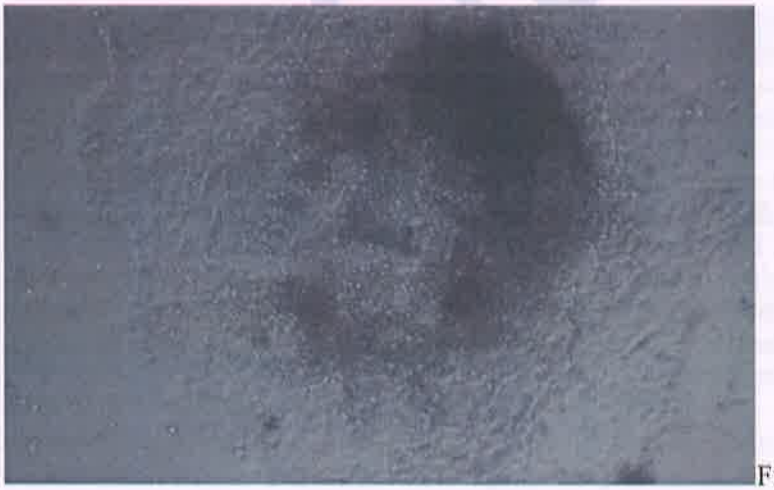


Figure 1

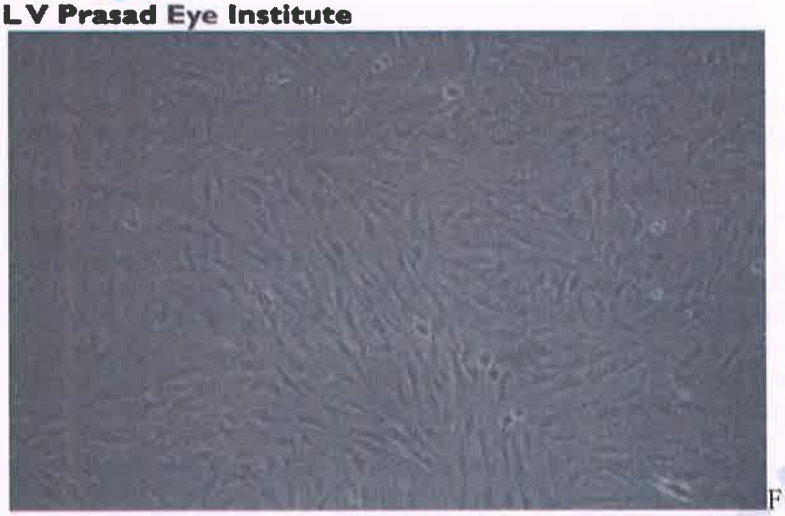


Figure 2

Preparing cells for Transplantation

Cells adhered to surface of confluent culture flask are washed with 1x PBS and then subjected to trypsinization by adding 1ml of TrypLE™ (12563011, Gibco®) and incubating them at 37° C in a 5% CO_2 chamber, for about 2 minutes. Cells are then spin down at 1300rpm for 4 minutes, at room temperature and checked for viability using 0.4% sterile and filtered Trypan blue dye. Post viability check, cells in media is spin down again at 1000rpm for 3 minutes, at room temperature in a microcentrifuge tube. The resultant cell pellet and100µl of thrombin component of the commercially available TISSEEL Lyo™ is taken into a 1ml tuberculin syringe (needle gauze 24-27) and applied onto the defective portion of the patient's eye. Pure populations of stromal cells are suspended in the thrombin component so that they could form a matrix whereas a pool of limbal epithelial cells alone or mixed populations of both limbal epithelial and limbal stromal stem cell niche is mixed with the fibrin component. Subjects would then be transplanted with 1-5 x 10^5 stromal cells.

Sterility Checks

Prior to transplantation a sample volume of 20µl from the 1ml cell suspension were subjected to sterility test for bacterial, fungal pathogens by inoculating on certified contamination free blood agar and thiglycolate media (at Jhaveri Microbiology Center, LVPEI).
Absence of *Mycoplasma* species is confirmed via biochemical assay using luminescence method, also pH, Bacterial Endotoxin tested for spent media.

Duration of Study: All participants will remain in the study for active follow-up 12 months and final follow-up at 24 months with frequent monitoring for first 3 months (Day 1, Day 7, Day 30, Day 90, Day 180 and Day 720 post-surgery) and then follow-up assessment at Month 12 and final follow-up at Month 24 post-surgery. In case the participant decides to discontinue, or if there are reports of unexpected toxicity or no clinical benefit and/or patient requires alternate therapeutic approach as per investigator's discretion the patient will be withdrawn from the study. The patient will be included in efficacy analysis when patient completes 3 months' post-surgery.

Study Endpoints:

Safety will be assessed through collection of adverse events (AEs), any local or systemic toxicities as determined by clinical and laboratory evaluations. Safety endpoints will be:

1. Local toxicities will be assessed clinically by the presence of increased inflammation and vascularization at the surgery site more than expected because of surgery.
2. Change in corneal thickness from baseline (on the day of surgery) as measured using pachymetry and subsequently evaluated from Month 3 onwards at every visit.
3. Increase in intra-ocular pressure, can be done digitally at every visit and by applanation tonometry from Month 3 onwards at every visit.
4. Systemic toxicities will be determined using clinical laboratory assessments covering hematology, blood chemistry and urinalysis work-up at baseline, Months 3, 6, 12 and 24.

Efficacy Endpoints:

1. Proportion of eyes showing two-line improvement in BCVA (Best Corrected Visual Acuity) from baseline to last follow-up visit using ETDRS Vision Charts.
2. Change in the corneal haze after treatment using clinical photography and scheimpflug imaging.

Statistical Plan and Methods:

Non-randomized Open Labeled Pilot Clinical Trial

Sample Size Determination

The primary objective of the study is to evaluate the clinical safety and efficacy of Ex-vivo Cultivated Allogenic Limbal Stem Cell in superficial corneal pathologies.

The sample size of this study has been computed to estimate the proportion of patients who achieve complete restoration of ocular surface with sufficient precision. With 20 evaluable patients in the study, and assuming a 50% chance of complete restoration at the end of the 6 months' study period, the half-width of the 90% confidence interval around the proportion of patients with complete restoration will be 0.26. Since the lower limit of the confidence interval will be above 0, the proportion of patients with complete restoration of ocular surface can be estimated with sufficient precision in this study.

Analysis Populations

Safety Population

Patients who undergo surgery successfully will be followed-up for all safety and toxicity assessments for the duration of the time they are on study.

Efficacy Population

All patients who have slit lamp biomicroscopy done at baseline undergo surgery successfully and complete the month 6 visit, will be evaluated for restoration of ocular surface, 2-line improvement in

BCVA and other efficacy assessments. Missing month 6 data may be imputed using previous post-surgery efficacy data, if appropriate.

Patients who undergo surgery and complete at least Month 3 evaluation visit will be considered evaluable for efficacy.

Safety Analysis

Safety will be determined by assessment of local and systemic toxicities on the patients and summarized appropriately.

Efficacy Analysis

Primary Efficacy Analysis

The primary efficacy endpoint of restoration of ocular surface will be assessed by computing the proportion of patients who achieve complete restoration after 6 months of transplantation. A 2-sided 90% confidence interval will be constructed around this proportion to compute the precision of the estimate. A Kaplan-Meier survival curve will be plotted to assess the outcome of the transplantation after 6 months.

Secondary Efficacy Analysis

The proportion of patients showing 2-line improvement in BCVA from baseline until last follow-up visit will be computed along with 90% CIs. The proportions of patients with other improvements or deterioration in BCVA will also be computed.

Data Imputation

Missing efficacy and safety data may be imputed in certain cases, if found appropriate. Details on imputations, data derivations and transformations, etc., will be described in a statistical analysis plan which will be prepared prior to the final analysis.

Date of Original Approved Protocol:

Date of Most Recent Protocol Amendment (if applicable):

N/App

1. INTRODUCTION

1.1 Background

Keratocytes, normally quiescent cells of the corneal stroma, transform into fibroblasts in response to injury or inflammation and repair the damaged cornea by laying down scar tissue [1, 2]. The presence of altered extracellular matrix components in such stromal scars renders the cornea optically opaque, resulting in visual loss [3]. Visual impairment and blindness due to corneal scarring affects millions worldwide [4, 5] and is the commonest indication for corneal transplantation in the developing world [6]. Although replacing the scarred tissue with a clear corneal allograft is usually effective in improving vision [7], the global demand for donor corneal tissue vastly exceeds its availability. Moreover, post-operative complications like immune-rejection, astigmatism and infection often restrict the functional survival of corneal allografts [8], especially in developing countries [9]. As such, there is increasing interest in the development of therapeutic alternatives to corneal transplant, including stem cell therapy, cell-free collagen scaffolds, and bioengineered constructs [10-12]. The recent discovery of multi-potent stem cells in the corneal stroma has opened up the possibility of developing a cell-based approach to treating corneal scars as an alternative to keratoplasty [13, 14]. In a murine model of corneal opacity, human stromal stem cells were effective in regenerating normal corneal extra-cellular matrix and repairing collagen fibril defects [15]. Subsequently other groups have independently confirmed the existence of these adult stem cells of mesenchymal lineage in the human peripheral corneal and limbal stroma [16-18]. These stromal stem cells are immune-suppressant and may have a role not only in remodeling but also in preventing corneal stromal scars [19].

As the next step towards translating this research into clinical therapy, we proposed to investigate the transplantation of ex-vivo cultivated Allogenic limbal stromal cells for the treatment of the corneal pathologies. The limbus is an ideal source as the stem cells are numerous and located very superficially in the tissue [17]. Pre-clinical work suggests human corneal stromal stem cells can be isolated from the cadaveric tissues, cultivated in conditions suitable for cell based therapy and used to prevent fibrosis in a murine model of corneal stromal scarring. Further, these cells are able to successfully engraft, differentiate, and mediate wound healing in the corneal stroma such that the tissue remains healthy, free of fibrotic tissue, and optically transparent. The clinical implications of these findings are substantial in that it represents the potential to lessen the burden on donor tissue necessary for corneal allografts by using cultured cells to regenerate tissue. We foresee the ability of a clinician to and grow and expand the cells in number and after surgically removing the scar tissue from the wounded eye, apply the cultured limbal stem cells to regenerate healthy, transparent tissue.

1.2 Determine Stability and Storage *Ex vivo* Stromal Stem cells

Therapeutically accepted and serologically tested cadaveric corneas, within four days of collection, are obtained from *The Ramayamma International Eye Bank* (LVPEI, Hyderabad, http://eyebank.lvpei.org). These corneas are washed with 1.25mM penicillin-streptomycin with Phosphate Buffer saline, pH (7.4), for 3mins followed by another wash *with* PBS. Complete 360° limbal rims are isolated using a surgical blade in buffer saline and chopped for fifteen minutes using a small, curved corneal scissors, in incomplete media (plain DMEM media, Sigma-Aldrich, D0567). The tiny limbal tissues pieces are subjected to collagenization by adding 40µL of reconstituted Collagenase-IV

(17104019,Thermofischer, at the rate of 20µL of Collagenase-IV per every 1 ml of incomplete media) to the incomplete media. Incubation was carried out for16hours at 37°C in 5% CO2 chamber. Post 16-hour incubation, the enzymatic digestion was stopped by adding 2ml of complete media (with 2% FBS). The collagenised tissue fragments are then spinned down thrice at 1000rpm for 3minutes, at room temperature, in saline. 3ml complete media (plain DMEM media, Sigma-Aldrich, with 2% fetal bovine serum, with added epidermal growth factor and insulin) was added to the pellet and kept to culture with media being replaced for every 2days. (Detailed process explained in Figure.1). Further sub-culturing under controlled growth conditions of low serum (2%), gave a pure populations of the human limbus-derived stromal/ mesenchymal stem cells (hLMSCs). These cells are analysed for their characteristic biomarkers. Pure population of the hLMSCs of P2 are seeded at 20000/cm2 and are culture till confluency. Cultured cells are stained for stem cell and mesenchymal-origin markers by immunostaining.

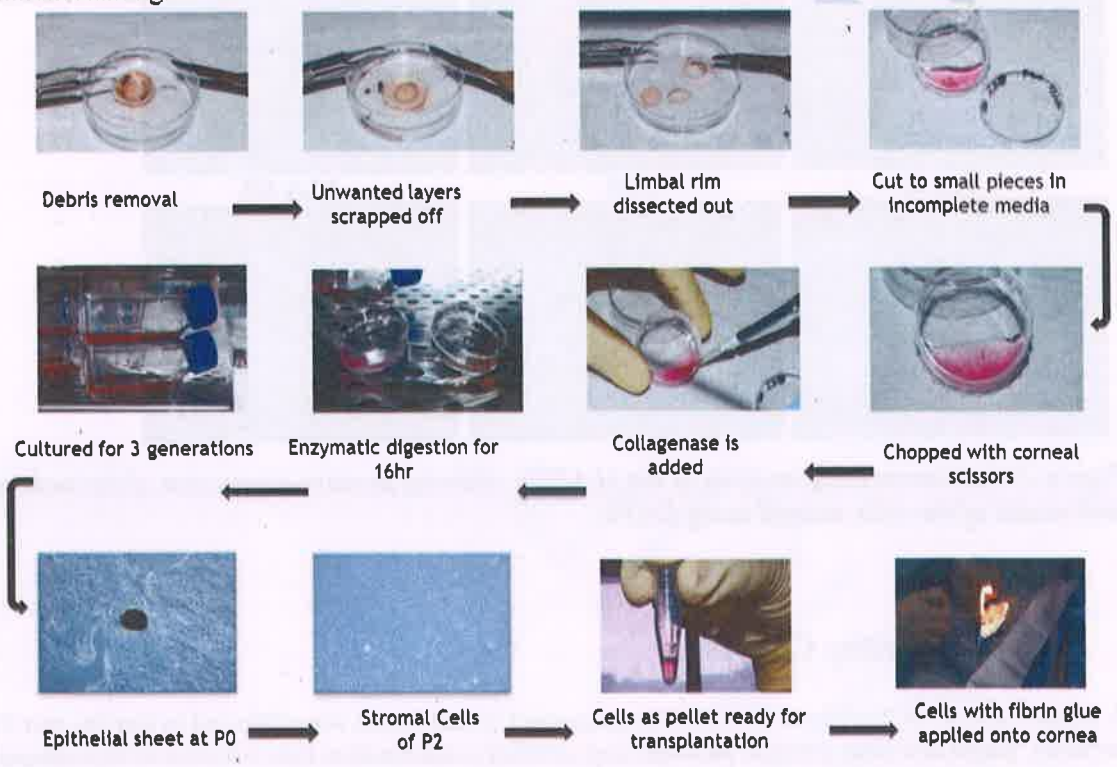


Figure.1: Schematic diagram of the hLMSCs cultivation technique.

The hLMSCs are checked for stemness property by using the markers ABCG2, Pax-6 and p-63α and are confirmed for the mesenchymal origin using the markers Vimentin, CK3, CK19, CD105 and CD34 (Figure.2). The non-antigenic nature of the hLMSCs of not eliciting an immune response was confirmed from the non-expression of the marker HLA-DR. All the other markers in the panel are shown to be expressed by hLMSCs, proving their characteristic features.

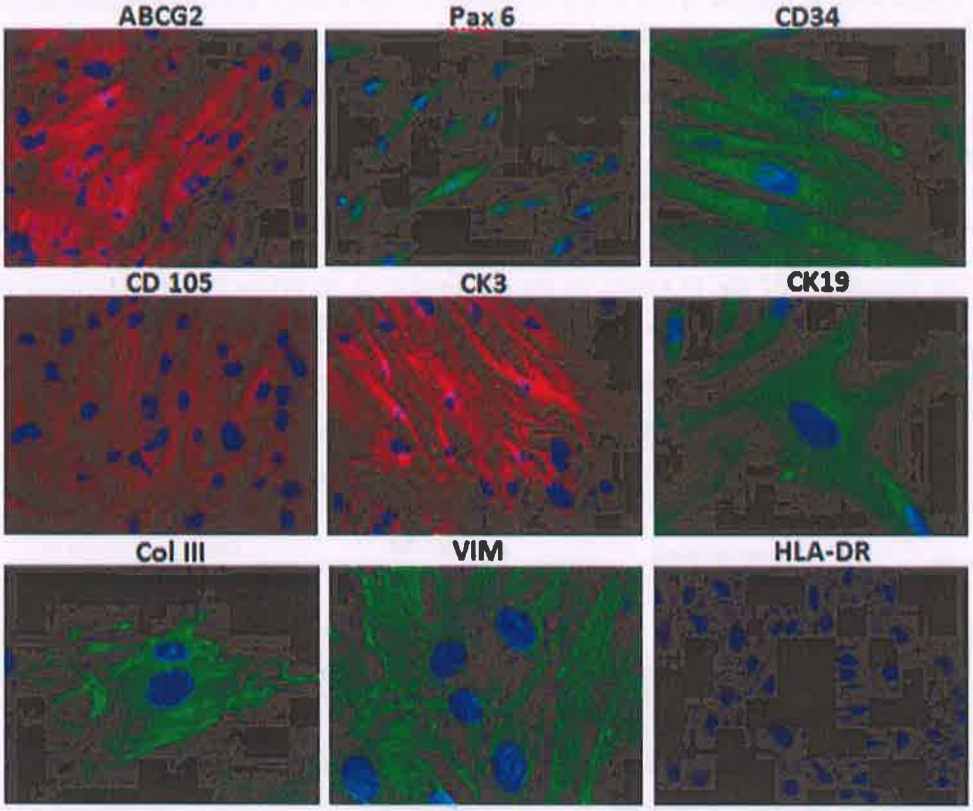


Figure.2: Immunostaining analysis of the hLMSCs, showing positive expression of the makers; with nuclei of the cells stained using DAPI

1.3 Sterility Checks

A sample volume of 20µl from the hLMSCs suspended in 1ml media was subjected to sterility test for bacterial, fungal and other parasitic pathogen and certified contamination free. Absence of Mycoplasma species was confirmed via biochemical assay using luminescence method.

1.4 Endotoxin Test:

Number of endotoxins present in the cell composition was determined using Lonza PYROGENT gel-clot based assay, which was found to be 0.09 EU/mL. This is within the permissible limits of ophthalmic devices (0.5 EU/mL) as per US FDA regulations.

1.5 Other tests:

The characteristics of the limbal stromal stem cells, towards determining their safety, was assessed at molecular level as well; via transcriptomics. Experiments included:

1.5.1 RNA Extraction & DNase Treatment

Total RNA was isolated from tissues (conjunctiva &limbus) and cell cultures (primary culture& passage 3 of limbal stromal cell cultures) via using TRI reagent (Sigma-Aldrich, USA). About cell cultures, medium was removed from 80-90% confluent cell culture and washed with PBS-1x. Appropriate volume of TRI reagent was added to the cells. Cell lysate was passed several times through pipette and transferred to a sterile 1.5ml microcentrifuge tube. To the lysate, 0.2ml of chloroform was added per 1ml of TRI reagent, mixed by vortex and kept at room temperature for 15minutes followed by centrifugation at 12000g for 15minutes at 4°C. Aqueous phase was collected in a fresh tube and 0.5ml of 2-proponal was added per 1ml of TRI reagent, mixed by vortex and kept at room temperature for 10minutes followed by centrifugation at 12000g for 10 minutes at 4°C. RNA pellet was washed with 75% ethanol, air dried and dissolved in 20µl of nuclease free water. RNA was quantified in spectrophotometer and checked by 1% agarose gel stained with EtBr. RNA was treated with DNase I (RNase free; Ambion) according to manufacturers' protocol. Briefly, a 30µl reaction volume containing 30µg of total cellular RNA, 1x reaction buffer, 6U of DNase I (RNase free) and nuclease free water. Reaction was incubated at 37°C for 30minutes. After incubation 70µl DEPC water was added to the reaction and the RNA was purified by 100µl TRI reagent as described earlier.

1.5.2 Next Generation Sequencing

RNA-Seq analysis of all 4 samples have been done by RNAseq library preparation by using the TruSeq® Stranded Total RNA LT - (with Ribo-Zero Human/Mouse/Rat) kit (catalog # RS-122-2201) from Illumina as per given protocol along with all QC checks. We have performed a paired end sequencing run (50 x 2 cycles) on illuminaHiSeq 2500 platform. The Fastq file obtained from sequencer after trimming the adapter sequences using Bcl2fastq software (https://support.illumina.com/downloads/bcl2fastq_conversion_software_184.html). Fastq data have been used for alignment with hg19 version of human genome using Tophat (http://ccb.jhu.edu/software/tophat/manual.shtml) program with options provided as transcript annotation file. The alignment data have been used for guided transcript assembly using Cufflinks (http://cole-trapnell-lab.github.io/cufflinks/cufflinks/index.html) program. Transcripts are then merged across samples by using Cuffmerge (http://cole-trapnell-lab.github.io/cufflinks/cuffmerge/index.html) program to make a reference transcript assembly. This merged transcript assembly have been used as reference to compare for differential gene expression between a pair of samples with use of Cuffdiff (http://cole-trapnell-lab.github.io/cufflinks/cuffdiff/index.html) program. The Cuffdiff outfile provided us the normalized expression of genes/transcript in the form of FPKM and the fold differences converted into log2 values. In data analysis part, the counts obtained for each sample are analyzed using EBSeq for differential expression by setting the conjunctival tissue type as the control sample. After obtaining the list of differentially expressed genes for the tissues under an FDR of 0.05, we did a set of theoretic analyses to find genes that are exclusive in up-regulation or down-regulation to each tissue type or overlapping across tissue-types in case of oncogenes and tumor suppressor genes.

