Study Title	A Phase I/II Randomized, Double-Masked Placebo-Controlled Study for Determining the Safety of Processed Amniotic Fluid (PAF) Drops after Photorefractive Keratectomy
ClinicalTrials.gov ID (NCT Number)	NCT04281004
Principal Investigator (PI)	Mark Mifflin, MD
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A PHASE I/II RANDOMIZED, DOUBLE-MASKED PLACEBO-CONTROLLED STUDY FOR DETERMINING THE SAFETY OF PROCESSED AMNIOTIC FLUID (PAF) DROPS AFTER PHOTOREFRACTIVE KERATECTOMY (PRK)

STATISTICAL ANALYSIS PLAN

Protocol Title (Number):	The PAF after PRK Trial
Protocol Date:	
SAP Author:	Richard Holubkov, Ph.D.
SAP Version:	0.1 (for review by DSMB)
SAP Date:	January 9, 2020

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ABBREVIATIONS

ABBREVIATION	DEFINITION
pAF	Processed Amniotic Fluid
PRK	Photorefractive Keratectomy
ICH	International Conference on Harmonization
SRI	Surface Regularity Index
CTRM	Cell Therapy and Regenerative Medicine Program
SAP	Statistical Analysis Plan
ITT	Intent-To-Treat Population
mITT	Modified Intent-to-Treat Population
FDA	United States Food and Drug Administration
DSMB	Data Safety Monitoring Board
SAS	Statistical Analysis System
MedDRA	Medical Dictionary for Regulatory Activities
AE	Adverse Event

1. PREFACE

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for the Phase I/II Randomized, Double-Masked Placebo-Controlled Study for Determining the Safety of Processed Amniotic Fluid (pAF) Drops after Photorefractive Keratectomy (PRK) Trial. This study is referred to as the "pAF after PRK" trial in the remainder of this document.

This single-center randomized trial assesses the safety and efficacy of using processed pAF in patients following PRK. More specific safety and efficacy objectives are described in this document.

The structure and content of this SAP provides sufficient detail to meet the requirements identified by the FDA and International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH): Guidance on Statistical Principles in Clinical Trials.[1] All work planned and reported for this SAP will follow internationally accepted guidelines, published by the American Statistical Association[2] and the Royal Statistical Society,[3] for statistical practice.

The following documents were reviewed in preparation of this SAP:

- Trial Protocol
- ICH Guidance on Statistical Principles for Clinical Trials.

The reader of this SAP is encouraged to also read the clinical protocol for details on the conduct of this study, and the operational aspects of clinical assessments and timing for completing a patient in this study.

It is possible that, due to updates or identification of errors in specific statistical software discussed below, the exact technical specifications for carrying out a given analysis may be modified. Such modification is considered acceptable as long as the original, prespecified statistical analysis approach is completely followed in the revised technical specifications.

2. PURPOSE OF SAP

The purpose of this SAP is to outline the planned analyses to be completed for the pAF after PRK trial. The planned analyses identified in this SAP will be included in future study abstracts and manuscripts. Also, exploratory analyses not necessarily identified in this SAP may be performed. Any *post hoc*, or unplanned, analyses not explicitly identified in this SAP will be clearly identified as such in any published reports from this study.

This SAP may be updated in response to additional developments, either within or outside the trial. In such circumstances, an updated version number will be assigned to the revised SAP. Previous SAP versions will be archived.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

3.1.1 Primary Objective

The primary objective of the pAF after PRK trial is to determine the safety of using processed Amniotic Fluid (pAF) in patients following PRK.

3.1.2 Secondary Objectives

The secondary objectives of the pAF after PRK trial are the following:

- To determine if pAF hastens re-epithelialization following PRK compared to placebo.
- To determine if pAF reduces post-operative pain following PRK compared to placebo.
- To determine if pAF affects visual outcome following PRK compared to placebo.
- To determine if pAF affects ocular surface staining and corneal regularity following PRK compared to placebo.