1.5.3 Real Time qPCR

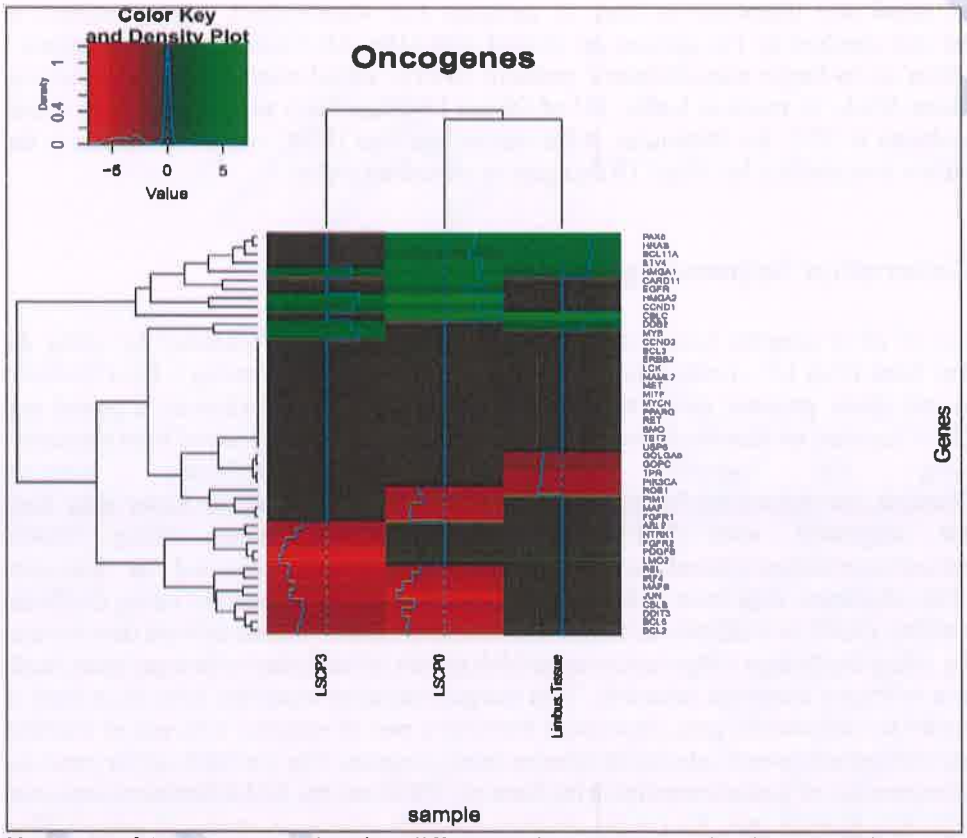
RNA (2µg) was reverse-transcribed using Superscript III (Invitrogen) according to the manufacturer's instructions. Then, cDNA (0.5 µL) was used for qPCR in a final volume of 10 µL with SYBR Green Reaction Mix (Invitrogen) and a 0.2 µM primer concentration. The qPCR was performed using Step One (Applied Biosystems, Life technologies) hardware and software. The expression level of target

genes (CD90, ABCB5, PAX6, Vimentin, CK3 and CK12) was represented as relative expression by using 2− pri formula.

1.5.4 Statistical Analysis

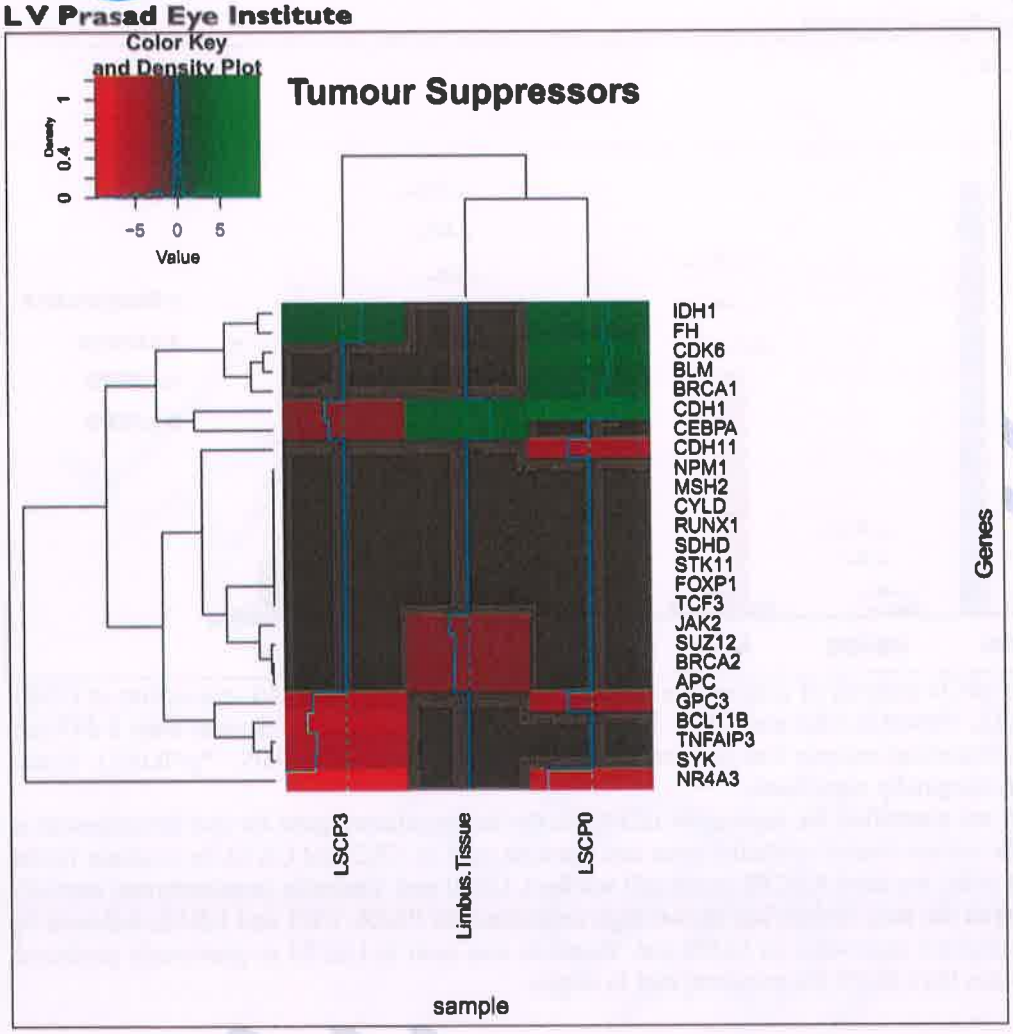
Experiments are performed in duplicate, at least 3 times. Student's two-tailed, paired, t- Test was run and p values ≤ 0.05 are considered statistically significant. All results are presented as the mean ± standard deviation (MD ± SD).

Results:

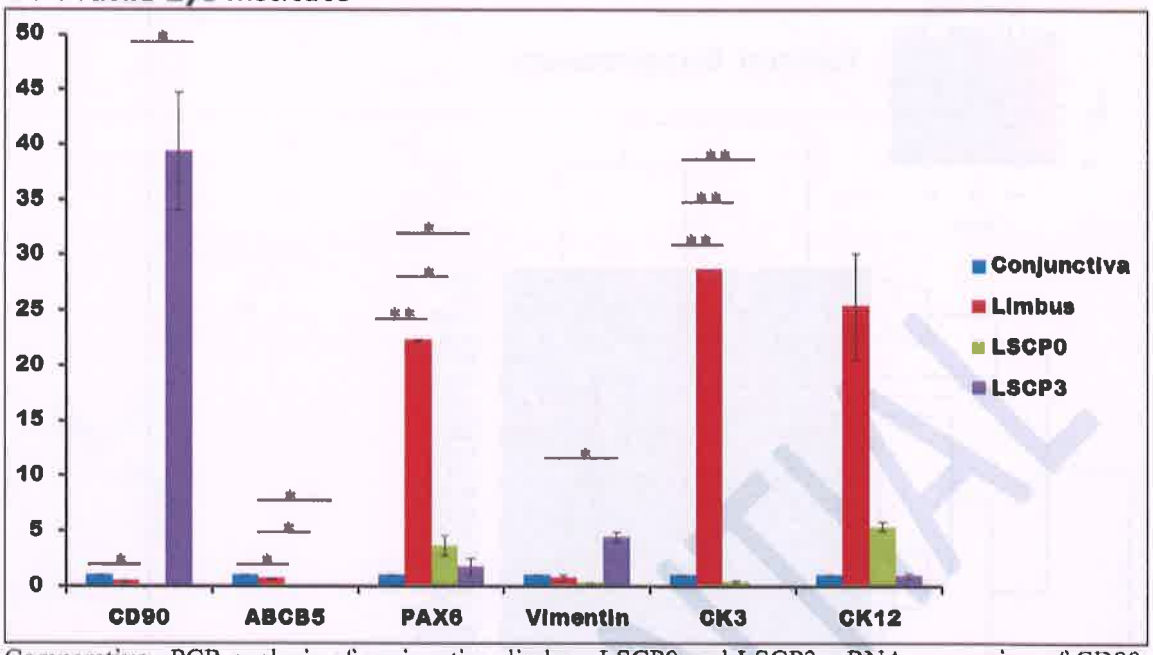


Heatmap of 47 oncogenes showing differences in gene expression between Limbus tissue, LSCP0 and LSCP3 represented in 3 different columns. The colors of the heat map representing single genes changing from blue to red across the column indicate enrichment of gene expression related to oncogenes. Highlighted green boxes in the table represent significant up regulated expression, red boxes represent significant down-regulate de expression and black color indicates no significant deference between any of limbus, LSCP0 and LSCP3 with respect to conjunctiva.

The above heat map shows most of the genes that belong to oncogenic pathway are not significantly expressed with few genes being significantly down regulated and few genes being significantly up regulated. The up regulation is around 2-4-fold change, whose fold of increase is not enough to be said that these genes lead to a tumorogenic pathway, leaving us to conclude that the cultures of Limbal tissue, LSCP0 and LSCP3would be safe for transplantation in terms of tumorigenicity.



RNA-Seq analysis of all 3 samples with respect to 25 tumor suppressor genes shows that most of these genes are not significantly expressed or showing down regulation, inferring that there is not a chance for tumor formation, which usually requires a high expression of tumor suppressor genes against the tumor inducing oncogenes.



Comparative qPCR analysis of conjunctiva, limbus, LSCP0 and LSCP3.mRNA expression of CD90, ABCB5, PAX6, Vimentin, CK3 and CK12. Results are presented as MD ± SD from **at least 3** different experiments. Statistical analysis was performed using two-tailed **t**-Tests (*p<0.05, **p<0.001). Values without * are marginally significant.

In qPCR part we quantified the expression of PAX6, the key regulatory gene for eye development as well as ocular surface limbal epithelial stem cell markers such as CK3 and CK12.To evaluate limbal stromal stem cells, we used ABCB5 (stem cell marker), CD90 and Vimentin (mesenchymal marker). When compared the rest, limbus has shown high expression of PAX6, CK3 and CK12; followed by LSCP0. The highest expression of CD90 and Vimentin was seen in LSCP3 as previously predicted, which concludes the LSCP3 are mesenchymal in origin.

2. ANIMAL STUDIES

Conventional allograft therapy for corneal scarring is widespread and successful, but donor tissue is not universally available, and some grafts fail owing to rejection and complications such as endothelial failure. We investigated direct treatment of corneal scarring using allogenic stem cells, a therapy that, if successful, could reduce the need for corneal grafts. Mesenchymal cells were expanded from small superficial, clinically replicable limbal biopsies of human cadaveric corneo-scleral rims. Limbal biopsy–derived stromal cells (LBSCs)expanded rapidly in media containing human serum, were highly clonogenic, and could generate spheres expressing stem cell genes (ABCG2, Nestin, NGFR, Oct4, PAX6, and Sox2). Human LBSCs differentiated into keratocytes expressing characteristic marker genes (ALDH3A1, AQP1, KERA, and PTGDS) and organized a thick lamellar stroma-like tissue containing aligned collagen and keratan sulfate proteoglycans when cultured on aligned

Nanofiber substrata. When engrafted into mouse corneal wounds, LBSCs prevented formation of light-scattering scar tissue containing fibrotic matrix components. The presence of LBSCs induced regeneration of ablated stroma with tissue exhibiting lamellar structure and collagen organization indistinguishable from that of native tissue. Because the limbus can be easily biopsied from either eye of an affected individual and LBSCs capable of corneal stromal remodeling can be expanded under xeno-free allogenic conditions, these cells present a potential for allogenic stem cell–based treatment

of corneal stromal blindness. The Study was conducted by scientist from Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, USA, Hyderabad Eye Research Foundation, L V Prasad Eye Institute, Hyderabad India and Department of Molecular Pharmacology and Physiology, Morsani College of Medicine, University of South Florida, Tampa, FL 33612, USA (20).

2.1 Study Design:

The purpose of this study was to determine if mesenchymal cells present in human corneal limbal biopsies (LBSCs) differentiate into corneal keratocytes in vitro and whether they can prevent corneal scarring in a murine model in vivo. In vitro, stem cell potential was
assessed using clonogenic potential, sphere formation, and expression of stem cell genes. Keratocyte differentiation was examined by gene and protein expressions and by secretion of typical stromal ECM. In vivo, LBSCs were introduced into mouse corneal debridement wounds, and the effect on corneal transparency, fibrotic ECM expression, and ECM ultra structural organization was assessed.

2.2 Statistical Analysis

Statistical analyses used two-sided tests and two-way ANOVA. In vivo experiments were designed to provide a power of 0.8 on the basis of results from our previous study [21] with animals randomized as to treatment. Collection and analysis of in vivo data were carried out with observers masked as to treatment groups. No adverse events were observed, and no data points were excluded from analyses.

2.3 Summary of Results

The summary of the pre-clinical test proves that Stromal cells can be obtained from limbal biopsies, LBSCs exhibit stem cell–like properties, LBSCs differentiate into keratocytes in vitro, Human LBSCs engraft in murine cornea in vivo, LBSCs promote regeneration of native stromal tissue during wound repair and LBSC treatment reduced corneal vascularization in mice.

Our data demonstrate that corneal stromal stem cells can be obtained from a small surface ocular biopsy. In the current study, we emulated a biopsy procedure, but clinical use of such biopsies to obtain limbal epithelial stem cells is well established [25, 26, 27]. The presence of mesenchymal cells in limbal biopsy tissue has been described [21]. The current study confirms that mesenchymal stem cells obtained from libel biopsies are functionally equivalent to corneal stromal stem cells we have previously described, on the basis of sphere forming ability (fig. S2), gene expression patterns (Fig. 2), and the ability to organize stromal ECM in vitro (Fig. 3). The importance of this finding is that we are now able to use a clinically established procedure to obtain autologous stem cells with regenerative potential. These cells also grow rapidly in HS, thus allowing production of a fully autologous, xenobiotic-free cell-based reagent. Recent reports have described mesenchymal stromal cells proximal to the epithelial basement membrane in limbal regions near epithelial stem cells. These mesenchymal "niche cells" are thought to help maintain the phenotype of limbal epithelial stem cells in vivo. Niche cells can form spheres in vitro and express stem cell genes, including ABCG2, SOX2, Nanog, OCT4, and Nestin. LBSCs isolated in this study from human limbal biopsies closely resembled niche cells. They grew more rapidly when only collagenase was used in their isolation and they were highly clonogenic, formed spheres, and

expressed genes characteristic of both adult and pluripotent cells. By these criteria, LBSCs, corneal stromal stem cells, and limbal niche cells appear indistinguishable and probably represent the same population of neural crest–derived mesenchymal stem cells.

An important advance contributed by this study is observation that LBSCs induced position of a native stromal tissue rather than scar tissue in healing wounds. Previous in vivo studies have used lumican-null mice, which develop stromal haze owing to abnormal collagen fibrillogenesis. Although the disruption of stromal matrix in these mice resembles that in scars, it is not evident that restoration of transparency tolumican-null cornea is fully analogous to remediation of corneal scars. In the current study, we found that human LBSCs in the healing wound allowed regeneration of a fully transparent native stromal tissue. The new tissue had no expression of fibrotic mRNA or proteins, had no change in light scatter, and had highly organized stromal ECM indistinguishable from that of normal mouse cornea.

Few mammalian tissues heal by regenerating native tissue. Scarring provides a strong and rapid structural repair, but tissue functionality can be impaired by scar tissue. This is particularly true for cornea in which collagen fibril diameter, parallel alignment, packing, and lamellar organization are essential for vision. Traumatic damage to stroma typically heals, leaving scar tissue with disorganized collagen that scatters light and produces long-term disruption of vision. The ability of LBSCs to induce the replacement of ablated tissue with transparent ECM containing native components with collagen organization indistinguishable from that of normal stroma is an exciting finding that points to the clear potential for use of these cells in clinical applications to treat human corneal scars. Here, LBSCs were engrafted only in the most anterior portion of the stroma and were in low abundance in comparison to the mouse stromal cells. Amelioration of fibrotic matrix, light scatter, and disruption of the stromal organization occurred both where LBSCs were located and in more posterior regions of the stoma. The lack of co localization of LBSCs and scarring suggests that LBSCs exert their effects indirectly. Rather than simply replacing mouse stromal ECM with that produced by the differentiated LBSC, these cells are likely exerting a paracrine influence on the mouse stromal cells repopulating the wounded region. The mechanism of this regenerative effect is unknown. The ability of the stem cells to work at a distance may be an advantage in cell-based therapy of existing scars, in that it may not be necessary to saturate scar tissue with stem cells but rather only to deliver them proximal to the region needing to be regenerated. Whether existing scars can be treated with these cells remains an open question. Much work over the previous decade has focused on corneal limbal epithelial stem cells for treatment of limbal stem cell deficiency, a potentially blinding but rare condition. Autologous limbal epithelial stem cells have been successfully used to correct this condition in several human trials. Corneal epithelium and stroma are structurally and functionally distinct tissues, and epithelial stem cells are not suitable for stromal therapy. However, the positive results of pioneering clinical trials with epithelial stem cells and the data presented in our current study strongly support the idea that autologous mesenchymal stem cells (the LBSCs) may be successful in treating stromal scarring, the major cause of corneal blindness in the world. Because these cells can be obtained and cultured in an autologous, xenobiotic free fashion and because fibrinogen-based adhesives are currently approved for ocular applications, barriers to bringing this treatment to clinical trial may be modest.

In summary, we have found a population of mesenchymal cells expanded from human limbal biopsy tissue with the potential to differentiate into keratocytes, to generate stromal tissue in vitro, to block corneal scarring, and to stimulate regeneration of transparent stromal
tissue in murine healing wounds in vivo. The ability to obtain cells from clinically reliable biopsies and to expand them in HS presents the opportunity to use these cells clinically to remediate cornea wounds and scars.