3.2 Study Endpoints

3.2.1 Primary Efficacy Endpoint

The primary efficacy endpoint of the pAF after PRK trial is the number of calendar days from PRK to complete re-epithelialization, evaluated in all PRK-treated eyes among enrolled patients.

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

3.2.1.1 Eligibility for Primary Analysis

The primary endpoint is to be evaluated in all PRK-treated eyes among consented and enrolled patients.

3.2.2 Secondary Efficacy Endpoint

The secondary efficacy endpoint of the pAF after PRK trial is uncorrected visual acuity, as assessed in each PRK-treated eye on Study Day 30.

3.2.2.1 Eligibility for Secondary Analysis

The secondary endpoint is to be evaluated in all PRK-treated eyes among consented and enrolled patients.

3.2.3 Exploratory Endpoints

Exploratory endpoints of the pAF after PRK trial, and the populations eligible for each tertiary analysis, include:

i. Dimensions of epithelial defects as assessed on Days 1 to 8 except Day 2
ii. Pain as assessed on 0-10 point scale on Days 1 to 7
iii. Uncorrected visual acuity on Day 8and at 1, 3, 6, and 12 Months
iv. Best corrected visual acuity at 1, 3, 6, and 12 Months
v. Ocular surface staining at 1, 3, 6, and 12 Months
vi. Corneal Regularity via Surface Regularity Index (SRI) at 1, 3, 6, and 12 months

3.2.4 Safety Endpoints

Safety endpoints of the pAF after PRK trial include:

i. Ocular adverse events, occurring from randomization until end of follow-up

ii. Opthalmic Examination Findings

iii. Need for Rescue Therapy

While adverse events will be collected and survival tracked for all randomized subjects, the population of patients used in key safety analyses will be those randomized patients who received treatment with study agent for at least one study day.

4. STUDY METHODS

4.1 Overall Study Design and Plan

The pAF after PRK trial will randomize approximately 60 eligible and consented patients to post-PRK treatment for 7 days starting on the day of surgery, with pAF or placebo delivered in a double-blind fashion, in a 1:1 ratio. Treatment with the assigned therapy is to commence immediately after surgery, with the patient receiving the first dose prior to leaving the surgery center.

4.2 Selection of Study Population

Patients will be eligible for enrollment if they meet all of the following inclusion criteria:

A. Patient is at least 21 years of age

B. Patient is undergoing PRK for visual correction in both eyes

C. Patient is willing and able to give consent for study participation and comply with study procedures, including follow-up visits.

Additionally, patients must not meet any of the following exclusion criteria:

D. Any active eye disease, including keratoconus or any other ectactic disorders

E. Documented uncontrolled diabetes

F. Known to be pregnant or nursing, or planning to become pregnant during the

study

G. Severe dry eye as measured by corneal staining

H. Calculated PRK treatment resulting in residual stromal bed <300 um

I. History of previous eye surgery or refractive laser procedures

J. Any active collagen vascular disease; OR

K. Visual potential less than 20/20 in either study eye.

4.3 Method of Treatment Assignment and Randomization

Randomization to intervention (pAF or placebo drops) will be performed in a 1:1 ratio, without additional stratification. Randomized blocks of varying lengths will be used to generate the randomization scheme. Smaller block lengths will be used preferentially at the beginning of the generated randomization sequence. Specific block length probabilities are not included in this SAP but will be kept on file at the Coordinating Center, in order to limit predictability of subsequent treatment assignments in case of unblinding. Randomization specifics will be as follows:

1. Two sequences of blocks will be generated using blocks of length 2 and/or length 4, so that the total number of randomizations across the two sequences will be sufficient to achieve the number of randomizations expected in the trial (at least 80). The first sequence will preferentially use smaller block lengths.

2. Within each sequence, the blocks will be permuted as necessary to achieve a fully random permutation of blocks.

3. Each block will then be assigned a random permutation of treatments (for block length 2, a sequence of one of each treatment assignment will be permuted; for block length 4, a sequence containing two of each treatment assignment will be randomly permuted).