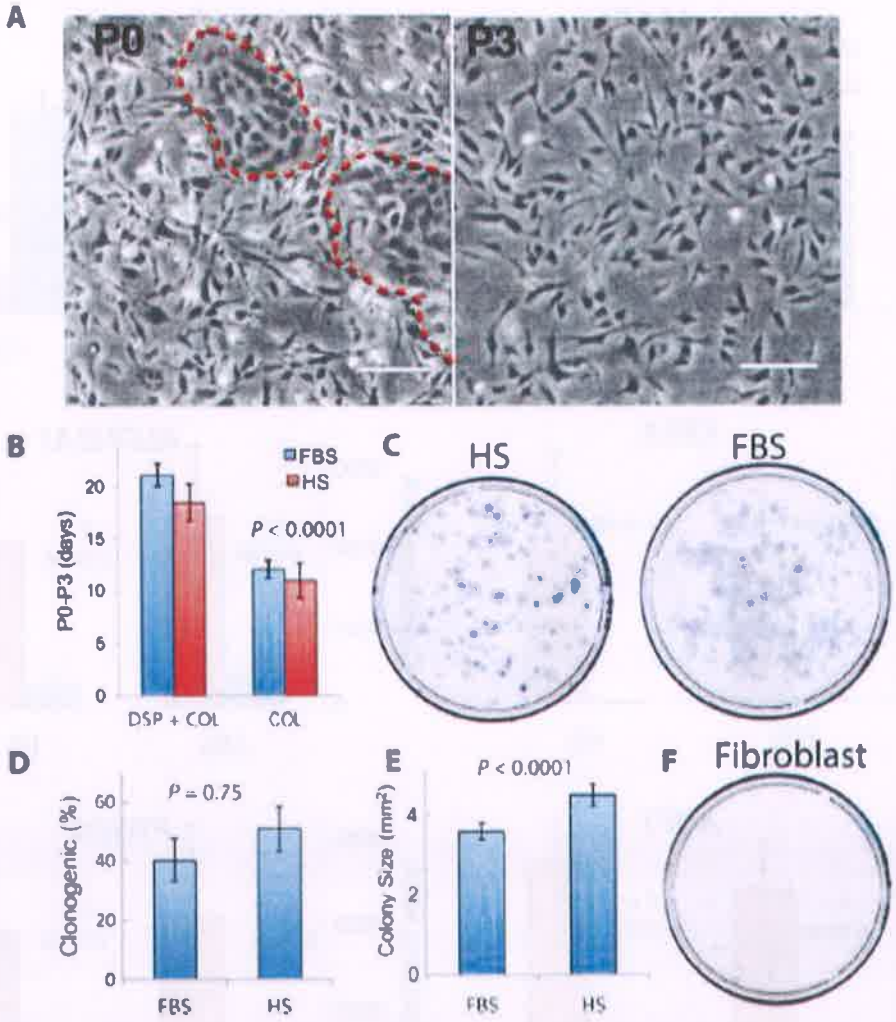


Fig. 1. Ex vivo expansion and clonogenicity of limbal biopsy stromal cells. (A) Phase-contrast images of primary cells cultured from limbal biopsy tissue prepared by digestion with collagenase only. In initial plating (P0), red dashed lines mark islands of epithelial cells. Scale bars, 50 μm. (B) The length of time (in days) required to expand cells from P0 to P3 was compared for LBSCs prepared with dispase and collagenase (DSP + COL) or collagenase only (COL), expanding cells in either HS or FBS. Data are means ± SD (n = 4). P value determined by two-way analysis of variance (ANOVA). (C) Clonal growth of LBSCs in HS and FBS. (D) Percentage of clonogenic cells in P3 cultures. Data are means ± SD (n = 4). (E) Colony size was calculated with Fiji image analysis software. Data are means ± SD (n > 400). P values in (D) and (E) were determined by a two-sided t test. (F) Corneal fibroblasts in FBS did not exhibit clonal growth (n = 4).

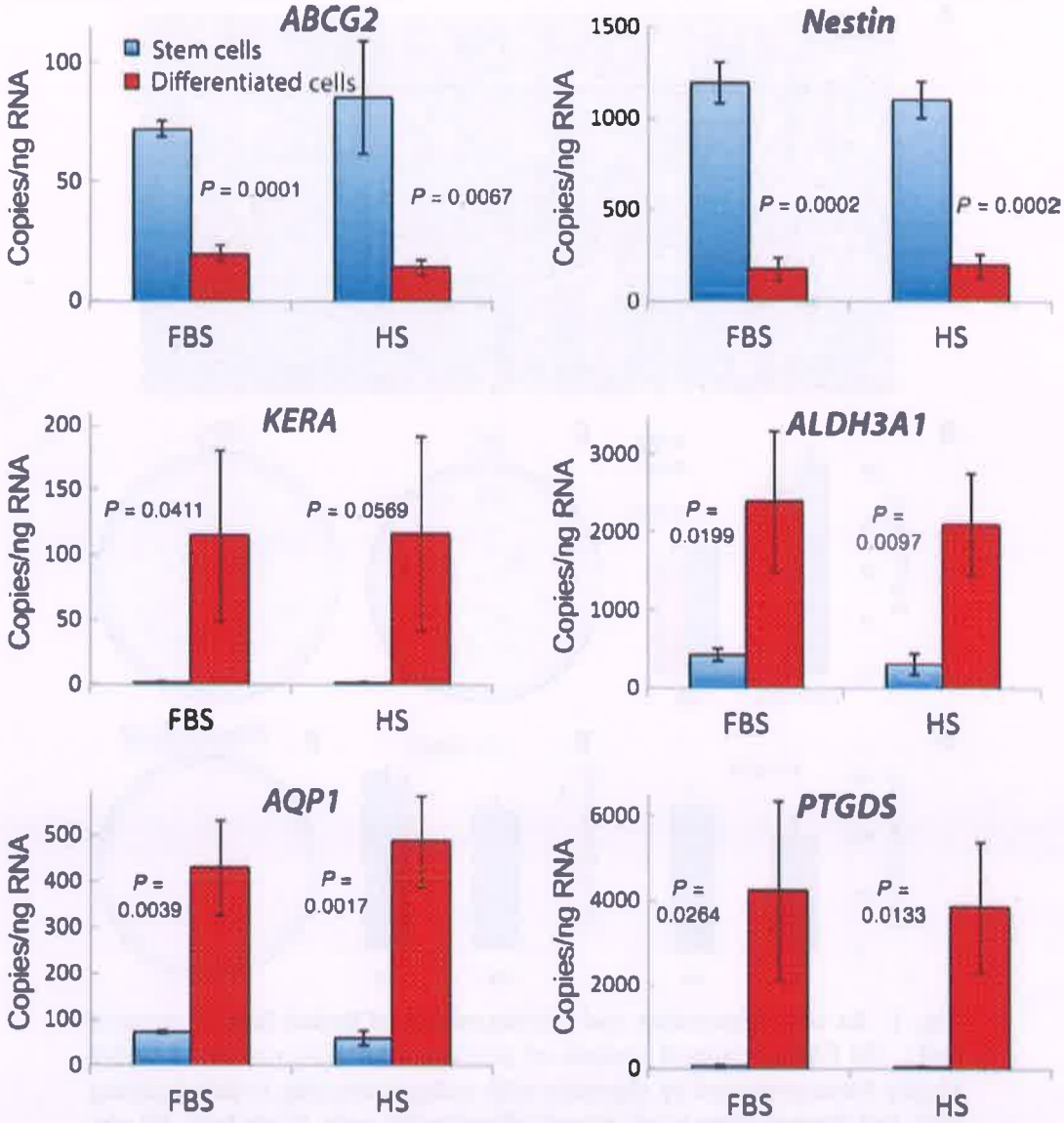


Fig. 2. Gene expression during ex vivo differentiation of LBSCs. LBSCs expanded to P3 in FBS or HS were cultured in differentiation conditions on collagen gels for 1 week. mRNA was quantified as copies per nanogram of total cellular RNA, determined by quantitative polymerase chain reaction (qPCR). Data are averages ± SD from four different cell lines, each obtained from a different donor cornea. *P* values were determined by two-sided *t* test.

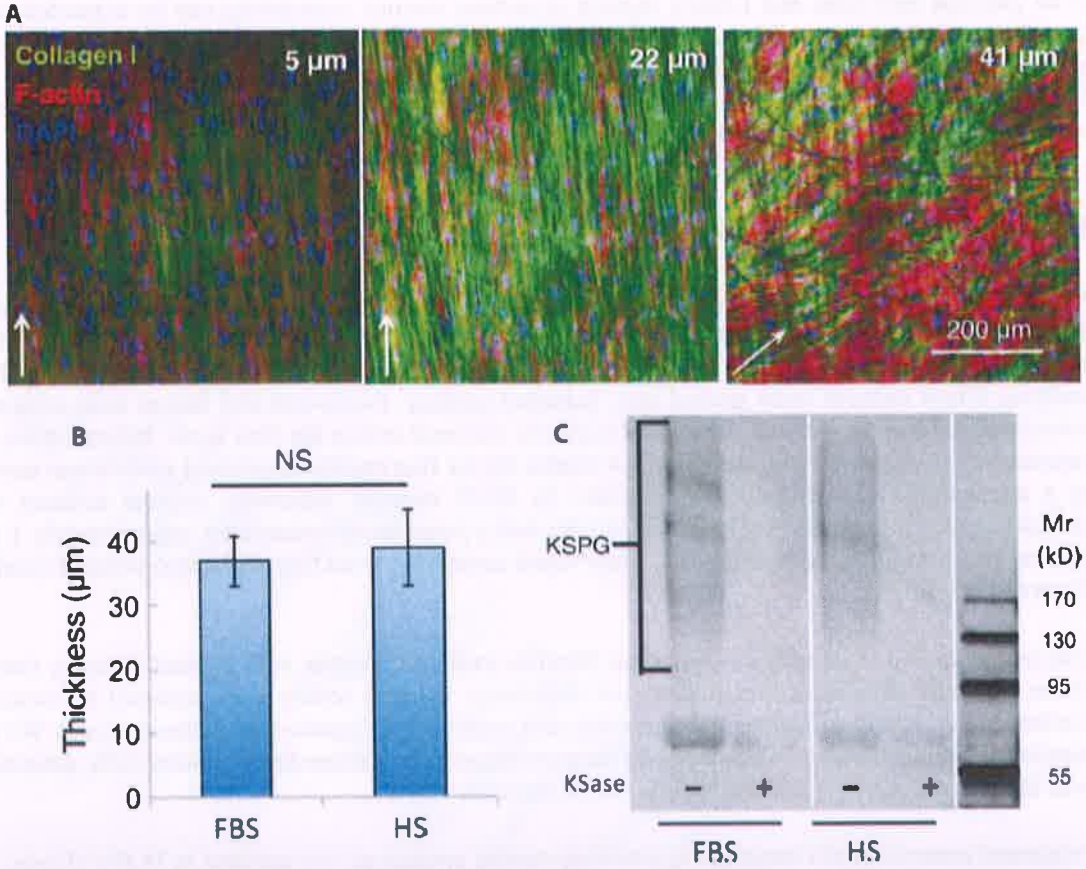


Fig. 3. Generation of a stroma-like three-dimensional matrix ex vivo. ECM produced by LBSCs cultured on aligned nanofiber substrate for 4 weeks was imaged by confocal microscopy capturing optical sections at different *z* levels above the substratum. (A) Type I collagen fibrils (green) and keratocytes (nuclei, blue; F-actin, red) are shown at different depths of the construct. (B) Thickness of collagenous matrix at 4 weeks in HS or FBS was determined from confocal analysis. Data in (B) show averages ± SD from cell lines from four different donors. Lack of significance (NS; $P > 0.05$) was determined by a two-sided *t* test. (C) Cornea-specific keratan sulfate proteoglycan (KSPG) was detected by immunoblotting. Alternate lanes show sensitivity of the heterogeneous KSPG band (130 to 300 kD) to keratanase (KSase). M_r, relative molecular mass.

1. The direct treatment of corneal scarring using autologous stem cells, a therapy that, if successful, could reduce the need for corneal grafts, was investigated earlier. Mesenchymal cells were expanded from small superficial, clinically replicable limbal biopsies of human cadaveric corneo-scleral rims. Limbal biopsy–derived stromal cells (LBSCs) expanded rapidly in media containing human serum, were highly clonogenic, and could generate spheres expressing stem cell genes (**ABCG2**, **Nestin**, **NGFR**, **Oct4**, **PAX6**, and **Sox2**). Human LBSCs differentiated into keratocytes expressing characteristic marker genes (**ALDH3A1**, **AQP1**, **KERA**, and **PTGDS**) and organized a thick lamellar stroma-like tissue containing aligned collagen and keratan sulfate proteoglycans when cultured on aligned nanofiber substrata. When engrafted into mouse corneal wounds, LBSCs prevented formation of light-scattering scar tissue containing fibrotic matrix components. The presence of LBSCs induced regeneration of ablated stroma with tissue exhibiting lamellar structure and collagen organization indistinguishable from that of native tissue. Because the limbus can be easily biopsied from either eye

of an affected individual and LBSCs capable of corneal stromal remodeling can be expanded under xeno-free autologous conditions, these cells present a potential for autologous stem cell–based treatment of corneal stromal blindness.

Ref: Basu, Sayan, et al. "Human limbal biopsy–derived stromal stem cells prevent corneal scarring." Science translational medicine 6.266 (2014): 266ra172-266ra172.

3. CLINICAL EXPERIENCE

A homogeneous group of patients whose limbal cell deficiency was evaluated by scoring the gravity of the clinical picture and the keratin expression pattern. Stem cells, obtained from the limbus of the contralateral eye, were cultivated onto a fibrin substrate and their preservation was evaluated by clonal analysis. Fibrin cultures were grafted onto damaged corneas. Fibrin-cultured limbal stem cells were successful in 14 of 18 patients. Re-epithelialization occurred within the first week. Inflammation and vascularization regressed within the first 3-4 weeks. By the first month, the corneal surface was covered by a transparent, normal-looking epithelium. At 12-27 months' follow-up, corneal surfaces were clinically and cytologically stable. Three patients had a penetrating keratoplasty approximately 1 year after restoration of their corneal surface. Their visual acuity improved from light perception or counting fingers to 0.8-1.0. [18]

Autologous limbal stem cells cultivated on fibrin to treat 112 patients with corneal damage, most of whom had burn-dependent limbal stem-cell deficiency. Clinical results were assessed by means of Kaplan–Meier, Kruskal–Wallis, and univariate and multivariate logistic-regression analyses. We also assessed the clinical outcome according to the percentage of holoclone-forming stem cells, detected as cells that stain intensely (p63-bright cells) in the cultures.

Permanent restoration of a transparent, renewing corneal epithelium was attained in 76.6% of eyes. The failures occurred within the first year. Restored eyes remained stable over time, with up to 10 years of follow-up (mean, 2.91±1.99; median, 1.93). In post hoc analyses, success — that is, the generation of normal epithelium on donor stroma — was associated with the percentage of p63-bright holoclone-forming stem cells in culture. Cultures in which p63-bright cells constituted more than 3% of the total number of clonogenic cells were associated with successful transplantation in 78% of patients. In contrast, cultures in which such cells made up 3% or less of the total number of cells were associated with successful transplantation in only 11% of patients. Graft failure was also associated with the type of initial ocular damage and postoperative complications. Cultures of limbal stem cells represent a source of cells for transplantation in the treatment of destruction of the human cornea due to burns.

Ref: Rama, Paolo, et al. "Limbal stem-cell therapy and long-term corneal regeneration." New England Journal of Medicine 363.2 (2010): 147-155

4. PRODUCT INFORMATION

4.1 GENERAL INFORMATION

Therapeutically accepted and serologically tested cadaveric corneas, within four days of collection, are obtained from The Ramayamma International Eye Bank (LVPEI, Hyderabad, http://eyebank.lvpei.org). These corneas were washed with 1.25mM penicillin-streptomycin with Phosphate Buffer saline, pH (7.4), for 3mins followed by another wash *with* PBS. Iris and endothelial layer were scrapped for visibility. Complete 360° limbal rims will be isolated using a surgical blade in buffer saline and chopped for using a small, curved corneal scissors, in incomplete media (plain DMEM media, Sigma-Aldrich, D0567). The tiny limbal tissues pieces will be subjected to collagenization by adding 20µL of reconstituted Collagenase-IV (17104019, Thermofischer, at the rate of 10IU/µL of Collagenase-IV; per every 1 ml of incomplete media) to the incomplete media. Incubation was carried out for16hours at 37°C in 5% CO_2 chamber.

Post 16-hour incubation, the enzymatic digestion will be stopped by adding 2ml of complete media (with 2% FBS). The collagenised tissue fragments would then spin down three times at 1000rpm for 3minutes, at room temperature, in saline. 3ml complete media (plain DMEM media, Sigma-Aldrich, with 2% fetal bovine serum, with added epidermal growth factor and insulin) was added to the pellet and kept to culture with media being replaced for every 2days. P_0 cells (as shown inFig1) are purely limbal epithelial cells. No stromal cells grow in passage P_0. Stromal cells initiate at P_1 generation and pure culture of stromal cells is obtained from P_2 onwards (as shown in Fig2).

Figure 1

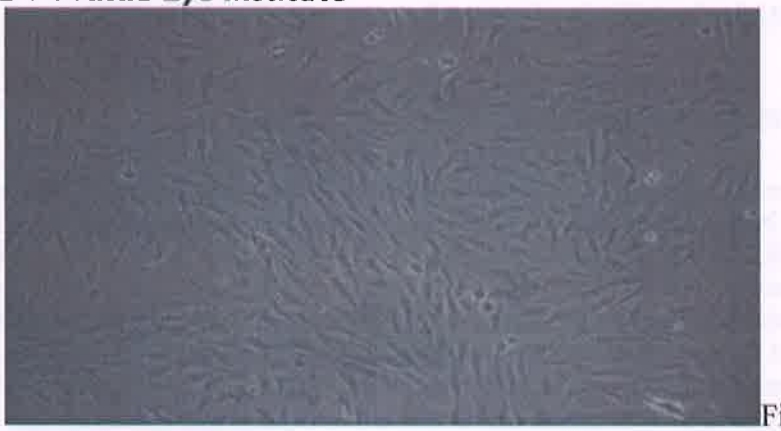


Figure 2

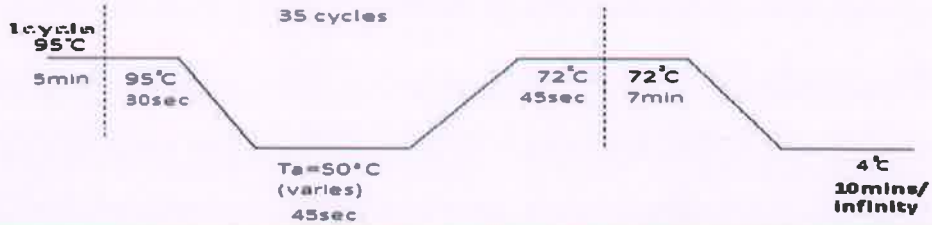
4.2 PREPARING CELLS FOR TRANSPLANTATION

Cells adhered to surface of confluent culture flask are washed with 1x PBS and then subjected to trypsinization at by adding 1ml of TrypLE™ (12563011, Gibco®) and incubating them at 37° C in a 5% CO_2 chamber, for about 2 minutes. Cells are then spinned down at 1300rpm for 4 minutes, at room temperature and checked for viability using 0.4% sterile and filtered Trypan blue dye. Post viability check, cells in media is spin down again at 1000rpm for 3 minutes, at room temperature in a microcentrifuge tube. The resultant pellet is 100µl of thrombin component of the commercially available TISSEEL Lyo™ will be taken into a 1ml tuberculin syringe (needle gauze 24-27) and applied onto the defective portion of the patient's eye. Pure populations of stromal cells are suspended in the thrombin component so that they could form a matrix whereas a pool of limbal epithelial cells alone or mixed populations of both limbal epithelial and limbal stromal stem cell niche is mixed with the fibrin component. Subjects would then be transplanted with 100-500 thousand stromal cells.

Flow chart for the production of allogenic ex vivo stromal stem cells are included in Section 21.1

4.3 STERILITY CHECKS

Prior to transplantation a sample volume of 20µl from the 1ml cells suspension were subjected to sterility test for bacterial, fungal pathogens by inoculating them on contamination free certified blood agar and thiglycolate media, (at Jhaveri Microbiology Center, LVPEI).
Absence of *Mycoplasma* species was confirmed via PCR, by generating template DNA from the cells in media of the confluent flasks. The set of primers used are Pro16sF5' GGTAATACATAGGTCGCAAG 3'; Pro16sR 5' GTAAGA GGCATGATT



4.4 RELEASE CRITERIA

S. No.	Test	Test Method	Specification
1.	Description	Microscopic Observation	Cells shall be spindle/dendritic shape in active growing condition. Cells shall be intact and round in shape after detachment from the adhered surface
2.	Cell Count	Dye Exclusion using Hemocytometer	0.5 X 10^6 cells /mL + 20%
3.	Cell Viability	Dye Exclusion using Hemocytometer	≥ 70 %
4.	Bacterial Endotoxin*	Gel clot	≤ 0.125EU/mL
5.	Mycoplasma*	Biochemical	Not Detected
6.	Bioburden Test*	Direct Inoculation	No CFU

* Performed on spent medium.

5. STUDY RATIONALE:

To evaluate the safety and efficacy of ex-vivo cultivated allogenic limbal stromal stem cells for the treatment of visually significant superficial corneal stromal scarring and other pathologies.

This study proposes to investigate the transplantation of ex-vivo cultivated allogenic limbal stromal cells for the treatment of the corneal pathologies. The limbus is an ideal source as the stem cells are numerous and located very superficially in the tissue [17]. Pre-clinical work suggests human corneal stromal stem cells can be isolated from the cadaveric tissues, cultivated in conditions suitable for cell based therapy and used to prevent fibrosis in a murine model of corneal stromal scarring. Further, these cells are able to successfully engraft, differentiate, and mediate wound healing in the corneal stroma such that the tissue remains healthy, free of fibrotic tissue, and optically transparent. The clinical implications of these findings are substantial in that it represents the potential to lessen the burden on donor tissue necessary for corneal allografts by using cultured cells to regenerate tissue. We foresee the ability of a clinician to and grow and expand the cells in number and after surgically removing the scar tissue from the wounded eye, apply the cultured allogenic limbal stem cells to regenerate healthy, transparent tissue.

6. STUDY OBJECTIVES:

6.1 Primary Objective:

To evaluate the safety of ex-vivo cultivated allogenic limbal stromal stem cells for the treatment of visually significant superficial corneal stromal scarring and other pathologies including any ocular or systemic adverse effects at the various post-operative time points.

6.2 Secondary objective (s):

The secondary outcome measures are:

1. Visual improvement using ETDRS Vision Chart.
2. Change in the density and appearance of the corneal scarring and other pathologies after treatment.

7. ENDPOINTS:

7.1 Safety Endpoints

Safety will be assessed through collection of adverse events (AEs), any local or systemic toxicities as determined by clinical and laboratory evaluations. Safety endpoints will be:

1. Local toxicities will be assessed clinically by the presence of increased inflammation and vascularization at the surgery site more than expected because of surgery.
2. Change in corneal thickness from baseline (on the day of surgery) as measured using pachymetry and subsequently evaluated from Month 3 onwards at every visit.
3. Increase in intra-ocular pressure, can be done digitally at every visit and by applanation tonometry from Month 3 onwards at every visit.
4. Systemic toxicities will be determined using clinical laboratory assessments covering hematology, blood chemistry and urinalysis work-up at baseline, Months 3, 6, 12 and Month 24.

7.2 Efficacy Endpoints:

1. Proportion of eyes showing two-line improvement in BCVA (Best Corrected Visual Acuity) from baseline to last follow-up visit using ETDRS Vision Chart.
2. Change in the corneal haze after treatment using clinical photography and scheimpflug imaging.

8. STUDY DETAILS

8.1 Study Design:

This would be a single-center prospective, open labeled, non-randomized interventional study. This study is an Investigator Initiated Study. The Ethics Committee of the LV Prasad Eye Institute,

Hyderabad would prospectively approve this study. This study would be conducted in strict adherence to the tenets of the Declaration of Helsinki, Indian GCP Guidelines and Schedule Y of Drugs and Cosmetics Act and associated amendments and Ethical Guidelines for Biomedical Research on Human Participants and current National Stem Cell Research Guidelines.

Once the participants are found to be suitable for limbal transplant surgery, the patients will be administered written informed consent and audio /visual consent as per regulations. Detailed ophthalmic examination will be done to ensure that the patient is eligible for the trial.

On Day 0 that is the date of surgery a Unique Participant Identification Number (UPIN) will be assigned to each patient and which would be in addition to hospital medical record number. The surgery will be done under local or general anesthesia (depending on age and patient preference).

In this prospective interventional study patients with unilateral superficial corneal scars will undergo a surgical procedure. Limbal ring from a cadaveric donor tissue, which is therapeutically accepted and serologically tested, is collected. This tissue will then be cultivated in the stem cell biology laboratory using standardized culture technique. Briefly the limbal tissue will be cut into small pieces and digested overnight using an enzyme (Collagenase I.). The cells obtained from the digest will be cultured on a petri-dish using 2% serum and growth factors. The cultured cells will be passaged three times to remove all epithelial cells from the culture and to obtain only stromal cells.

In the second procedure, the eligible patients will undergo corneal transplant surgery, in this procedure, first the central corneal epithelium will be removed using a surgical sponge and 0.1ml of stromal cells at a concentration of $5x10^3$ cells/µl diluted in the thrombin component of fibrin glue (TISEEL, Baxter) will be applied to the debrided corneal stroma. A soft bandage contact lens will be placed over the cornea at the end of the procedure. The patient will receive topical antibiotic and steroid eye drops in the post-operative period. Periodic comprehensive ophthalmic evaluation along with anterior segment optical coherence tomography (ASOCT) scanning and slit-lamp photography will be done at Day 1, Day 7, Day 30, Day 90, Day 180, Day 360 and Day 720 post-surgery. The primary outcome measure of this study is to note any ocular or systemic adverse effects of this intervention at the various post-operative time points. The secondary outcome measures are visual improvement and change in the density and appearance of the corneal scarring and other pathologies after treatment.

8.2 Study Assessments:

8.2.1 Safety Assessments

Safety variables to be assessed will be adverse events (AEs), clinically significant changes in laboratory values (hematology, clinical chemistry and urine examination), changes in ocular surface (corneal erosions, vascularization or conjunctivalization), change in corneal thickness and IOP, and eye pain.

All AEs whether considered procedure related or not, will be recorded in source documentation and on the CRF with a diagnosis, start/stop dates, action taken, whether study drug was discontinued. For all events, the relationship to treatment and the severity of the event will be determined by the Investigator, using the terms and definitions given in Section 12 of this protocol.

All observations pertinent to the safety of the investigational membrane will be recorded in source documentation and on the CRF and included in the final report. Parameters that will be assessed are:

8.2.1.1 Corneal Edema:

Corneal swelling will be measured by pachymetry at every visit from month 3 follow up. The baseline corneal thickness measurement will be taken on the day of surgery after removal of the fibrovascular pannus.

8.2.1.2 Intra-Ocular Pressure:

Intraocular pressure (IOP) is the fluid pressure inside the eye. Tonometry is the method eye care professionals use to determine this. IOP is an important aspect in the evaluation of patients at risk from glaucoma. Most tonometers are calibrated to measure pressure in millimeters of mercury (mmHg).

Intraocular pressure is measured with a tonometer as part of a comprehensive eye examination. Standard applanation tonometry will be used.

For this study, considering the clinical status of the patients, it will not be always possible to measure the intra-ocular pressure at baseline, therefore it will be done digitally (to get a rough estimate) at every visit starting at baseline and additionally by applanation tonometry from Month2 onwards at every visit. Digital tonometry is done by pressing gently against the eyeball with the person looking down. Although not quantitative like the applanation tonometer, it allows the clinician to identify the presence of abnormally high ocular tension.

8.2.1.3 Ocular Pain:

Magnitude of pain will be assessed as patient reported outcome using Visual Analogue Scale (VAS) as using tool in Appendix 3 at every visit.

8.2.1.4 Schirmer's test (5 minute) without anesthesia

Schirmer's test uses paper strips inserted into the eye for several minutes to measure the production of tears. Both eyes are tested at the same time. The test consists of placing a small strip of filter paper on the outer 1/3rd of the lower eyelid (conjunctival sac). The eyes are closed for 5 minutes. The paper is then removed and the amount of moisture is measured. This technique measures the total tear secretion. This test will be performed at screening and at every visit after that month 3.
A young person normally moistens 15 mm of each paper strip. Because hypolacrimation occurs with aging, 33% of normal elderly persons may wet only 10 mm in 5 minutes. Persons with Sjögren's syndrome moisten less than 5 mm in 5 minutes which is considered to be dry eye.

How to read results of the Schirmer's test:

Normal	≥15 mm wetting of the paper after 5 minutes.
Mild dry eye	14-9 mm wetting of the paper after 5 minutes.
Moderate dry eye	8-4 mm wetting of the paper after 5 minutes.
Severe dry eye	<4 mm wetting of the paper after 5 minutes.

8.2.1.5 Laboratory Evaluations

Blood and urine samples will be collected for the analysis of laboratory parameters (hematology, biochemistry and complete urine examination) from all patients at screening, Day 180 and Day 360and Day 720 or End of Study Visit. The volume of blood collected will depend on the institutional local laboratory requirement. The volume of biological sample collected from each participant will be included in the Patient Information Sheet and Informed Consent Form.

Hematology assessments will include CBC (red blood cell [RBC], hemoglobin, hematocrit, platelet count, and WBC count with differential [neutrophil, lymphocyte, monocyte, basophil and eosinophil counts]).

Biochemistry assessments will include serum creatinine, blood urea, random blood glucose and electrolyte panel (sodium, potassium, and chloride).

8.2.1.6 Vital Signs

Vital signs and demographics will be noted during screening visit to ensure eligibility of the participant for the study.

Brief Physical Examinations will be done at Screening and End of Study Visit.

A comprehensive medical history including demographics, prior surgery, prior history of corneal transplant, allergy, and baseline signs and symptoms will be collected at Screening from all participants.

Detailed ophthalmic examinations at screening and at every follow-up visit

Any worsening of the baseline symptoms and change in patient's clinical status will be checked at all visits.

8.2.1.7 Electrocardiogram

Patients who are ≥40 years and who would require to be operated under general anesthesia will undergo 12-lead electrocardiogram to rule out any cardiac problems that would interfere with the surgical procedure prior to surgery.

8.2.2 Efficacy Assessments

8.2.2.1 AS-OCT

AS-OCT helps cornea and glaucoma specialists follow, diagnose and treat their patients. Cross-sectional images are most commonly used to review the cornea, angle and anterior chamber. Images of the cornea are performed on patients with keratoconus, corneal scars and corneal dystrophies. Both manual and automatic corneal pachymetry measurements quantify corneal disease. Images of the angle are commonly performed to quantify the angle for angle closure glaucoma and attempt to identify the scleral spur, Schlemm's canal, Schwalbe's line and trabecular meshwork. Anterior chamber biometry is helpful for refractive surgery. Imaging the iris can document iris cysts, iris nevus, iris melanoma and iridoschesis.

AS-OCT is an excellent preoperative and postoperative tool to evaluate and manage patients with: blebs, intrastromal corneal rings, full-thickness penetrating keratoplasty (PK), descemet-stripping endothelial keratoplasty (DSEK), deep lamellar endothelial keratoplasty (DLEK), IOLs and laser-assisted in situ keratomileusis (LASIK) etc.

In this study AS-OCT will be performed at baseline and at from Month 3 onwards at all visits.

8.2.2.2 Slit Lamp Biomicroscopy

Slit lamp biomicroscopy will be done at baseline and at every subsequent visit to examine the ocular surface of the patient's eye.

The procedure to be followed will be as detailed below:

Patient will be seated on the examination chair and rest his/her chin and forehead on a support to steady the head. Using the bio-microscope, the ophthalmologist or optometrist will then examine the patient's eye for transparency. Each layer (epithelium, Bowman's membrane, stroma, Descemet's, and endothelium) of the cornea will be systematically examined for focal opacities and scars, edema or haze, and any other abnormalities (e.g., endothelial keratic-precipitates: KPs).

The following scale will be used to describe this aspect of the cornea:

0	Absence of active inflammation
1	Stable (old) scar or structural change
2	Presence of active inflammation (corneal stroma) or evolving structural change in the corneal stroma including focal scarring (deep, mid, or superficial corneal stroma) with or without corneal surface change/irregularity.

Further examination of the ocular surface will be conducted using fluorescein dye by instilling the dye into the inferior conjunctival cul-de-sac of each eye. Ocular staining will be evaluated between 2 and 4 minutes after the instillation of the fluorescein at a slit lamp magnification of 16X using cobalt blue illumination.

No dye will be taken up when the epithelium is intact. In the presence of epithelial erosions, the underlying stromal layer will take up the dye thus clearly marking the regions from where epithelium is lost. The presence of fibrovascular tissue can be identified by the stippled appearance of the dye on the ocular surface. The dye is naturally rinsed out of the eye by tears.

The following scale will be used to describe the fluorescein staining of the cornea.

0	Normal	No epithelial fluorescein staining is present. A sharply focused light reflex is present over the entire corneal surface. There is no stromal haze (iris details clearly seen) or increase in corneal thickness
1	Mild	Minimal diffuse or focal epithelial fluorescein staining is present. A slightly irregular light reflex is present. There is no stromal haze or increase in corneal thickness. Fine, diffuse punctuate epithelial keratitis (PEK)

2	Moderate	Minimal diffuse or focal epithelial fluorescein staining more than 50% of corneal surface is present and associated with an irregular surface light reflex. Stromal haze may be present and corneal thickness may be increased. Fine and coarse PEK, involvement of deep epithelium.
3	Severe	Minimal diffuse or focal epithelial fluorescein staining more than 50% of corneal surface is present and associated with an irregular surface light reflex. And or focal sub epithelial immune infiltrates (SEIs) may be visible in the anterior cornea. And or stromal haze is definitely present with some blurring of iris details and corneal thickness is increased. Coarse, granular infiltrates (PEK) within deep epithelium.

A subsequent test may involve placing drops in the eye in order to dilate the pupils. The drops take about 15 to 20 minutes to work, after which the examination is repeated, allowing the back of the eye to be examined. It will not be possible to conduct this examination preoperatively due to the corneal but will be done at baseline and from Month 3 onwards in all patients.

8.2.2.3 Slit Lamp Photography

Slit lamp photography is used to document the microscopic and obscure details of the transparent, translucent and opaque structures of the anterior segment and surrounding areas of the eye.
Often conditions affecting the anterior segment of the eye are of a subtle nature and can only be documented using a Slit Lamp Biomicroscope with an attached camera. Slit Lamp Photography utilises a variety of magnifications, angles of view and types of illumination to highlight the areas of interest. This is especially useful in following progression or changes in specific pathology such as new vessels, cataracts and pterygium.
The slit lamp photography will be performed at baseline and at every visit after the first follow up at Dat 7 onwards.

8.2.2.4 Best Corrected Visual Acuity:

BCVA (Best Corrected Visual Acuity) at baseline and Month 1 to Month 6 at all follow-up visits using ETDRS will be assessed.

8.2.3 Study Flow Chart

Activity	Screening (within 14±7 days prior to surgery)	Day 0(Day of surgery)	Day 1 (Post-Surgery)	Day 7 (± 3) Post-surgery	Day 30 (± 15) Post-Surgery	Day 90 (± 15) days Post-Surgery	Day 180 (± 15) days Post-Surgery	Day 720 (± 15) days Post-Surgery
Informed Consent	X							
Demographics	X							
Vitals	X						X	X
Eligibility Criteria	X	X						
12-lead ECG[1]								
Medical History/Con Meds	X							
Change in Medical history/Con Meds/baseline signs and symptoms		X	X	X	X	X	X	X
Detailed Ophthalmic Examination	X				X	X	X	X
Pain using VAS-Scale	X		X	X	X	X	X	X
BCVA	X				X	X	X	X
Slit lamp biomicroscopy	X			X	X	X	X	X
Slit lamp photography	X		X	X	X	X	X	X
IOP by Applanation Tonometry	X		X	X	X	X	X	X
Corneal densitometry	X		X	X	X	X	X	X

[1]Prior to surgery if the patient is ≥ 40 years to rule out any cardiac ailments will be done once prior to surgery

AS-OCT	X	X	X	X	X	X	X	X
Review Discontinuation Criteria		X	X	X	X	X	X	X
Assign UPIN		X						
Surgery		X						
Discharge from Hospital			N/A					
Safety Monitoring		X	X	X	X	X	X	X
Clinical Laboratory Assessments: Hematology/Biochemistry/Urinalysis	X						X	X
Record Final clinical assessment								X

8.3 STUDY PROCEDURES

8.3.1 Screening Period-Within 14±7days prior to surgery

The Informed Consent Document must be signed prior to any study-related procedures being performed. Entry in the study is defined as the point when Informed Consent form is signed-off by the participant. If a patient is found to be ineligible for the study based on results of the screening procedures, then that patient will be considered as screening failure and will be replaced. Patients enrolled to participate in the study are eligible for surgery. Once patients are enrolled they will not be replaced.

All participants screened and enrolled into the study will be listed on the Screening and Enrollment log.

Clinical assessment prior to surgery for corneal epithelial transplant:
Assessments outlined below should be done during the Screening period. Prior to the surgery, the following procedures will be performed for all participants and the results are to be available and reviewed to confirm continued eligibility:

1. Administer informed consent
2. Collect Demographic Data
3. Complete detailed Ophthalmic examination
4. Record Vitals
5. 12-Lead ECG will be done for patients ≥ 40 years of age to rule out any cardiac ailments
6. Record any clinically significant baseline signs and symptoms
7. Collect Complete Medical History, Concomitant Medication and OTC (Over–the –Counter) medicines data
8. Baseline AS-OCT
9. Slit lamp biomicroscopy
10. Schirmer's Test
11. Slit Lamp photography
12. Ocular pain by VAS
13. IOP
14. Baseline Visual Acuity (BCVA) Testing for corrected uncorrected vision using ETDRS
15. Confirm diagnosis for Superficial Corneal Scar and eligibility of the patient
16. Baseline Laboratory tests

Patients who are eligible for the study and agree to participate will undergo comprehensive systemic evaluations within 28-42 days prior to the surgery. The following additional tests will be administered:

1. Collect vital signs data: Temperature, Blood pressure, heart rate etc.
2. Reconfirm Eligibility criteria

8.3.2 Day 0 -Day of Surgery

The following procedures will be performed:

1. Confirm eligibility criteria.
2. If eligible, assign Unique Patient Identification Number (UPIN).
3. Any change in baseline symptoms medical history and concomitant medication
4. Ascertain discontinuous criteria
5. Surgery will be performed under local or general anesthesia in accordance with the patient's age, preference and general condition. Below surgical steps will be followed for corneal transplant surgery:
   - Central corneal epithelium will be removed using a surgical sponge
   - 0.1ml of stromal cells in a concentration of $5x10^3$ cells/uL diluted in the thrombin component of fibrin glue (TISEEL, Baxter) will be applied to the debrided corneal stroma.
   - A soft bandage contact lens will be placed over the cornea at the end of the procedure.
   - The patient will receive topical antibiotic and steroid eye drops in the post-operative period.
6. Participants will be observed and monitored for one-day post-surgery in the hospital and then discharged.
7. The follow-up visits post-surgery will be on Day 7 (± 3), Day30 ((± 15), Day 90(± 15) days,180 (± 15) and 360 (± 15). Final follow-up visit (End of Trial) will be scheduled at Month 12/DAY 360 (± 15) days post-surgery.

8.3.3 Day 1 -Post surgery

The following procedures will be performed:

1. Monitor the post-surgical progress
2. Review discontinuation criteria
3. Record any change in medical history, concomitant medications and baseline signs and symptoms
4. Perform ophthalmic examination whatever examination may be possible
5. Slit Lamp Photography
6. IOP if possible
7. Ocular Pain by VAS
8. Review Discontinuation criteria
9. Record any safety concerns

10. Discharge the patient if stable post-surgery

8.3.4 Days7 (± 3)

The following procedures will be performed:

1. Monitor post-surgical progress
2. Review discontinuation criteria
3. Record any change in medical history, concomitant medications and baseline signs and symptoms
4. Complete Ophthalmic examination
5. AS-OCT if possible
6. IOP if possible
7. Ocular Pain by VAS
8. Slit lamp biomicroscopy
9. Slit lamp Photography
10. BCVA Visual Acuity if possible
11. Record any safety concerns
12. Check Density of scarring and other ocular pathologies

8.3.5 Days 30 (± 15), Day 90 (± 15 days)

1. Monitor post-surgical progress
2. Review discontinuation criteria
3. Record any change in medical history, concomitant medications and baseline signs and symptoms
4. Complete Ophthalmic examination
5. AS-OCT if possible
6. IOP by Tonometry
7. Pachymetry
8. Schirmer's test
9. Ocular Pain by VAS
10. Slit lamp biomicroscopy
11. Slit lamp Photography
12. BCVA Visual Acuity if possible
13. Record any safety concerns
14. Check Density of scarring and other ocular pathologies

8.3.6 Day 180 (± 15 days), Day 360 (± 15 days)

1. Monitor post-surgical progress
2. Review discontinuation criteria
3. Record any change in medical history, concomitant medications and baseline signs and symptoms
4. Complete Ophthalmic examination
5. AS-OCT if possible
6. IOP by Tonometry
7. Pachymetry
8. Schirmer's test
9. Ocular Pain by VAS
10. Slit lamp biomicroscopy
11. Slit lamp Photography
12. BCVA Visual Acuity if possible
13. Laboratory parameters
14. Record any safety concerns
15. Check Density of scarring and other ocular pathologies

8.3.7 End of Study/ Month 24 (±15 days)

1. Monitor post-surgical progress
2. Record any change in medical history, concomitant medications and baseline signs and symptoms
3. Complete Ophthalmic examination
4. AS-OCT
5. Slit lamp biomicroscopy
6. Slit lamp Photography
7. BCVA Visual Acuity
8. IOP by Tonometry
9. Pachymetry
10. Schirmer's test
11. Ocular Pain by VAS
12. Record any safety concerns
13. Record final clinical assessment
14. Laboratory parameters

15. Record any safety concerns
16. Check Density of scarring and other ocular pathologies

9. STUDY POPULATION

This study will include 20 (Male and Female) participants, aged between 18-60 years, who have unilateral blindness due to various corneal pathologies like superficial (defined as involving the anterior 200μM of the corneal stroma on ASOCT imaging) corneal ulcers, burns, scars. All the patients who will be included in this study would need to undergo stem cell transplantation and they who would not require any second intervention.

Patient excluded from the study would be those with bilateral corneal disease, Corneal scars with limbal dysfunction (clinically defined as absent limbal palisades or conjunctivalization of the cornea) or ocular surface disease including dry eye disease (defined as a Schirmer's test of less than 10mm at 5 minutes), Unknown etiology, post-herpetic eye disease or eyes with active intra-ocular inflammation, Children (<18 years of age), Less than 3 months after documented clinical resolution of acute disease and Inability/refusal to give written informed consent or to undergo any of the anterior segment imaging tests. Patient should have not participated in another clinical study within 30 days of their enrolment on this study.