4. The final randomization sequence for the trial will be the series of assignments in the first sequence, followed by the series of assignments in the second sequence.

Randomization seeds will be selected and recorded, along with all code used to generate the randomizations, to enable reproducibility of the treatment sequence if necessary.

Randomization sequences will be prepared by the pAF after PRK study biostatistician.

4.3.1 Handling of Incorrect Randomizations in Study Analyses and Reports

Any patients who are assigned the incorrect treatment will be given the assigned treatment and treated as such in all analyses (recognizing that extremely rare "good faith" errors of this type may occur and that the cause of any such errrors must be immediately rectified). Any such violations are to be reported to the DSMB as part of all reports.

4.3.2 Delivery of Randomization

The sequence of randomizations will be delivered to designated unblinded personnel at the University of Utah's Cell Therapy and Regenerative Medicine Program (CTRM), who will transcribe the randomization sequence in order to treatment boxes with a sequential masked ID, each of which contains the assigned treatment (study drug or placebo).

Each patient who is consented, and who successfully receives PRK and is fully eligible for the trial in all respects immediately after surgery, will then receive the next available treatment box, in numerical order. Therefore, in this trial, only patients who actually receive a treatment box after surgery will be considered to have been randomized and eligible for intention to treat analysis.

4.4 Treatment Masking (Blinding)

The pAF after PRK trial is performed in a double-blinded fashion. Patients, as well as surgeons and study personnel at participating centers involved in the patient's treatment and follow-up, will be blinded to the assigned treatment strategy.

While the pAF after PRK biostatistician will be unblinded to treatment assignments, knowledge of arm-specific treatment results will be limited to biostatisticians involved in the interim and final analyses. Moreover, for all interim analyses, such materials will be prepared with (for example) one arm randomly labeled as "Treatment A" and one arm as "Treatment B", with knowledge of arm identities limited to the biostatistician(s) presenting such materials to the Data Safety Monitoring Board (DSMB). The DSMB may request to be unblinded to treatment assignment at any time, including at the time of their initial look at the trial data.

5. SEQUENCE OF PLANNED ANALYSES

5.1 Interim Analyses

5.1.1 Frequency of and Timepoints for Interim Analysis

The pAF after PRK DSMB is a body of investigators not directly affiliated with the trial. The DSMB is primarily charged with the review of available safety data, including but not limited to adverse events reported in the trial. The DSMB is scheduled to meet three times during the trial, at timepoints after initial outcome data (for at least the first 8 days of follow-up) is available for approximately 5, 30, and 60 randomized patients.

The DSMB will also review other data relevant to the conduct of the trial such as enrollment and protocol adherence, during their within-study meetings. The DSMB will, at their discretion, be able to request analyses additional to those described in this SAP, and to request additional data review meetings on an *ad hoc* basis.

At all meetings, the DSMB is to review interim data and make recommendations regarding continuation or modification of the pAF after PRK trial.

5.1.2 Interim Efficacy Analysis

No interim analyses of efficacy are planned in this trial.

5.1.3 Subgroups for Monitoring and Final Analysis

No patient characteristics have been designated for formal assessment of subgroup effect have been identified in this trial. Exploratory analyses of various treatment effects within and across patient subgroups may be performed in this trial; these will be reported as exploratory in all publications.

5.1.4 Blinding in the Interim Analysis

As noted above, the number of Coordinating Center biostatisticians who are unblinded to results by treatment arm and identity of treatment arms will be limited as much as possible, while other Coordinating Center personnel shall be blinded to all safety and efficacy data, prior to final analysis or decision to unblind all investigators to study results.

All interim analysis tables and analyses involving treatment arms will have treatment not explicitly identified, but referred to in a coded fashion, for example as "Treatment A" and "Treatment B", consistently throughout the report presented to the DSMB. The DSMB will have the option of being unblinded to treatment arm identity at any time including at the time of their initial data review.

5.2 Final Analyses and Reporting

All final, planned analyses identified in the protocol and in this SAP will be performed only after all randomized patients have completed the trial protocol and results of all significant queries have been resolved. A blinded data review meeting will be held prior to final database lock and completion of the final analyses. In addition, no database may be locked, random code unblinded, or analyses completed until this SAP has been approved.