Those who sign informed consent and meet all the entry criteria will be enrolled to the study. Participants will be screened for eligibility for study entry based on their ophthalmic presentation. Thorough ophthalmic screening will be done prior to enrollment in the study which will include a detailed clinical examination to ascertain the ocular disease status and other underlying causes for reduced vision. Screening for systemic conditions and health of the participant will be done prior to the surgery as per Institute practice. All the screening procedure should be accomplished within 14±7 days from the date the participant signs the informed consent.

9.1 Subject Eligibility

9.1.1 Inclusion Criteria

1. Male and female participants who are ≥18 and ≤ 60 years of age.
2. Patients having unilateral superficial corneal pathologies (defined as involving the anterior 200μM of the corneal stroma on ASOCT imaging)
3. Corneal burns, ulcers and scars
4. No prior history of corneal transplantation
5. No ongoing and other active ocular pathology
6. Candidate for stem cell transplant
7. No severe pathological and psychological conditions that might interfere with the patient's participation in the study
8. Able to provide written and audio-visual informed consent prior to any study specific screening procedures with the understanding that the patient has the right to withdraw from the study at any time, for any reason without prejudice.

9.1.2 Exclusion Criteria:

1. Bilateral corneal disease,
2. Corneal scars with limbal dysfunction (clinically defined as absent limbal palisades or conjunctivalization of the cornea),
3. Ocular surface disease including dry eye disease (defined as a Schirmer's test of less than 10mm at 5 minutes),
4. Unknown etiology, post-herpetic eye disease or eyes with active intra-ocular inflammation,
5. Children (<18 years of age),
6. Less than 3 months after documented clinical resolution of acute disease,
7. Inability/refusal to give written informed consent,
8. Undergo any of the anterior segment imaging tests,
9. Patient should have not participated in another clinical study within 30 days of their enrolment on this study,
10. History or evidence of cardiac disease: congestive heart failure; New York Heart Association (NYHA) class 2 or greater (see Appendix 6); active coronary artery disease; unstable angina, cardiac arrhythmias requiring anti-arrhythmic therapy, atrio-ventricular block of second or third degree, or uncontrolled hypertension, patients with recent (less than 6 months) myocardial infarction (MI) or coronary revascularization,
11. Pregnant and lactating patients, positive urine pregnancy test in women of childbearing potential,
12. Reproductive age patients not practicing effective and adequate birth control measures,
13. Previous participation in this study.

9.1.3 Patient Discontinuation

A patient has the right to discontinue participation and withdraw his/her consent at any time for any reason without prejudice to future medical care by the Investigator or other physician at the institution. It is recommended that participants remain assigned to the study until the Investigator believes that doing so is no longer beneficial. The Principal Investigator should decide when the patient should be discontinued from the study pre-maturely.

The Investigator has the right to discontinue a patient's participation in this study in the event of an intercurrent illness, protocol violation, or other reasons including, but not limited to, those described below:

- Any severe local or systemic toxicity observed
- Any AE which, in the Investigator's opinion, requires discontinuation
- At the request of the patient and withdrawal of consent
- Participants get pregnant. Pregnancy will not be reported as a SAE but specific reporting requirements must be followed as stated in Section12.9

- Use of illicit drugs or other substances that may, in the opinion of the Investigator, contribute to toxicity.
- The patient is lost to follow-up.
- Safety concerns as directed by the investigator

The Investigator will make every reasonable effort to keep each patient on the study, unless it is in the patient's best interest to discontinue. Every reasonable effort will be made to follow the patient to measure study outcomes as End of Study (EOS) Visit

Participants who are not enrolled, but sign informed consent and undergo at least some of the screening procedures will be considered screening failures. A record of these participants will be maintained by the study site.

If any patient should die or suffer any life-threatening adverse event meeting the "Serious" criteria during the trial or within 60 days of surgery, Investigator will inform the Institution Ethics Committee, Institute Committee of Stem Cell Research as per process detailed in Section 12 of this protocol.

In all cases, the reason for withdrawal must be recorded in the CRF and in the patient's medical records.

Participants who discontinue after enrolment will not be replaced.

10. PROTOCOL WAIVERS

Every effort should be made to ensure that the protocol required tests and procedures are completed as described. However, it is anticipated that from time to time there may be circumstances, outside of the control of the Investigator, which may make it unfeasible to perform the test/procedure as per schedule. In such cases the Investigator will take all steps necessary to ensure the safety and well-being of the patient. When a protocol required test cannot be performed the Investigator will document the reason for this and any corrective and preventive actions which he/she has taken to ensure that normal processes are adhered to as soon as possible.

11. DATA SAFETY MONITORING BOARD

The Data Safety Monitoring Board (DSMB) will act in an advisory capacity to the LV Prasad Eye Institute (LVPEI) to monitor participant safety, data quality and evaluate the progress of the study.

The DSMB will concentrate on progress of the trial, adherence to the protocol, patient safety and consideration of new information of relevance to the research question, with safety and well-being of the trial participants their foremost consideration. The DSMB Members should be such so as to have at least 2 members independent of the study. The independent members should be clinicians with experience of involvement with clinical trials, of which one should be an ophthalmologist. Each member will have appropriate training and experience to clinically and/or statistically assess safety data or adverse events. The CVs of all DSMB members should be on file, should indicate the relationships between the independent members to ensure an independence of views, and their role in the DSMB. The Chair should be independent member for the LVPEI/Hyderabad Eye Research Foundation to review and approve.

The DSMB will make recommendations to the study sponsor (LVPEI/HERF) on continuation, modification, or cessation of the study in conjunction with the Medical Monitor/Safety Officer and Statistician

DSMB members should be bound by confidentiality obligations.

A plan for the meeting frequencies for the DSMB has been included in a Charter.

Study Stopping Rule:

Safety Analysis will be performed at each study visit. The stopping rule would be to not recruit a second patient until the first patient is followed up for safety concerns for at least one-month post-surgery. Follow the same procedure for the first three patients. Abort the study in consultation with the DSMB if there are severe toxicities observed at one month follow up or earlier post-surgery for any of these first three patients.

12. ADVERSE EVENTS REPORTING

12.1 Adverse Events

The Investigator is responsible for the monitoring of participants' safety and reporting of all AEs and SAEs.

The study personnel will review participants' volunteered information related to subjective complaints, but in addition, will review all laboratory findings and query the patient specifically for visits requiring hospitalization, procedures, laboratory follow-up or unscheduled visits to see their physician or specialists. Each trial patient will be questioned about AEs during the study.

Adverse events, whether reported in response to a query, observed by site personnel, or reported spontaneously by the patient will be documented in the patient's source documents as well as on the appropriate CRF. The Investigator will assess and record any AE in detail on the AE CRF/CRF pages including a description of the event, the date of onset, severity, time course, duration and outcome, relationship of the AE to study procedure, whether the relationship to synthetic scaffold was related, possibly related, probably related, or not related and any actions taken. All AEs should be followed to resolution or stabilization.

12.2 Adverse Event Definitions

An adverse event is any untoward medical occurrence in a patient or clinical investigation patient subject to any procedure or investigational agent, which could be a drug or device. The AE does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational agent, whether or not considered related to that product.

Adverse events associated with the use of an investigational product in humans, whether or not considered product related, include the following:

- An AE occurring in the course of the use of an investigational product in professional practice.
- An AE occurring from an overdose whether accidental or intentional.
- An AE occurring from drug abuse.
- An AE occurring from drug withdrawal.

An AE where there is a reasonable possibility that the event occurred purely as a result of the participants' participation in the study (e.g., AE or SAE due to discontinuation of anti-hypertensive drugs during washout phase) must also be reported as an AE even if it is not related to the investigational product.

The clinical manifestation of any failure of expected pharmacological action is not recorded as an adverse event if it is already reflected as a data point captured in the CRF. If, however, the event fulfills any of the criteria for a "serious" AE, it must be recorded and reported as such.

12.3 Serious AEs (SAE) Definition

An SAE is any untoward medical occurrence that:

- Results in death.
- Is life threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly or birth defect.
- Is an important medical event.

Life threatening: The term "life threatening" in the definition of "serious" refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an AE which hypothetically might have caused death if it were more severe.

Hospitalization: Any AE leading to hospitalization or prolongation of hospitalization will be considered as serious, UNLESS at least 1 of the following exceptions are met:

- The admission results in a hospital stay of less than 12 hours.

OR

- The admission is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), and documented as such in the patient's screening source documents.

OR

- The admission is not associated with an AE (e.g., social hospitalization for purposes of respite care).

However, it should be noted that invasive treatment during any hospitalization may fulfill the criteria of 'medically important' and as such may be reportable as an SAE dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.

Disability means a substantial disruption of a person's ability to conduct normal life's functions.

Important medical event: Any adverse event may be considered serious because it may jeopardize the patient and may require intervention to prevent another serious condition. As guidance for determination of important medical events refer to the World Health Organization Adverse Reaction Terminology – Critical Terms List (WHOART). These terms either refer to or might be indicative of a serious disease state.

Such reported events warrant special attention because of their possible association with a serious disease state and may lead to more decisive action than reports on other terms.

An isolated laboratory abnormality that is assigned Grade 4, according to Common Toxicity Criteria (CTC) definition, is not reportable as an SAE, unless the Investigator assesses that the event meets standard International Council on Harmonization (ICH) criteria for an SAE. CTC Grade 4 baseline laboratory abnormalities that are part of the disease profile should not be reported as SAEs, specifically when they are allowed or not excluded by the protocol inclusion/exclusion criteria. In addition to the above the following will not be considered as SAEs:

i. An admission to the hospital for respite care;

ii. Hospitalization planned before entry into the clinical study for elective treatment of a condition unrelated to the study indication or its treatment; hospitalization occurring on an emergency/outpatient basis and not resulting in admission);

iii. Part of the normal treatment regime or monitoring for the study indication and not associated with any deterioration in condition (. e.g. hospitalization to avoid skin photosensitivity);

iv. Hospitalization not requiring formal admission.

v. Hospitalization for routine diagnostic procedures for a pre-existing condition, which is recorded on the history of the case report form (CRF).

12.4 Unexpected Adverse Event

An unexpected AE is any adverse product or protocol related event, the specificity or severity of which is not consistent with the current IB (or Package Insert) for the Allogenic ex vivo stromal Stem cells, or other concomitant medication. Also, reports which add significant information on specificity or severity of a known, already documented AE constitute unexpected AEs. For example, an event more specific or more severe than described in the IB would be considered "unexpected". Specific examples would be; (a) acute renal failure as a labeled AE with a subsequent new report of interstitial nephritis and (b) hepatitis with a first report of fulminant hepatitis.

12.5 Relationship of Adverse Event to Investigational Product

The Investigator's assessment of causality must be provided for all AEs (serious and non-serious); the Investigator must record the causal relationship in the CRF, as appropriate, and report such an assessment in accordance with the SAE reporting requirements if applicable. An Investigator's causality assessment is the determination of whether there exists a reasonable possibility that the investigational product caused or contributed to an AE. If the Investigator's causality assessment is

"unknown but not related to investigational product," this should be clearly documented on study records.

Adverse events will be assessed as related, possibly related, probably related, or not related to Study Drug.

Related	The AE follows a reasonable temporal sequence from treatment and is consistent with the IB.
Probably	The AE follows a reasonable temporal sequence from study treatment, and cannot reasonably be explained by the known characteristics of the participant's clinical state.
Possibly	The AE follows a reasonable temporal sequence from study treatment, but could have been produced by the participant's clinical state or by other modes of therapy administered to the participant.
Not Related	The AE is definitely produced by the participant's clinical state or by other modes of therapy administered to the participant.

Factors to be considered in assessing the relationship of the AE to study drug include:

- The temporal sequence from drug administration: The event should occur after the drug is given. The length of time from drug exposure to event should be evaluated in the clinical context of the event.
- Recovery on discontinuation (de-challenge), recurrence on reintroduction (re-challenge): Patient's response after drug discontinuation (de-challenge) or participant's response after drug re-introduction (re-challenge) should be considered in the view of the usual clinical course of the event in question.
- Underlying, concomitant, intercurrent diseases: Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the patient may have.
- Concomitant medication or treatment: The other drugs the patient is taking or the treatment the patient receives should be examined to determine whether any of them may be suspected to cause the event in question.

12.6 Severity of the Adverse Event

Severity is a clinical determination of the intensity of an adverse event. The severity assessment fora clinical adverse event should be completed using the following definitions as guidelines:

Mild:	Awareness of sign or symptom, but easily tolerated
Moderate	Discomfort enough to cause interference with usual activity
Severe:	Incapacitating with inability to work or do usual activity
Not applicable	In some cases, an adverse event may be an "all or nothing" finding, which cannot be graded

12.7 Adverse Event Collection Period

All AEs occurring after signing of informed consent will be fully recorded in the patient's AE/SAE tracking worksheets. AEs/SAEs should be collected and reported after the patient has signed the Informed consent and until 60 days' post-surgery or until resolved

Documentation must be supported by an entry in the patient's file. A laboratory test abnormality considered clinically relevant, e.g., causing the patient to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged relevant by the Investigator, should be reported as an adverse event. Each event should be described in detail along with start and stop dates, severity, relationship to investigational product, action taken and outcome.

12.8 Clinical Trial Related Injury or Death

Any injury caused to or death of the participant due to his/her participation in clinical trial will be considered as clinical trial related injury or death and the participant or his/her nominee(s), as the case may be will be entitled for financial compensation for such injury or death. The definition of clinical trial related injury or death will be in accordance with the prevailing compensation law issued by the government of India.

12.9 Reporting Serious Adverse Events

Since this is an Investigator Initiated Study, the primary responsibility of reporting Serious Adverse Events generated in the clinical study lies with the Lead Principal Investigator. As per the existing safety reporting guidance issued by the Indian regulatory authority, irrespective of causal relationship to therapy all Serious Adverse Events have to be reported to the DCGI (Drug Controller General (India) using the prescribed format (Appendix XI and XII of Schedule Y of drugs and Cosmetics Act of 1940 (31). The format is included as Appendix 3 of this protocol)

12.9.1 Reporting of Fatal SAEs:

Any SAE, including laboratory test abnormalities, clinical trial related injury or death, regardless of causal relationship, must be immediately recorded in CRF AE/SAE forms within 24 hours after the Investigator becomes aware of the SAE. The SAE will be reported to the Institutional Ethics Committee Chairman and Institute Stem Cell Research Committee Chairman, Sponsor and RA (DCGI) within **24 hours**.

Investigator to reportfatalSAEwithin**24 hours** of becoming aware [as per APPENDIX XI and XII of Schedule Y] to

i. Sponsor/CRO,
ii. Chairman of EC/IEC /IC-SCR
iii. DCGI

Investigator to submit the analysis report (causality assessment) within **10 days** of becoming aware of the event to [as per APPENDIX XI & XII of Schedule Y]:

i. Sponsor/CRO (if applicable)

ii. Chairman of EC/IEC/IC-SCR

iii. Head of Institute

iv. DCGI

v. Chairman of Expert Committee

12.9.2 Reporting of Non-Fatal SAEs

Investigator to report non-fatal SAEwithin**24 hours** of becoming aware [as per APPENDIX XI and XII of Schedule Y] to:

i. Sponsor/CRO,

ii. Chairman of EC/IEC/IC-SCR

iii. DCGI

Investigator/Sponsor to submit the analysis report (causality assessment) within **10 days** of becoming aware of the event to [as per APPENDIX XI & XII of Schedule Y]:

i. Chairman of EC/IEC,

ii. Head of Institute

iii. DCGI

The contact details sending notifications:

Sponsor:

Dr. Sayan Basu, MBBS, MS
Consultant Corneal Surgeon and Scientist
Hyderabad eye Research Foundation
LV Prasad Eye Institute,
Kallam Anji Reddy Campus
Banjara Hills
Hyderabad -500034
Telangana
Phone: +91 9989479969
e-mail-sayanbasu@lvpei.org

Dr. Vivek Singh, Ph.D.,
Scientist), Prof. Brien Holden Eye Research Center,
Champalimaud Translational Centre for Eye Research
Hyderabad eye Research Foundation
LV Prasad Eye Institute,
Kallam Anji Reddy Campus
Banjara Hills
Hyderabad -500034

LV Prasad Eye Institute
Telangana
Phone: +91 90522 04108
e-mail-viveksingh@lvpei.org

EC Chairman:

Justice. C. Rangarajan
Former Judge,
High Courts of Madras and Andhra Pradesh
Blue Lotus #103,
Road No. 3, Banjara Hills
Hyderabad 500 034
Phone: +91402354 6787 Mobile: +91939 100 5635
Email: justicerangarajan@gmail.com

DCGI Contact details:

The Drugs Controller General (India)
Directorate General of Health Services
Central Drugs Standard Control Organization
FDA Bhawan, Kotla Road, New Delhi – 110 002
Fax +911123236973 email dci@nb.nic.in

Detailed and complete Follow-up information relating to a SAE must be reported similarly to the DCGI and Institute EC and IC-SCR within **10 days** of its occurrence to the at the address provided above along with pre-screening check-list (Appendix XII of Schedule y of D &C (1940)) and procedure defined gazette notification dated February 8th 2013. The patient should be observed and monitored carefully until the condition resolves or stabilizes.

All deaths are to be thoroughly investigated and reported. Autopsy reports are to be obtained, if possible.

The Lead Investigator is also responsible for reporting SAEs to the Ethics Committees overseeing the study within the required timeframe.

In case it is established by the EC, DCGI Expert Committee and DCGI, that the injury is related to the Investigational Product or study procedure, the sponsor will provide compensation to the trial participant and intimate the details of the compensation provided within **30 days** of release of such order by the DCGI on the quantum of compensation to be provided.

12.10 Reporting Pregnancy occurring in the Clinical Trial

All pregnancies occurring in the clinical trial in participants or partners of participants will be reported in the same manner and within the same timelines as serious adverse events.

Report of In-utero Investigational Exposure must be completed for all pregnancies and sent to the IEC and the lead investigator must follow each pregnancy to term and report outcome to IEC and DCGI, even if the birth and baby are "normal".

13. ETHICS

13.1 Ethical Conduct of the Study

Documented approval from appropriate IRB/IEC will be obtained for participating clinical site prior to study start, according to GCP, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the IRB approval must be obtained.

This clinical study will be conducted in accordance with the principles of the Declaration of Helsinki, and in compliance with the International Conference on Harmonization (ICH) E6 Good Clinical Practice (GCP) Consolidated Guideline and current Schedule Y of Indian Drugs and Cosmetics Act (1940) other regulations and guidelines as applicable. The Investigator and all clinical study staff will conduct the clinical study in compliance with the protocol. The Investigator will ensure that all personnel involved in the conduct of the study are qualified to perform their assigned responsibilities through relevant education, training, and experience.

Before clinical study initiation, this protocol, the informed consent form (and assent form, if applicable), any other written information given to Participants, and any advertisements planned for patient recruitment must be approved by an Institution Ethics Committee/Institutional Review Board/Institute Committee of Stem Cell Research (IEC/IRB/IC-SCR). The Investigator must provide documentation of the IEC/IRB approval to the Sponsor. The approval must be dated and must identify the applicable protocol, amendments (if any), informed consent form, assent form (if any), all applicable recruiting materials, written information for patient, and patient compensation programs.

The IEC/IRB/IC-SCR must be provided with a copy of the Investigator's Brochure, any periodic safety updates, and all other information as required by local regulation and/or the IEC/IRB/IC-SCR. At the end of the study, the Investigator will notify the IEC/IRB/IC-SCR about the study's completion. The IEC/IRB/IC-SCR also will be notified if the study is terminated prematurely. Finally, the Investigator will report to the IEC/IRB/IC-SCR on the progress of the study at intervals stipulated by the IEC/IRB/IC-SCR.

The written approval of the IEC/IRB/IC-SCR together with the approved patient information/Informed Consent Forms must be filed in the study files.

If translated versions of Subject Informed consents are being used, translations should be done by certified and qualified translators and translated versions have to be approved by IEC/IRB/IC-SCR before use.

Written informed consent must be obtained before any study specific procedure takes place. Participation in the study and date of informed consent given by the patient should be documented appropriately in the patient's files.

13.2 Patient Informed Consent:

Voluntary informed consent will be obtained from every patient (and/or legal representative, as applicable) prior to the initiation of any screening or other study-related procedures. The Investigator must have a defined process for obtaining consent. Specifically, the Investigator, or designee, will explain the clinical study to each potential patient and the patient must indicate voluntary consent by signing and dating the approved informed consent form. The patient must be provided an opportunity to ask questions to the Investigator, and if required by local regulation, other qualified personnel. The

Investigator must provide the patient with a copy of the consent form written in a language the patient understands. The consent document must meet all applicable local laws and will provide participants with information regarding the purpose, procedures, requirements, and restrictions of the study, along with any known risks and potential benefits associated with the investigational product, the available compensation, and the established provisions for maintaining confidentiality of personal, protected health information. Participants will be told about the voluntary nature of participation in the study and will be provided with contact information for the appropriate individuals should questions or concerns arise during the study. The patient also will be told that their records may be accessed by regulatory authorities and Sponsor-designated personnel. The Investigator must keep the original, signed copy of the consent and must provide a duplicate copy to each participant.