Any *post hoc*, exploratory analyses completed to support planned study analyses, which were not identified in this SAP, will be documented and reported as such in all study publications.

6. SAMPLE SIZE DETERMINATION

Eliacik et al[4] found a mean time to re-epithelization of 3.1 ± 0.6 days for the Comfilcon Group and 3.6 ± 0.5 days for the Lotrafilcon Group. We used this 0.5-day difference as an estimate of treatment effect, and considered within-group standard deviations in the range of 0.5 to 0.7 days. Another key parameter in the estimation of statistical power is the intraclass correlation between eyes in the same patient. This correlation was estimated to be in the range of 0.5 to 0.75, as within-patient healing times have been reported to be nonuniform to a modest extent in the study PI's experience. The table

below shows true treatment differences detectable with 80% and 90% power under different estimates of the variability of re-epithelization time and intraclass correlation, **assuming that 56 participants (28 participants and therefore 56 eyes in each treatment arm) complete the trial.** These values were calculated[5] treating the study as a cluster-randomized trial with clusters (each participant's eyes) of size two.

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

Based on numerous power estimates (the above and others), a sample size of 56 patients completing the trial, yields acceptable power to find a significant treatment difference, if the true treatment difference is at least 0.5 days for the time to re-epithelialization outcome.
In order to collect additional safety data and allow for a small number of patients being lost to follow-up, the study will enroll a total of 60 participants.

7. ANALYSIS POPULATIONS

The following analysis populations are planned for the pAF after PRK trial:

- **Eligible Population:** The Eligible Population includes all subjects who are screened for eligibility into the trial, and meet the trial inclusion and exclusion criteria. This population, which will be equivalent to all subjects who received a study identification number, will be used for reporting of study flow per CONSORT guidelines.
- **Intention to Treat Population (ITT):** The Intention-to-Treat Population includes all subjects who provide informed consent and who are randomized into the trial, regardless of whether treatment was initiated or adherence to the protocol.
- **Modified Intention to Treat Population (mITT):** The Modified Intention-to-Treat Population includes all subjects in the ITT population

who receive PRK, and who have analyzable primary outcome data for at least one PRK-treated eye.

- **Safety Population:** The Safety Population includes all patients who received PRK, and who receive treatment with at least one drop of study agent during the post-treatment follow-up.
- **Per-Protocol Efficacy:** The PP-Efficacy population includes patients in the ITT population who are verified to meet all study inclusion and exclusion criteria, and who receive the full assignment treatment, as specified in the protocol, throughout the duration of follow-up.

8. GENERAL ISSUES FOR STATISTICAL ANALYSIS

8.1 Analysis Software

Analysis will be performed using SAS® Software version 9.4 or later whenever possible. Other software packages, including R and StatXAct, may be used in instances where a particular specialized procedure is not available in SAS®.

8.2 Methods for Withdrawals, Missing Data, and Outliers

Per the intention-to-treat principle, any subjects who withdraw from the study will have all available data used in the analysis, per outcome-specific analysis strategies specified elsewhere in this SAP. In the unexpected event that a substantial number of subjects are lost to follow-up or otherwise withdrawn, baseline characteristics and available information on components of efficacy outcomes will be reviewed and compared to subjects not withdrawn, to assess empirically if these subjects differ from those remaining in the study for the scheduled treatment and follow-up time.

Outliers will be reviewed for validity. Outliers that are valid (e.g., reports of extremely poor visual acuity during follow-up) will be included in all primary reports from this trial.

8.3 Multicenter Studies

This is designed as a single-center trial.

8.4 Multiple Comparisons and Multiplicity

As there is a single primary efficacy endpoint for this study, adjustment for multiple comparisons will not be required for the primary analysis.

As a single efficacy outcome has been formally designated as the secondary efficacy outcome, adjustment for multiple comparisons will not be required for the secondary efficacy analysis.

No formal adjustment will be made for multiple comparisons in the reporting of treatment differences for exploratory outcomes. Reported analyses of exploratory outcomes will explicitly note that such outcomes are exploratory.

Safety outcomes for this study will be reported using unadjusted *p*-values for each individual comparison. However, all reports of these outcomes, to the DSMB and in published reports, will explicitly note that multiple safety outcomes have been evaluated.

8.5 Planned Subgroups, Interactions, and Covariates

As noted above, there are no prespecified formal efficacy analysis subgroups for the pAF after PRK trial. Exploratory analyses may examine the association of treatment effect with patient characteristics prior to surgery.

As is noted below, the primary analysis of efficacy will not control for any patient characteristics; patient ID will be included in the analysis to model within-patient correlations of outcomes assessed in individual eyes.

8.6 Derived and Computed Variables

All derived and computed variables will be outlined in the analysis dataset specifications for this study. These datasets are independently programmed by two statisticians. The SAS COMPARE procedure will be used to verify that the dual programmed analysis datasets are identical for each variable and each observation.

9. STUDY SUBJECTS

9.1 Disposition of Subjects and Withdrawals

All eligible subjects who provide informed permission will be accounted for in this study. The frequency and percent of subjects in each population, study withdrawals, subgroups, and major protocol violations will also be presented. While the final definition of "major protocol violation" will be determined during the course of the trial, in all instances randomizations of subjects who were later found not to meet eligibility criteria, and instances where subjects received the opposite of the assigned treatment or did not receive PRK after randomization will be reported in the subject disposition reports.

10. EFFICACY ANALYSES

10.1 Primary Efficacy Variable Analysis

The analysis of the primary study outcome will include the following test of hypothesis:

H_0: time to re-epithelialization is not different among eyes receiving treatment with pAF after PRK, compared to eyes receiving placebo drops after pRK.

The alternative hypothesis is:

H_1: time to re-epithelialization is significantly different among eyes receiving treatment with pAF after PRK, compared to eyes receiving placebo drops after pRK.

For testing the primary hypothesis, a mixed effects linear model with time to re-epithelialization (number of calendar days from PRK to time to complete re-epithelialization) for each eye as outcome, and assigned treatment arm as a predictor will be fit using SAS PROC MIXED. This model will additionally include a random effect for each patient (formally included in the model as each patient's study ID, defined as a CLASS variable) to model correlation between eyes in the same participant. A two-sided probability value with an alpha level of 0.05 will be used to test the null hypothesis that time to re-epithelialization is not different between the two treatment arms. If the probability value is less than 0.05, regardless of the direction of treatment effect, the null hypothesis above will be considered to be rejected.

The primary analysis will be performed on an intention to treat basis.

All eyes with available outcome data will be included in the model. Any eyes that are not completely re-epithelialized by Day 8 will be assigned a value of 9 days to re-epithelialization in the primary analysis. Any eyes for which time to re-epithelialization is not fully known due to missed visits will be assigned the midpoint of the possible time in the primary analysis (for example, a patient not re-epithelialized in either eye on Day 2, who misses visits on Days 3 and 4 but has complete re-epithelialization in both eyes on Day 5, would be treated as having complete re-epithelialization in both eyes on Day 4 in the primary analysis). Eyes of patients who drop out of the study before Day 8, that do not achieve complete re-epithelialization before dropout, will be treated as missing in the primary analysis, but included in robustness analyses described below.

Results of the analysis above will be accompanied by appropriate presentation (e.g., histograms) of the distribution of number of calendar days to complete re-epithelialization among eyes in each treatment arm.