14. Benefit –Risk Evaluation:

14.1 Benefits:

1. Patient haze will get cleared, improving their visual acuity which finally leads to better vision.
2. These patients generally, have no cure or any clinical intervention other than going for Penetrating Keratoplasty (whole/partial corneal transplant depending on the region of damage).

14.2 Risks:

1. There are chances that haze may not get cleared and then patient has to go the regular/standard PK (which is the current method of clearing haze for these patients)
2. As it is well known, that cornea is immune privileged, and our treatment is confined to superficial layers only, there are no chances of any immune risks.

15. REGULATORY APPROVAL

This study is an Investigator Initiated Study (IIS). The LBSCs will be processed at the GMP facility of L.V. Prasad Eye Institute.

Approval will be obtained from the Institutional Ethics Committee and the Institute Committee for Stem Cell Research. Further regulatory approval will be obtained from the Biological Division of the Central Drugs Standard Control Organization (CDSCO), Ministry of Health and Family Welfare, Government of India, before study initiation and patient accrual.

The study will be registered with the Clinical Trials Registry India (CTRI).

This study will comply with Schedule Y of Drugs and Cosmetics Act (1940) including the recent amendments that have been introduced. It will be governed by the ICMR Ethical Guidelines for Biomedical Research on Human Participants issued in 2017. The study will also comply with the effective National Stem Cell Research Guidelines issued by ICMR 2017.

Manufacturing Licence will be obtained to manufacture small quantities for testing from the State FDA office in Form -29.

16. INSURANCE

This prospective Investigator Initiated Study (IIS) under the leadership of Principal Investigator, Dr. Sayan Basu and Dr. Vivek Singh. The study is being financially supported by Hyderabad Eye Research Foundation. The study will be conducted at L.V Prasad Eye Institute, Hyderabad, India.

All participants participating in the study will have clinical trial insurance coverage which will be procured by the Institute/Lead Investigator to cover any compensation/cost due to Clinical Trial related injuries or deaths. /Investigator, which is in line with applicable laws and/or regulations.

17. STUDY MONITORING AND SUPERVISION

This is an Investigator Initiated Study, where there is no provision of independent monitoring by a CRO. However, the lead investigator has the choice of hiring an independent clinical monitoring agency to monitor the study for quality and data integrity and compliance with regulations and guidelines mentioned above.

18. DATA COLLECTION AND STATISTICAL ANALYSIS

The Data Collection Forms called Case Report Forms (CRF) will be used to capture the data collected from the study. The management of all data collected and the statistical analysis will be performed by LV Prasad Eye Institute, Hyderabad.

18.1 Sample Size Determination

The primary objective of the study is to evaluate the clinical safety and efficacy of Ex Vivo Cultivated Allogenic Limbal Stromal Stem Cells used for the treatment of superficial Corneal Scar during corneal transplant surgery.

The sample size of this study has been computed to estimate the proportion of patients who achieve complete restoration of ocular surface with sufficient precision. With 20 evaluable patients in the study, and assuming a 50% chance of complete restoration at the end of the 12 months' study period, the half-width of the 90% confidence interval around the proportion of patients with complete restoration will be 0.26. Since the lower limit of the confidence interval will be above 0, the proportion of patients with complete restoration of ocular surface can be estimated with sufficient precision in this study.

18.2 Analysis Populations

18.2.1 Safety Population

Patients who undergo surgery successfully will be followed-up for all safety and toxicity assessments for the duration of the time they are on study.

18.2.2 Efficacy Population

All patients who have slit lamp biomicroscopy done at baseline undergo surgery successfully and complete the month 6 visit, will be evaluated for restoration of ocular surface, 2-line improvement in BCVA and other efficacy assessments. Missing month 6 data may be imputed using previous post-surgery efficacy data, if appropriate.

Patients who undergo surgery and complete at least Month 3 evaluation visit will be considered evaluable for efficacy.

18.2.3 Safety Analysis

Safety will be determined by assessment of local and systemic toxicities on the patients and summarized appropriately.

18.2.4 Efficacy Analysis

18.2.4.1 Primary Efficacy Analysis

The primary efficacy endpoint of restoration of ocular surface will be assessed by computing the proportion of patients who achieve complete restoration after 6 months of transplantation. A 2-sided 90% confidence interval will be constructed around this proportion to compute the precision of the estimate. A Kaplan-Meier survival curve will be plotted to assess the outcome of the transplantation after 6 months.

18.2.4.2 Secondary Efficacy Analysis

The proportion of patients showing 2-line improvement in BCVA from baseline until last follow-up visit will be computed along with 90% CIs. The proportions of patients with other improvements or deterioration in BCVA will also be computed.

18.2.5 Data Imputation

Missing efficacy and safety data may be imputed in certain cases, if found appropriate. Details on imputations, data derivations and transformations, etc., will be described in a statistical analysis plan which will be prepared prior to the final analysis.

19. QUALITY CONTROL AND QUALITY ASSURANCE

Lead Investigator/Stakeholders will decide to hire Independent Monitoring and Auditors to ensure compliance of the study to protocol and regulations. The coordinating clinical site will be visited at regular intervals by a monitor to ensure compliance. This will include on-site checking of the CRFs for

completeness and clarity, cross-checking with source documents, and clarification of administrative matters.

20. DATA HANDLING AND RECORD KEEPING

20.1 Access to Records

As required by the ICH/GCP guidelines and regulatory authorities, the Investigator will allow Monitors appointed by Sponsor to have direct access to all pertinent medical records in order to allow for the verification of data gathered in the CRFs or the electronic data forms and for the review of the data collection process. The records, including source documentation, must also be available for inspection by relevant regulatory health authorities.

20.2 Source Documents

Source documents may include, but are not limited to, laboratory reports, ophthalmic reports, Slit lamp photographs, clinic notes or pharmacy records and any other similar reports or records of any procedure performed in accordance with the protocol. Source documents may also include CRFs or electronic devices when information is recorded directly onto such forms or devices.

Whenever possible, the original recording of an observation should be retained as the source document; however, a photocopy is acceptable provided that it is a clear, legible, and exact duplication of the original document.

20.3 Record Retention

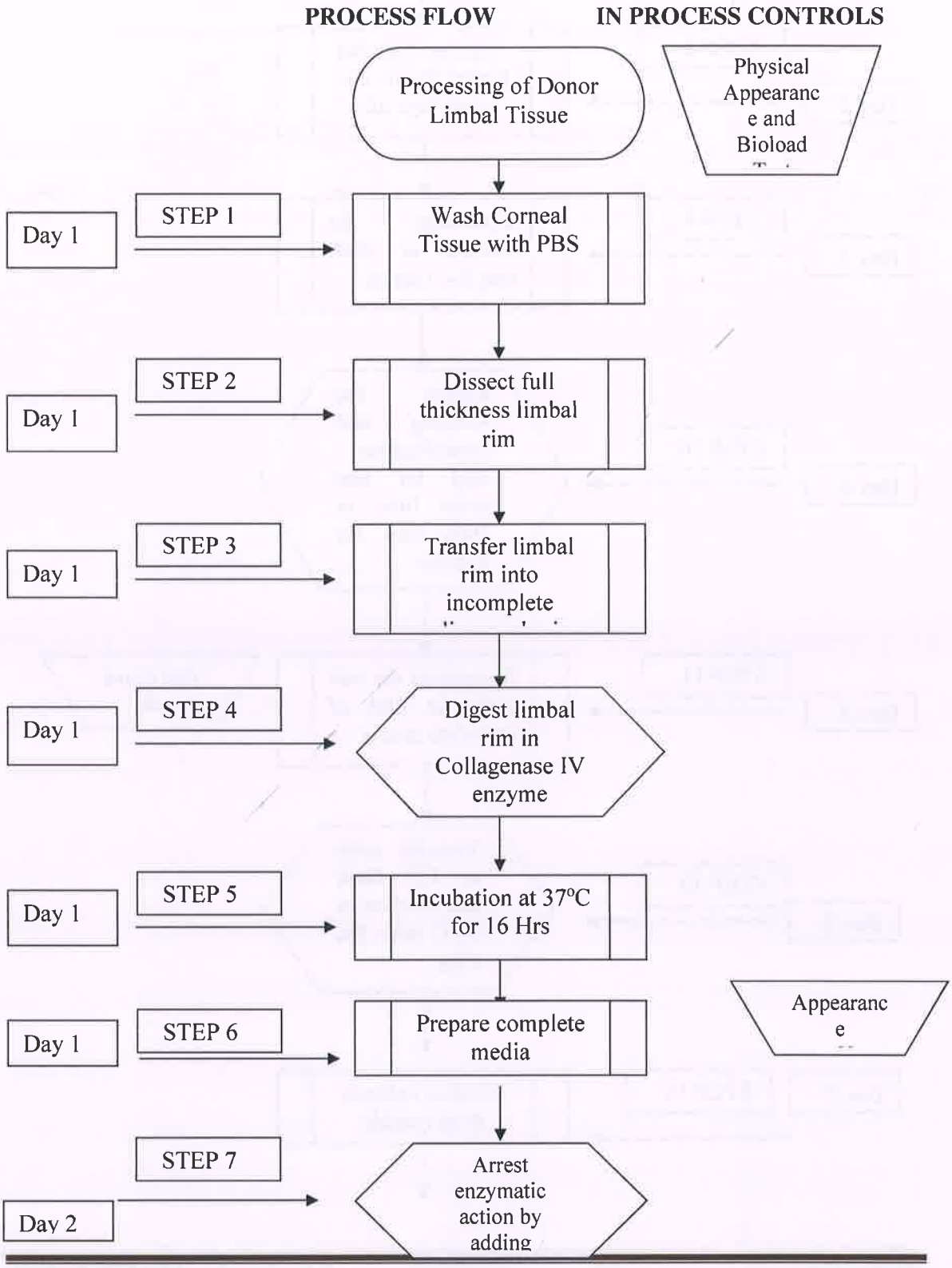
To enable evaluations and/or audits from regulatory authorities, the Investigator agrees to keep records, including the identity of all participating participants (sufficient information to link records, e.g., CRFs and hospital records), all original signed informed consent forms, copies of all CRFs, SAE forms, source documents, and detailed records of treatment disposition, and adequate documentation of relevant correspondence (e.g., letters, meeting minutes, telephone calls reports). The records should be retained by the Investigator according to ICH, local regulations, or as specified in the grant letter /contract, whichever is longer.

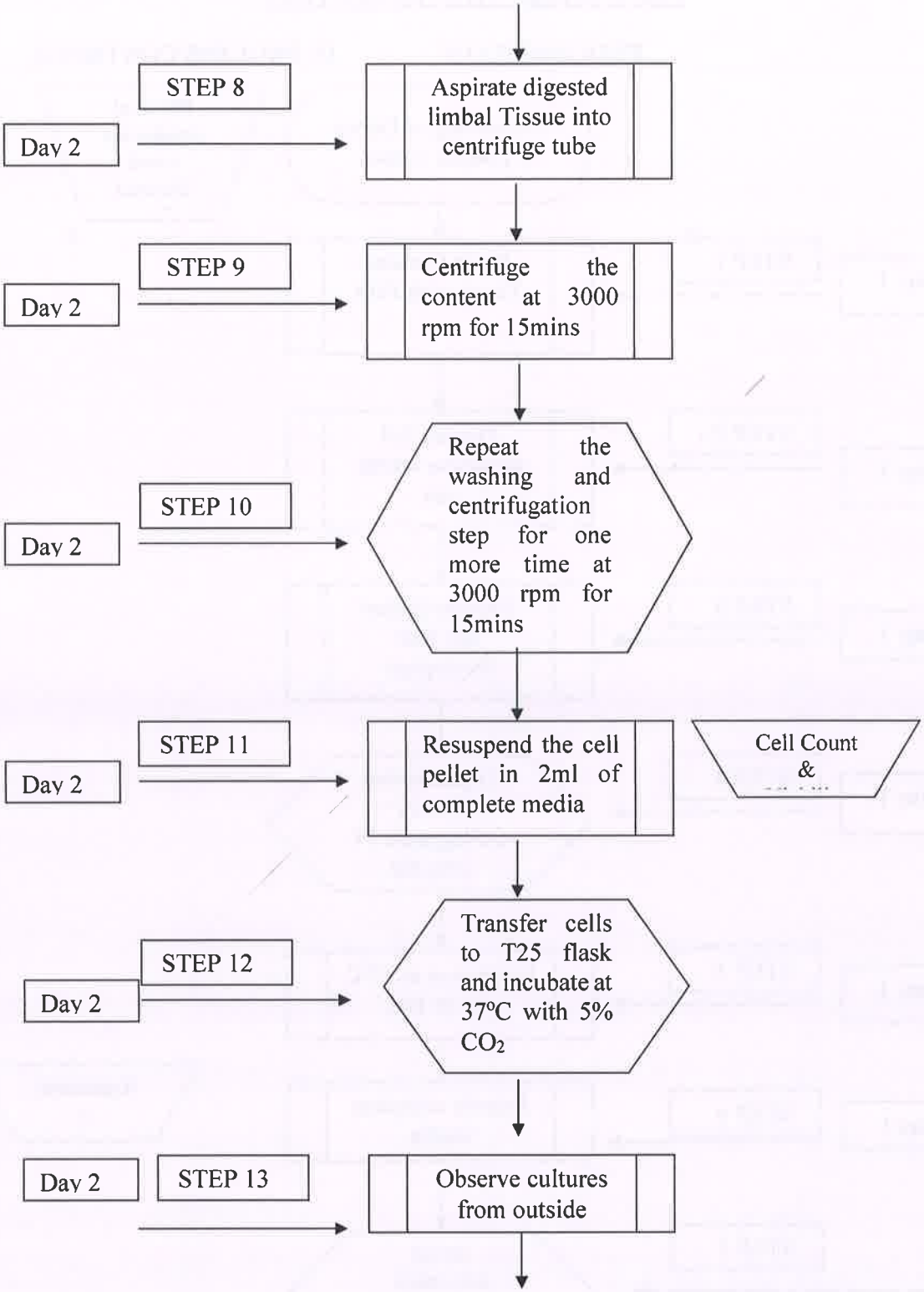
If the Investigator becomes unable for any reason to continue to retain study records for the required period (e.g., retirement, relocation) EC should be notified.

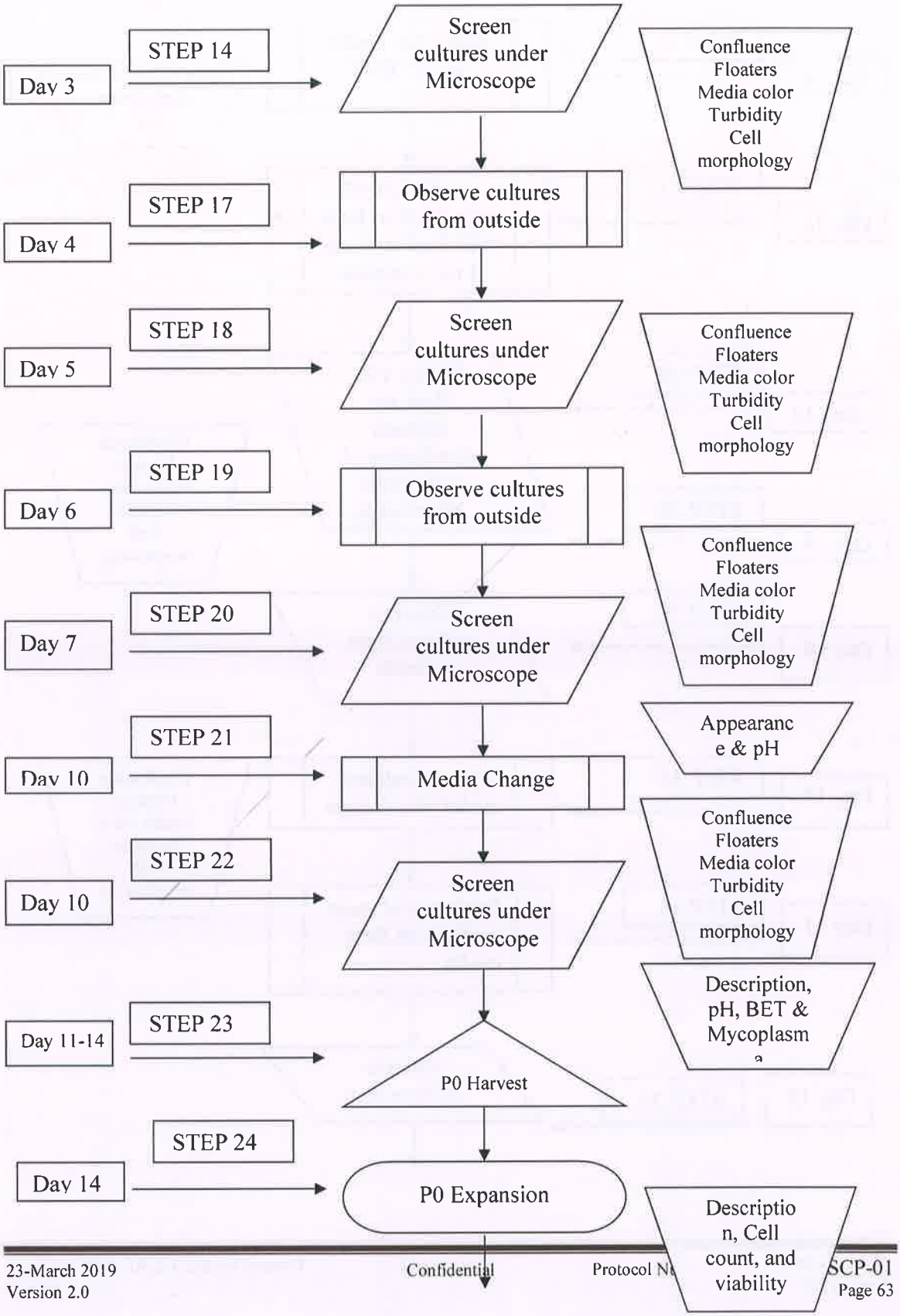
21. CLINICAL SUPPLIES MANAGEMENT

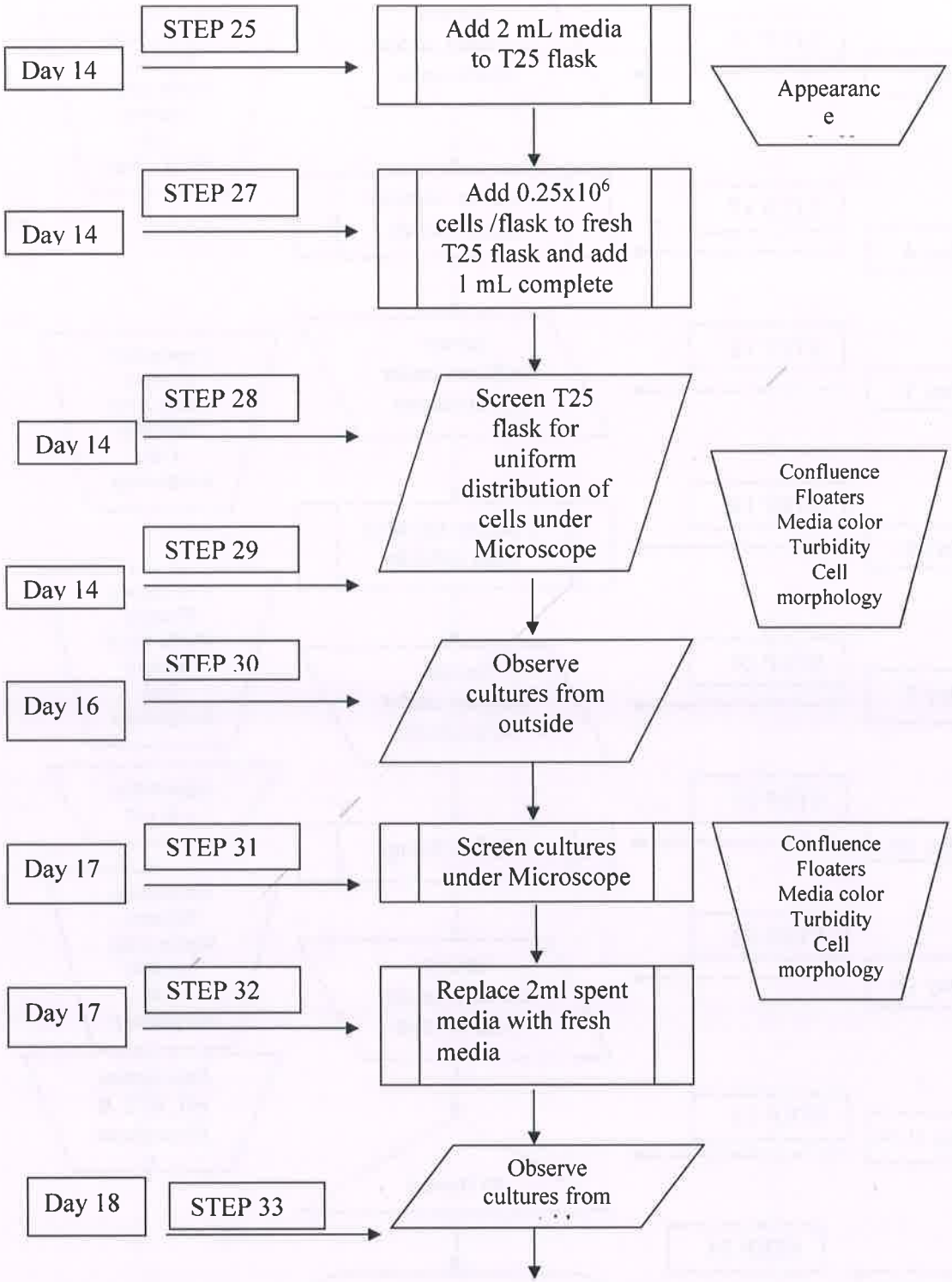
21.1 Production of *Ex vivo* Allogenic Limbal Stromal Stem Cells

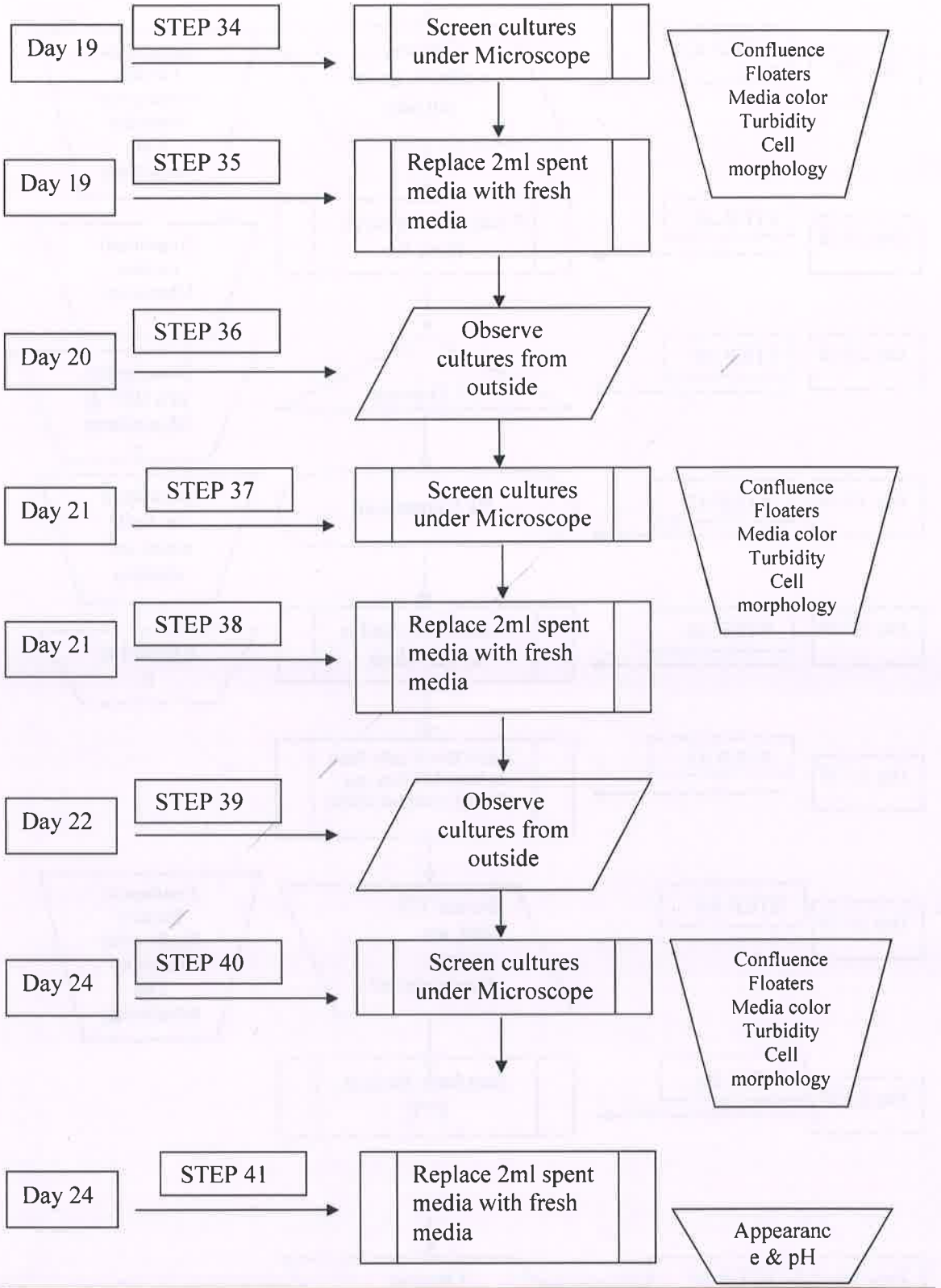
FLOW CHART FOR PRODUCTION

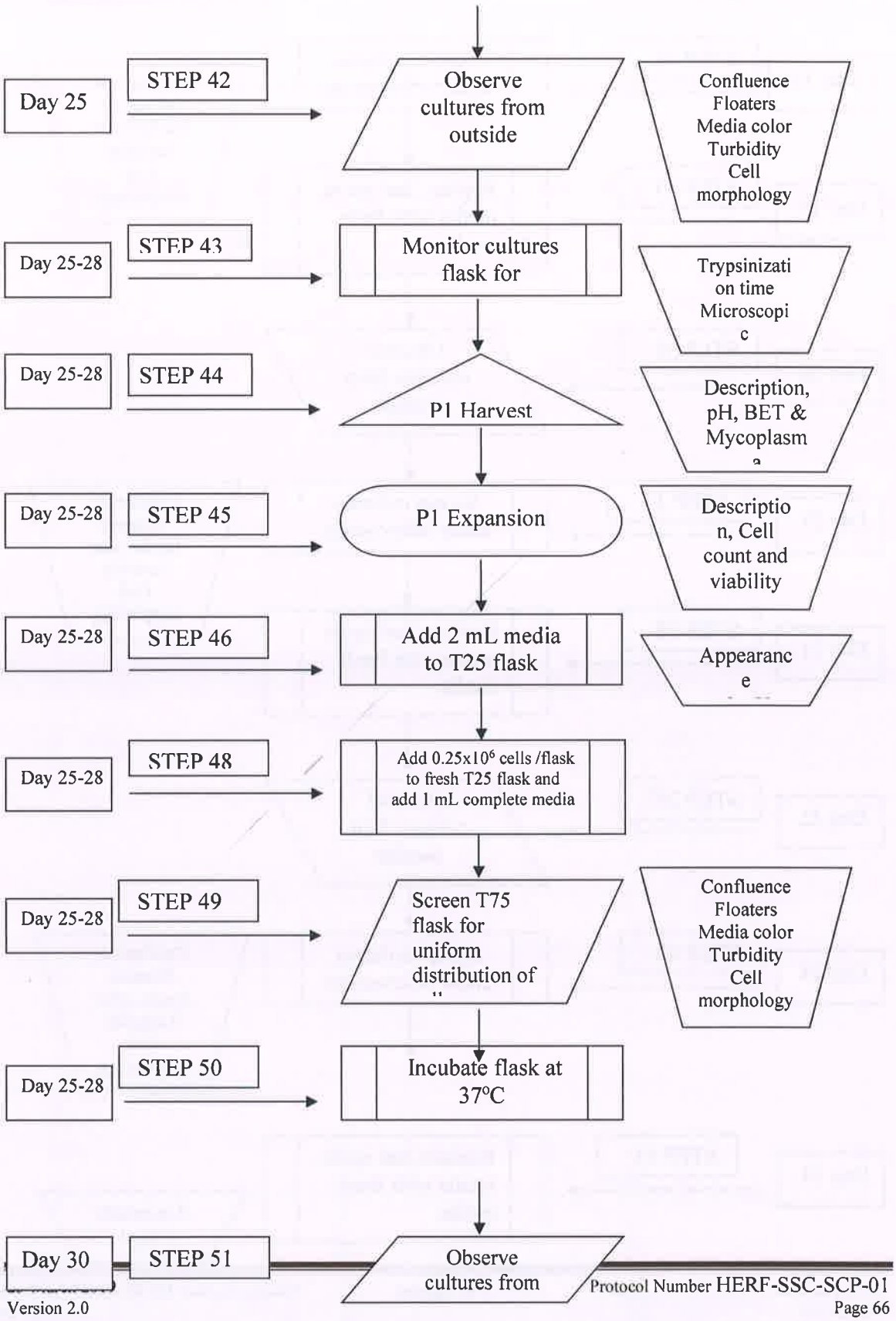


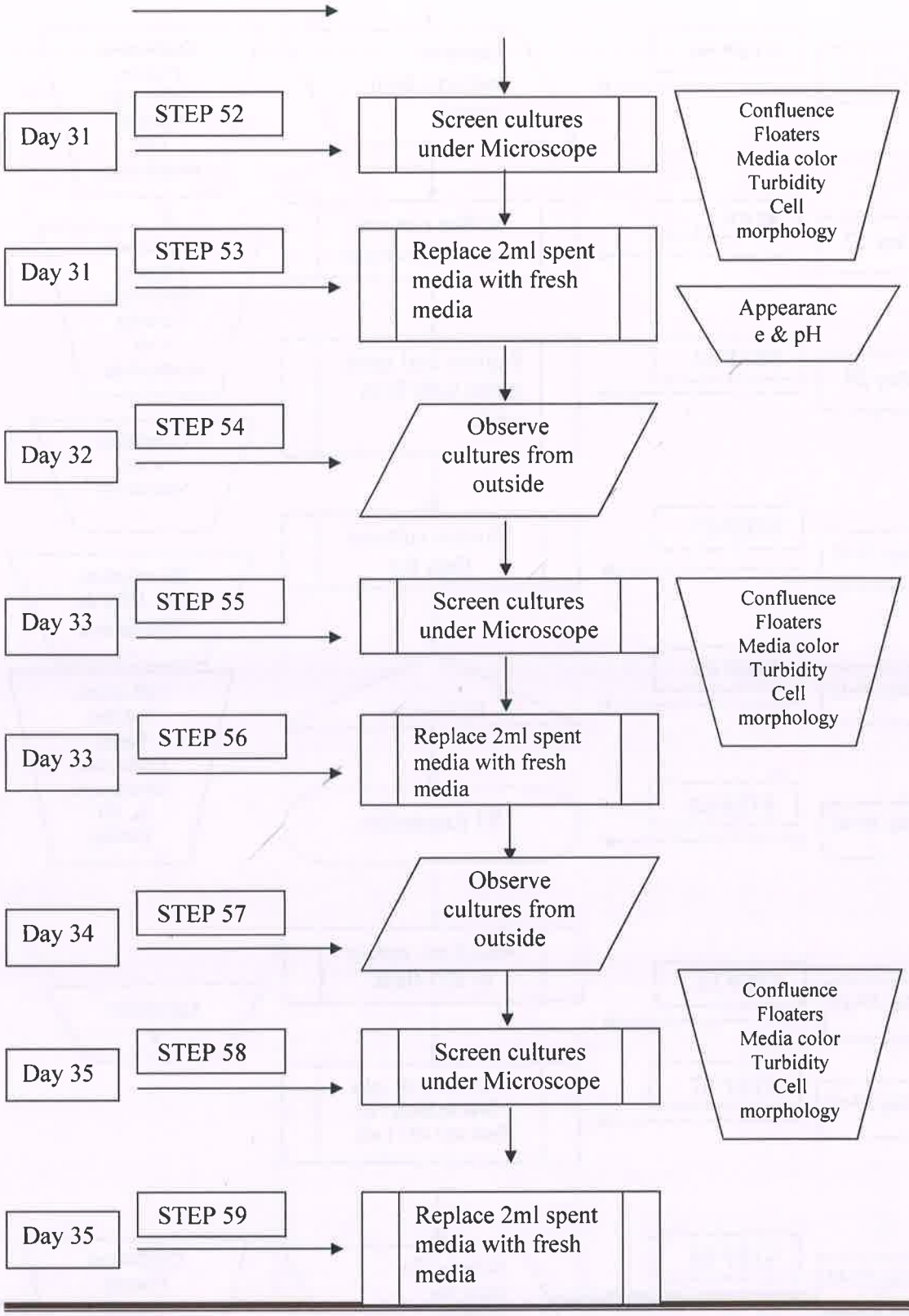

STEP 8
Day 2
Aspirate digested limbal Tissue into centrifuge tube
STEP 9
Day 2
Centrifuge the content at 3000 rpm for 15mins
Repeat the washing and centrifugation step for one more time at 3000 rpm for 15mins
STEP 10
Day 2
STEP 11
Day 2
Resuspend the cell pellet in 2ml of complete media
Cell Count
&
Transfer cells to T25 flask and incubate at 37°C with 5% CO_2
STEP 12
Day 2
Day 2
STEP 13
Observe cultures from outside

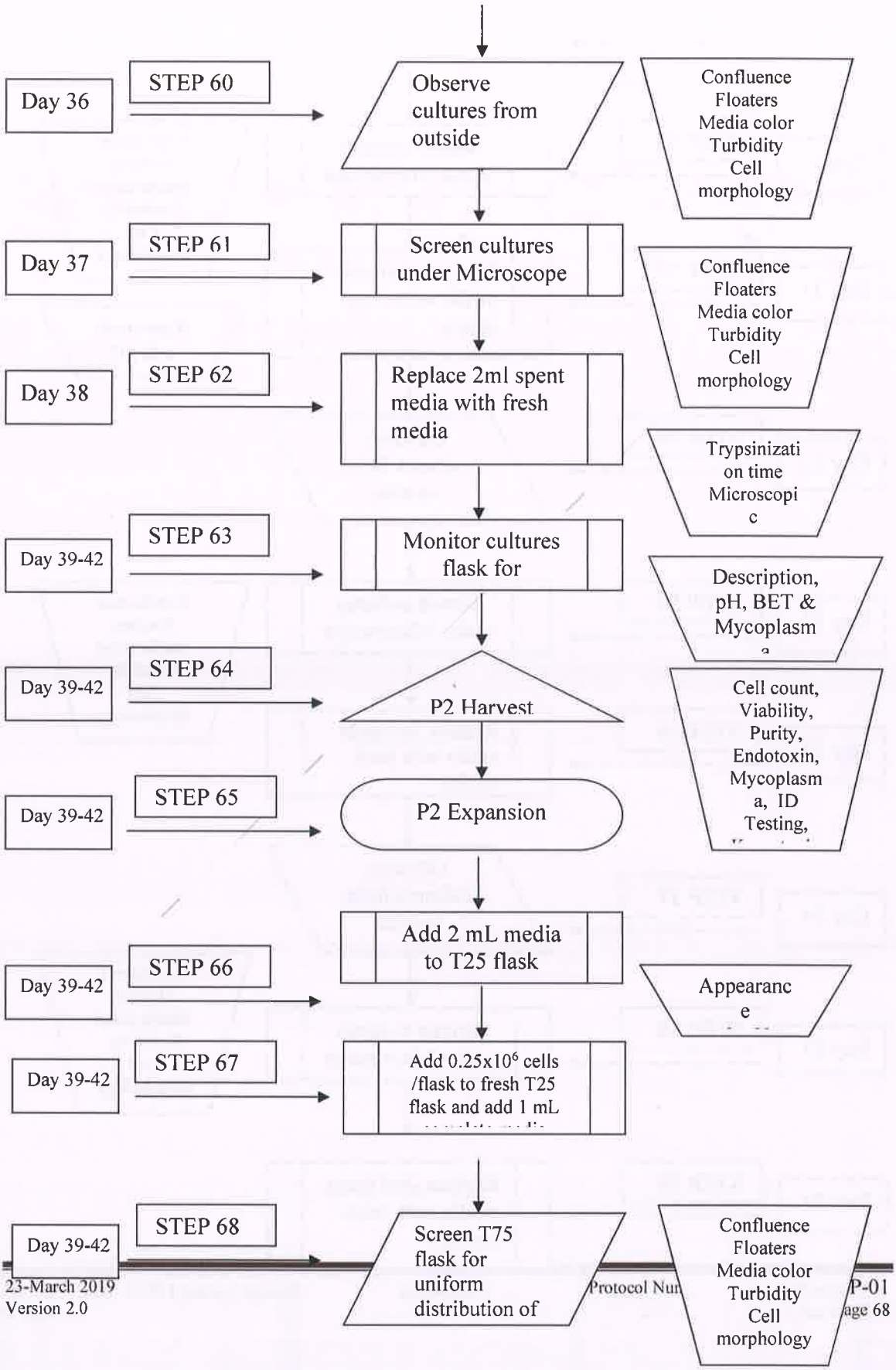

Day 3
STEP 14
Screen cultures under Microscope
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 4
STEP 17
Observe cultures from outside
Day 5
STEP 18
Screen cultures under Microscope
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 6
STEP 19
Observe cultures from outside
Day 7
STEP 20
Screen cultures under Microscope
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 10
STEP 21
Media Change
Appearanc e & pH
Day 10
STEP 22
Screen cultures under Microscope
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 11-14
STEP 23
P0 Harvest
Description, pH, BET & Mycoplasm a
Day 14
STEP 24
P0 Expansion
Descriptio n, Cell count, and viability

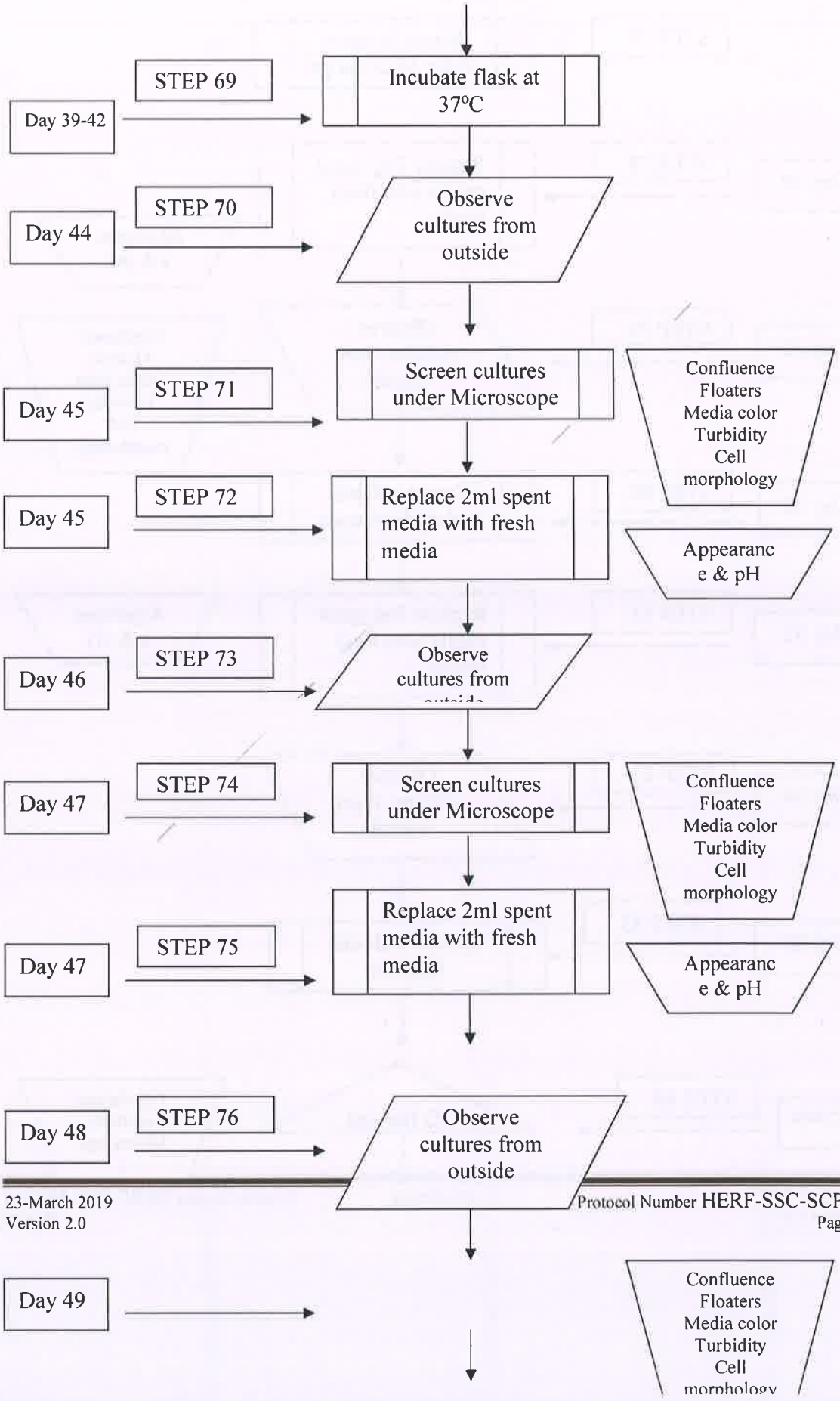

Day 14
STEP 25
Add 2 mL media to T25 flask
Appearanc e
Day 14
STEP 27
Add 0.25x10^6 cells /flask to fresh T25 flask and add 1 mL complete
Day 14
STEP 28
Screen T25 flask for uniform distribution of cells under Microscope
Confluence Floaters Media color Turbidity Cell morphology
Day 14
STEP 29
Day 16
STEP 30
Observe cultures from outside
Day 17
STEP 31
Screen cultures under Microscope
Confluence Floaters Media color Turbidity Cell morphology
Day 17
STEP 32
Replace 2ml spent media with fresh media
Day 18
STEP 33
Observe cultures from