10.1.1 Robustness Analyses

In this trial, it is possible that some patients may not be fully compliant with the drug administration regimen, some eyes may not achieve complete re-epithelialization by the time of the Day 8 evaluation, and/or that some patients may drop out prior to Day 8 without having achieved complete re-epithelialization in one or both eyes. If any of these circumstances occur, then the following robustness analyses will be carried out:

1. The two treatment arms will be compared by survival analysis approaches. Specifically, the number of calendar days to re-epithelialization will be treated as a time-to-event variable, and any eyes not exhibiting complete re-epithelialization at their final evaluation (including such eyes evaluated on Day 8) will be treated as censored at time of last evaluation. The time to re-epithelialization curves will be compared between the treatment arms using a two-sided log-rank test appropriate for the within-patient clustered nature of the trial data (REF Stedman R et al. A SAS Macro for a clustered logrank test. Computer Mathods and Programs in Biomedicine 2011; 104:266-70).

2. The primary analysis, as well as the survival analysis described immediately above, will be carried out as above among evaluable subjects who met all eligibility criteria for the trial and received their assigned treatment (per-protocol efficacy population), if this population differs from the mITT population.

As noted above in this document, in the event that any patients received the incorrect treatment assignment in the circumstances of an isolated "good faith" error, it is proposed that the treatment received be used in the primary reported analysis, although such errors are to be fully disclosed in the publication of trial results.

10.2 Secondary Efficacy Variable Analysis

The analysis of the secondary study outcome will include the following test of hypothesis uncorrected visual acuity, as assessed in each PRK-treated eye on Study Day 30:

H_0: at 30 days after surgery, uncorrected visual acuity is not different among eyes receiving treatment with pAF after PRK, compared to eyes receiving placebo drops after pRK.

The alternative hypothesis is:

H_1: at 30 days after surgery, uncorrected visual acuity is different among eyes receiving treatment with pAF after PRK, compared to eyes receiving placebo drops after pRK.

For testing this hypothesis, a mixed effects linear model with the logarithm of uncorrected visual acuity assessed on Day 30 for each eye as outcome, and assigned treatment arm as a predictor will be fit using SAS PROC MIXED. This model will additionally include a random effect for each patient (formally included in the model as each patient's study ID, defined as a CLASS variable) to model correlation between eyes in the same participant. A two-sided probability value with an alpha level of 0.05 will be used to test the null hypothesis that uncorrected visual acuity at 30 days is not different between the two treatment arms. If the probability value is less than 0.05, regardless of the direction of treatment effect, the null hypothesis above will be considered to be rejected.

Any eyes with uncorrected visual acuity less than 20/400 (*e.g.*, participant is able to detect hand motion only) will be treated as having 20/400 acuity in these analyses, with such (unexpected) instances being explicitly noted in the description of analysis results.

The secondary efficacy analysis will be performed on an intention to treat basis.

Robustness analyses will also be carried out and reported as appropriate (for example, patients with missing data on Day 30 who have not achieved 20/20

uncorrected visual acuity at their last study visit would have their last visual acuity value used in a supportive analysis).

10.3 Tertiary Study Outcomes

10.3.1 Visual Acuity on Other Study Days

The visual acuity outcome will be evaluated as above for other study timepoints at which acuity is assessed.

10.3.2 Dimensions of Epithelial Defects

Epithelial defect areas in each eye will be compared on each study day, graphically and analytically. The approach to assessing differences on each study day will be analogous to that used for the analysis of the primary and secondary study outcomes. We will also fit exploratory linear mixed models, accounting for within-eye correlation across timepoints as well as within-patient correlation between eyes, to model rate of change of defect areas over time.

10.3.3 Pain Scale on Days 1 to 7

The analysis of pain diary data will be an important tertiary analysis of this trial. Key quantities of interest include the duration of pain after treatment, and the most severe pain level after treatment. It is expected that some of the pain outcomes described below may show marked skewness; if this is the case, then rank-based analysis (Mann-Whitney Test with associated two-sided 95% Hodges-Lehmann confidence interval for difference between treatment arms) will be used instead of a *t*-test, which will be the preferred approach when appropriate.

Each patient's worst pain level in either eye will be compared for each of the study days that pain scores are evaluated. These distributions will also be shown graphically over time via an approach appropriate for the data distributions such as boxplots. As this is an exploratory outcome, comparisons will be performed and reported separately for each day unadjusted for multiple comparisons.