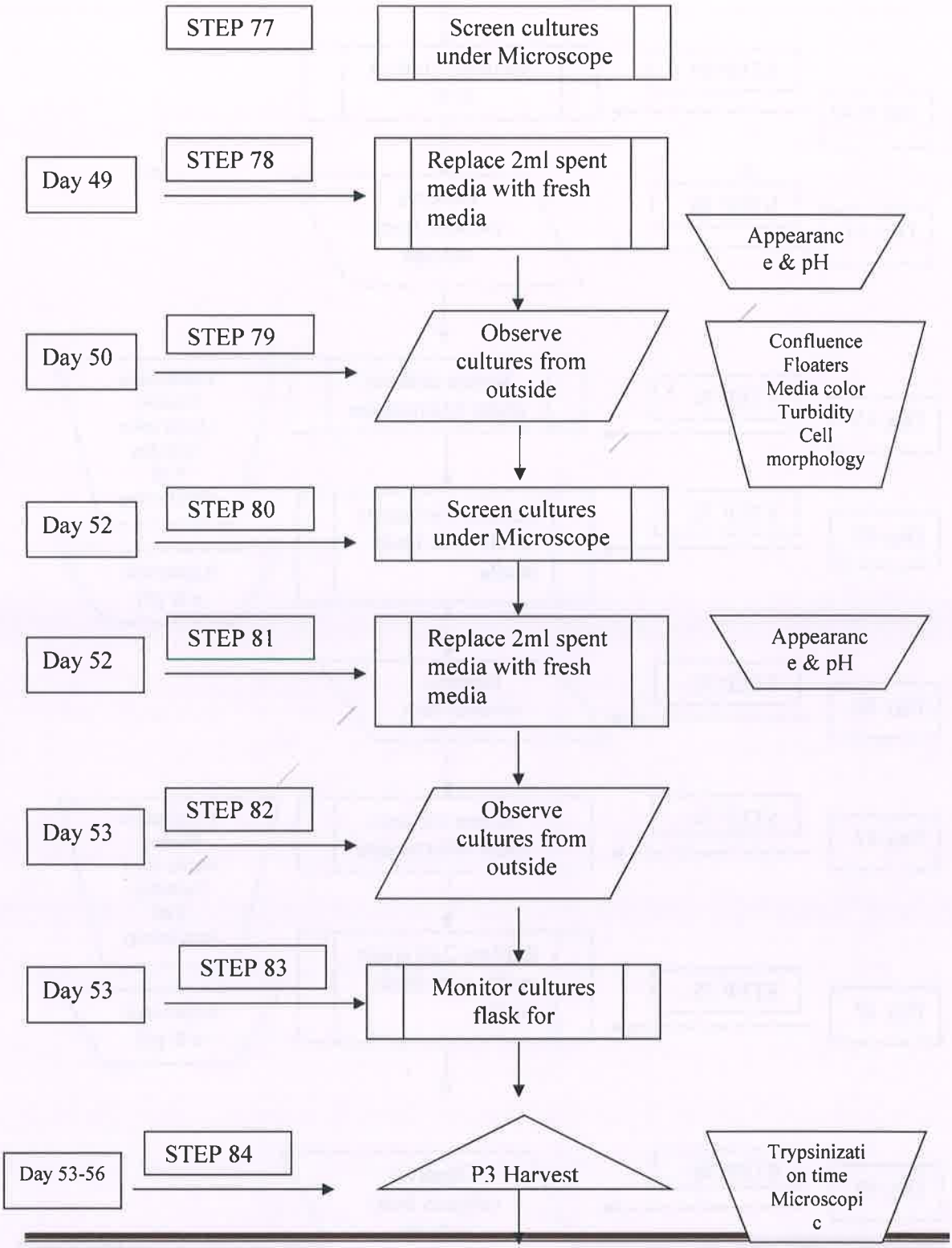

Day 19
STEP 34
Screen cultures under Microscope
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 19
STEP 35
Replace 2ml spent media with fresh media
Day 20
STEP 36
Observe cultures from outside
Day 21
STEP 37
Screen cultures under Microscope
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 21
STEP 38
Replace 2ml spent media with fresh media
Day 22
STEP 39
Observe cultures from outside
Day 24
STEP 40
Screen cultures under Microscope
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 24
STEP 41
Replace 2ml spent media with fresh media
Appearanc e & pH

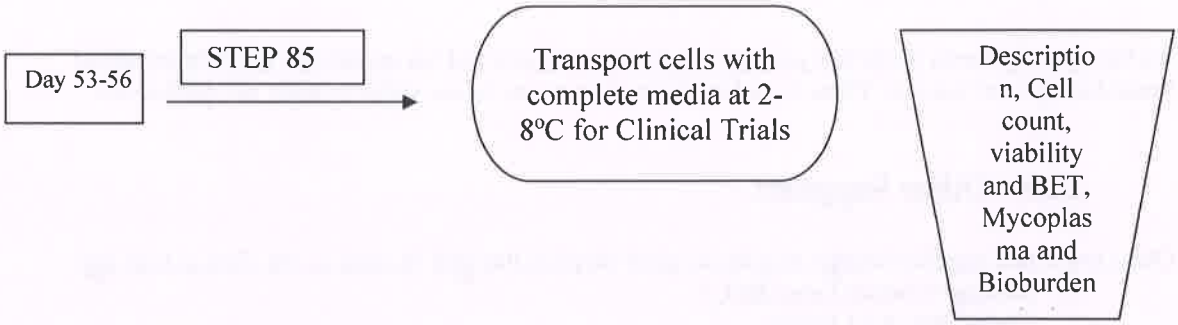

Day 25
STEP 42
Observe cultures from outside
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 25-28
STEP 43
Monitor cultures flask for
Trypsinization time
Microscopic
Day 25-28
STEP 44
P1 Harvest
Description, pH, BET & Mycoplasma
Day 25-28
STEP 45
P1 Expansion
Description, Cell count and viability
Day 25-28
STEP 46
Add 2 mL media to T25 flask
Appearance
Day 25-28
STEP 48
Add 0.25x10⁶ cells /flask to fresh T25 flask and add 1 mL complete media
Day 25-28
STEP 49
Screen T75 flask for uniform distribution of
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 25-28
STEP 50
Incubate flask at 37°C
Day 30
STEP 51
Observe cultures from


Day 31
STEP 52
Screen cultures under Microscope
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 31
STEP 53
Replace 2ml spent media with fresh media
Appearanc e & pH
Day 32
STEP 54
Observe cultures from outside
Day 33
STEP 55
Screen cultures under Microscope
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 33
STEP 56
Replace 2ml spent media with fresh media
Day 34
STEP 57
Observe cultures from outside
Day 35
STEP 58
Screen cultures under Microscope
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 35
STEP 59
Replace 2ml spent media with fresh media


Day 36
STEP 60
Observe cultures from outside
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 37
STEP 61
Screen cultures under Microscope
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 38
STEP 62
Replace 2ml spent media with fresh media
Trypsinizati on time
Microscopi c
Day 39-42
STEP 63
Monitor cultures flask for
Description, pH, BET & Mycoplasm a
Day 39-42
STEP 64
P2 Harvest
Cell count, Viability, Purity, Endotoxin, Mycoplasm a, ID Testing,
Day 39-42
STEP 65
P2 Expansion
Day 39-42
STEP 66
Add 2 mL media to T25 flask
Appearanc e
Day 39-42
STEP 67
Add 0.25x10⁶ cells /flask to fresh T25 flask and add 1 mL
Day 39-42
STEP 68
Screen T75 flask for uniform distribution of
Confluence
Floaters
Media color
Turbidity
Cell morphology


STEP 69
Day 39-42
Incubate flask at 37°C
STEP 70
Day 44
Observe cultures from outside
Day 45
STEP 71
Screen cultures under Microscope
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 45
STEP 72
Replace 2ml spent media with fresh media
Appearanc e & pH
Day 46
STEP 73
Observe cultures from
Day 47
STEP 74
Screen cultures under Microscope
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 47
STEP 75
Replace 2ml spent media with fresh media
Appearanc e & pH
Day 48
STEP 76
Observe cultures from outside
Day 49
Confluence
Floaters
Media color
Turbidity
Cell morphology


STEP 77
Screen cultures under Microscope
Day 49
STEP 78
Replace 2ml spent media with fresh media
Appearanc e & pH
Day 50
STEP 79
Observe cultures from outside
Confluence
Floaters
Media color
Turbidity
Cell morphology
Day 52
STEP 80
Screen cultures under Microscope
Day 52
STEP 81
Replace 2ml spent media with fresh media
Appearanc e & pH
Day 53
STEP 82
Observe cultures from outside
Day 53
STEP 83
Monitor cultures flask for
Day 53-56
STEP 84
P3 Harvest
Trypsinizati on time
Microscopi c



21.2 Packaging and Labeling

Content IMP is a cell pellet of 0.5 x 10^6 of Ex Vivo Cultured Allogenic Limbal Stromal Cells layered with 50 µL basal medium. The total volume of the suspension is 50 µL.

Caution: Cells shall not be used, if Eppendorf vial found to be open or not in Cool condition upon removing from Mini cooler.

"New Drug – Only for Investigational Use."

Storage: At 2 to 8 °C in Mini Cooler.

Batch No.: XXXYY-NN **Vial No.**:

Date of Mfg.: MMYY **Presentation:** 0.5 x 10^5 cells/50 µL

Shelf-Life: Up to 4 Hours

Manufactured by: CENTER FOR OCULAR REGENERATION (CORE)
Kallam Anji Reddy Campus, L.V Prasad Eye Institute, LV Prasad Marg, Road No.2, Banjara Hills, Hyderabad -500034

21.3 Distribution and Storage:

The investigational medicinal product will be distributed half an hour in advance to distribution site.

The investigational medicinal product before administration to patient is stored at 2 to 8 °C in refrigerated condition.

21.4 Stability Data

As the investigational medicinal product is a freshly cultured and harvested cell, and administrated immediately after harvest. There is no long term storage and hence stability study not performed.

21.5 Other Supplies:

Other important supplies besides regular surgical supplies that will be used in the clinical trial are:

1. Bandage Contact Lens (BCL)
2. Tisseel Kit (Ag 1.0 mL)
3. Suture material

These will be procured from reputed suppliers and stored and will be used as per instructions set for the manufacturer.

22. PROTOCOL AMENDMENTS

Modification of the protocol is prohibited without prior written agreement in the form of a protocol amendment. All amendments will be created by LVPEI and must be approved by the IEC/IRB/IC-SCR and CDSCO prior to implementation except when required to mitigate immediate safety risks or when the changes involve only logistical or administrative revisions.

23. STUDY DISCONTINUATION CRITERIA

The investigator has the right to close this study, and the Investigator has the right to close the centre, at any time, although this should occur only after consultation between involved parties. The IEC/IRB/IC-SCR must be informed. Should the study be closed prematurely, all study materials (except documentation that has to remain stored at site) will be destroyed on site according to the site's investigational product destruction guideline.

Documentation of investigational product destruction will be maintained by the site. The Investigator will retain all other documents. Events that may trigger premature termination of a study or closure of a center include, but are not limited to: new toxicity finding, results of any interim review of data, completed accrual and follow-up of patient, non-compliance with the protocol and change in development plans for the investigational product, slow recruitment, and/or poor quality data.

24. CONFIDENTIALITY

All records identifying the patient will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. Only the patient number, date of birth and patient initials will be recorded in the CRF, and if the patient name appears on any other document (e.g., pathologist report), it must be obliterated before a copy of the document is supplied to data management. Study findings stored on a computer will be stored in accordance with local data protection laws. The participants will be informed in writing that independent monitoring agencies, members IEC/IRB/IC-SCR, or Regulatory Authorities representatives may inspect their medical records to verify the

information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the patient's identity will remain confidential.

The Investigator will maintain a list to enable participants' records to be identified.

25. COMPLIANCE WITH LAW, AUDIT, DEBARMENT

By signing this protocol, the Investigator agrees to conduct the study in an efficient and diligent manner and in conformance with this protocol; generally accepted standards of GCP; and all applicable local laws, rules and regulations relating to the conduct of the clinical study.

The Investigator also agrees to allow independent monitoring agencies, audits, IRB review and regulatory agency inspection of trial-related documents and procedures and provide for direct access to all study-related source data and documents.

The Investigator shall prepare and maintain complete and accurate study documentation in compliance with GCP standards and applicable local laws, rules and regulations; and, for each patient participating in the study, provide all data, and upon completion or termination of the clinical study submit any annual reports as required by this protocol and prevailing regulations.

International Conference on Harmonization's GCP guidelines, Indian Good Clinical Practices Guidelines and the Indian regulations as specified in the Schedule Y of Drugs and Cosmetics Act 1940 (Third Amendment February 2013) recommendations have to be adhered to by the participating Investigators. The Investigator should inform the patient's primary physician about the patient's participation in the study if the patient has a primary physician and if the patient agrees to the primary physician being informed.

The Investigator will promptly inform all stakeholders of any regulatory agency inspection conducted for this study and provide the results (i.e., final observations and responses).
If any of the stakeholders, like sponsor including employees, subsidiaries and branches, their agents, contractors, subcontractors and investigators, fail to comply with any of the regulations and recommendations of licensing authority, the authority after giving an opportunity of show cause why such order was not complied with, can issue warning letter, terminate/suspend the study or debar investigators or any other stakeholders to conduct trials in future.

Persons debarred from conducting or working on clinical studies by any court or regulatory agency will not be allowed to conduct or work on this study. Any affected stakeholder will have 90 days from the date of order to appeal for reversal or modification of any such order.

Investigator will provide a "Letter of Undertaking" to the DCGI as per template provided in Appendix 1 of the Protocol along with the application for clinical trial approval to the DCGI.

26. PUBLICATION POLICY

If the investigators intend to pursue publication of the results of the study in cooperation with the lead Investigator, it will be done after discussions with stakeholders. Patient names and other personal data relating to an identified or identifiable patient (such as photographs, audio, videotapes, or other factors specific to physical, physiological, mental, economic, cultural or social identity) may not be disclosed in any publication.

Upon the study completion and finalization of the study report, the results of this study will be either submitted for publication with the intention to share these new scientific data with their peers at local and international journals.

27. APPENDICES

27.1 Appendix 1: Undertaking by the Investigator

1. Full name, address and title of the Principal Investigator (or Investigators when there is no Principal Investigator).
2. Name and address of the medical college, hospital or other facility where the clinical trial will be conducted:
   Education, training & experience that qualify the Investigator for the clinical trial (Attach details including Medical Council registration number, or any other statements of qualifications)
3. Name and address of all clinical laboratory facilities to be used in the study.
4. Name and address of the Ethics Committee that is responsible for approval and continuing review of the study.
5. Names of the other members of the research team (Co-or sub-Investigators) who will be assisting the Investigator in the conduct of the investigations.
6. Protocol Title and Study number (if any) of the clinical trial to be conducted by the Investigator.
7. Commitments:

   (i) I have reviewed the clinical protocol and agree that it contains all the necessary information to conduct the study. I will not begin the study until all necessary ethics committee and regulatory approvals have been obtained.
   (ii) I agree to conduct the study in accordance with the current protocol. I will not implement any deviation from or changes of the protocol without agreement by the Sponsor and prior review and documented approval or favourable opinion from the ethics committee of the amendment, except where necessary to eliminate an immediate hazard to the trial subject or when the changes involved are only logistical or administrative in nature.
   (iii) I agree to personally conduct or supervise the clinical trial at my site.
   (iv) I agree to inform all trial subject, that the drugs are being used for investigational purposes and I will ensure that the requirements relating to obtaining informed consent and ethics committee review and approval specified in the New Drugs and Clinical Trials Rules, 2019 and Good Clinical Practices guidelines are met.
   (v) I agree to report to the Sponsor all adverse experiences that occur in the course of the investigation(s) in accordance with the regulatory requirements and Good Clinical Practices guidelines.
   (vi) I have read and understood the information in the Investigator's brochure, including the potential risks and side effects of the drug.
   (vii) I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are suitably qualified and experienced and they have been informed about their obligations in meeting their commitments in the trial.
   (viii) I agree to maintain adequate and accurate records and to make those records available for audit or inspection by the Sponsor, ethics committee, Central Licencing Authority or their authorised representatives, in accordance with regulatory provisions and the Good Clinical Practices guidelines. I will fully cooperate with any study related audit conducted by regulatory officials or authorised representatives of the Sponsor.

(ix) I agree to promptly report to the ethics committee all changes in the clinical trial activities and all unanticipated problems involving risks to human subjects or others.

(x) I agree to inform all serious adverse events to the Central Licencing Authority, sponsor as well as the ethics committee within twenty-four hours of their occurrence. In case, of failure to do so, I shall furnish the reason for the delay to the satisfaction of the Central Licencing Authority along with the report of the serious adverse event.

(xi) The report of the serious adverse event, after due analysis, shall also be forwarded by me to the Central Licencing Authority, the Chairperson of the ethics committee and the Head of the institution where the trial has been conducted within fourteen days in accordance with the regulatory requirements.

(xii) I will maintain confidentiality of the identification of all participating subjects and assure security and confidentiality of study data.

(xiii) I agree to comply with all other requirements, guidelines and statutory obligations as applicable to clinical Investigators participating in clinical trials.

8. Signature of Investigator with date.

27.2 Appendix 2: DATA ELEMENTS FOR REPORTING SERIOUS ADVERSE EVENTS OCCURRING IN A CLINICAL TRIAL OR BIOAVAILABILITY OR BIOEQUIVALENCE STUDY

1. Patient Details:
   Initials and other relevant identifier (hospital or out-patient department (OPD) record number etc)*
   Gender
   Age or date of birth
   Weight
   Height

2. Suspected Drug(s)
   Generic name of the drug*
   Indication(s) for which suspect drug was prescribed or tested.
   Dosage form and strength.
   Daily dose and regimen (specify units - e.g., mg, ml, mg/kg).
   Route of administration.
   Starting date and time of day.
   Stopping date and time, or duration of treatment

3. Other Treatment(s):
   Provide the same information for concomitant drugs (including non-prescription or Over the Counter OTC drugs) and non-drug therapies, as for the suspected drug(s).

4. Details of Suspected Adverse Event(s)

   Full description of the event including body site and severity, as well as the criterion (or criteria) for considering
   the report as serious. In addition to a description of the reported signs and symptoms, whenever possible, describe
   a specific diagnosis for the event*
   Start date (and time) of onset of event.
   Stop date (and time) or duration of event.
   Dechallenge and rechallenge information.
   Setting (e.g., hospital, out-patient clinic, home, nursing home).

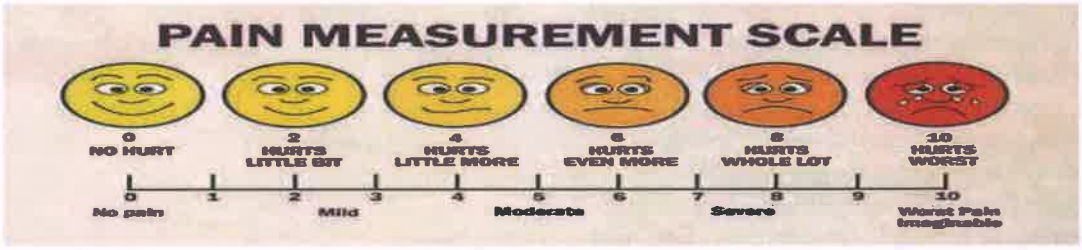
5. Outcome

   Information on recovery and any sequelae; results of specific tests or treatment that may have been
   conducted.
   For a fatal outcome, cause of death and a comment on its possible relationship to the suspected event; Any
   post-mortem findings.
   Other information: anything relevant to facilitate assessment of the case, such as medical history including
   allergy, drug or alcohol abuse; family history; findings from special investigations etc.

6. Details about the Investigator*

   Name and Address
   Telephone number
   Profession (specialty)
   Date of reporting the event to Central Licencing Authority:
   Date of reporting the event to ethics committee overseeing the site:
   Signature of the Investigator or Sponsor
   Note: Information marked * must be provided.

27.3 Appendix 3 Visual Analogue Scale (VAS) for Ocular Pain:



27.4 Appendix 4: New York Heart Association Functional Capacity

Class I: Patients with cardiac disease but without resulting limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.

Class II: Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitations, dyspnea, or angina pain.

Class III: Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitations, dyspnea, or anginal pain.

Class IV: Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the angina syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.

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