Using the worse pain level in either eye (assessed on a scale of 0 to 10), the number of days to full resolution of pain (no pain reported in either eye from that day forward) will be calculated, and compared between treatment arms. It is expected that this outcome may show marked skewness; if this is the case, then rank-based analysis (Mann-Whitney Test with associated two-sided 95% Hodges-Lehmann confidence interval for difference between treatment arms) will be used rather than *t*-tests.

Similar analyses will be carried out comparing patients between treatment arms with respect to the worst pain level in either eye at any time during evaluation in the pain diary, and with respect to the study day that peak pain level is reported. In the latter analysis, a patient whose highest pain level was reported on more than one study day would have the last such day used.

10.3.4 Use of rescue pain medication

Proportions of patients who required use of any pain medication during the first seven days of post-surgical follow-up will be reported and compared between treatment arms by chi-squared approaches. Analogous analyses will be performed for use of narcotics and use of NSAIDs at any time. In addition, the number of days that patients used pain medications will be reported and compared between treatment arms using rank-based tests, again for any type of pain medications as well as for narcotics and NSAIDs alone.

10.3.5 Corneal staining and surface irregularities

Corneal staining density, graded on a scale from 0 to 3, will be reported and compared using rank-based approaches between treatment groups at all evaluation times. To incorporate the effect of correlation between eyes in the same participant, a clustered version of the Wilcoxon test[4] will be used, using a SAS macro[5].

Corneal topography will be compared between treatment groups as well. More specifically, surface regularity index (SRI) at each evaluation timepoint will be compared between study arms using mixed models accounting for correlation within eyes in the same participant, using an approach analogous to the analysis of the secondary efficacy outcome of visual acuity above. Baseline SRI will be included in this model as a covariate.

11. SAFETY ANALYSES

11.1 Ocular Adverse Events

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

In general, all AE tables will be presented by treatment group, causality and severity. The following will be summarized and presented overall and by treatment group:

• The number and percentage of subjects experiencing an SAE

• The number and percentage of subjects experiencing an SAE by preferred term and by severity of event

• The number and percentage of subjects experiencing an SAE by preferred term and by relationship to study drug

• The number and percentage of subjects experiencing an AE

• The number and percentage of subjects experiencing an AE by preferred term and by severity of event

• The number and percentage of subjects experiencing an AE by preferred term and by relationship to study drug

• The number and percentage of AEs that cause a subject to discontinue study drug

• The number and percentage of AEs leading to death.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) central coding dictionary. Adverse events will be described in summary tables and in individual listings for the DSMB reports.

Primary reporting of Adverse Events will include the Safety Population, and reported overall as well as by the actual treatment that each subject received. Rates of adverse events will be compared between the two treatment groups using chi-squared type approaches. In most instances, exact approaches will be implemented that maximize the statistical power to detect a treatment difference. Specifically, when exact approaches are appropriate, the mid-*p*-value correction[14] will be implemented, whereby the probability value of obtaining a result at least as extreme as observed is reduced by one half of the probability of obtaining the specific result observed. This is directly calculable in SAS, for example, as the Fisher's exact test output gives these two probabilities directly.

11.2 Opthalmic Exam Findings

In addition to reporting of ocular AEs as above, numbers and proportions of participants in each study arm (if any) who had loss of best corrected vision in either eye by one line or more, compared to best corrected vision prior to treatment, at 6-month and 1-year evaluations will be reported. These numbers

will also be reported using eyes as numerator and denominator.

12. OTHER PLANNED EXPLORATORY ANALYSES

The discussion of exploratory analyses presented in this SAP is by no means exhaustive, as the pAF after PRK trials will generate data useful for exploratory analyses of clinical interest. We intend to use contemporary analytic approaches, including modification of existing approaches and derivation of novel techniques when appropriate, for such analyses. All exploratory, non-prespecified analyses will be clearly described as such in published reports. These exploratory analyses will be explicitly prespecified in advance, in separate Analysis Plans or in an updated version of this document as appropriate.

13. REFERENCES

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