Clinical Protocol Tittle: A Phase I/II Prospective, Randomized, Multicenter, Double-Masked, Vehicle-Controlled Clinical Trial to Evaluate the Safety and Efficacy of Corneal Collagen Cross-Linking of Keratoprosthesis Carrier Tissue in High-Risk Keratoprosthesis Implantation

Statistical Analysis Plan

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N = 68 subjects

Preliminary Results: Sixty-eight Kpros were implanted in 68 eyes (56 with previous sterile corneal ulceration) in 68 patients (15 with autoimmune disease) between 2017 - 2020 across 13 sites. The average age at the time of surgery was 62 [24-89] years, 42 (62%) subjects were male and 44 (65%) were white. The mean follow-up time was 91 weeks (SD= 47.6). A total of 20 KPros were removed with an average survival time of 70 [6-160] weeks.

Inclusion Criteria:

Previous sterile corneal ulceration	56
Autoimmune disease (ocular or other)	15
Ocular Cicatricial Pemphigoid	1
Steven Johnson's syndrome	7
Rheumatoid Arthritis	4
GVHD	2
Vogt-Koyanagi-Harada	1
Both autoimmune disease and previous ulceration	4
No autoimmune disease or previous ulceration	1

*Will include this subject for safety analysis but will remove for efficacy analysis

Primary endpoints or outcome measure(s)

- Time from surgery to device loss or replacement

Secondary endpoints or outcome measure(s)

- Twelve-month retention rate
- Incidence of delayed epithelial healing at day 30 (week 4 visit)
- Time from surgery to retroprosthetic membrane treatment (laser or surgical interventions)
- Time from surgery to occurrence of vitritis (sterile or infectious)
- Time from surgery to occurrence of ocular surgery to address melt, including partial graft.
- Cornea thickness metrics measured by AS-OCT; at week(s) 1, 4, 16, 24, 36, and 52***

Previous KPro

- Previous KPro in the study eye: yes/no
    - If yes, how many, how long were they in the eye for and why were they removed.
    - We don't have exact dates for all previous ones since some were done at other hospitals.

Reorganize the primary and secondary outcome measures into:

- Efficacy endpoints:
    - Removal or not of the study KPro
    - Rate of retention at 52 weeks
    - Time from KPro study surgery to ocular surgery done to address a melt, including partial graft

- Safety endpoints:
    - Incidence of delayed epithelial healing at day 30 (week 4 visit)
    - Time from surgery to retroprosthetic membrane treatment (laser or surgical interventions)
    - Time from surgery to occurrence of vitritis (sterile or infectious)

We will conduct an initial analysis to examine if distributions of baseline characteristics are balanced between the two treatment groups in our randomized study. Standard descriptive statistics will be reported, median (min-max) for numerical variables and frequency count (%) for categorical variables. Comparisons of numerical characteristics between treatment groups will be conducted using the Mann-Whitney U- test. Comparisons of categorical characteristics will be conducted using Chi-square, or Fisher's exact test as appropriate.

Primary Analysis

The primary efficacy analysis will follow an intent-to-treat analysis strategy including all randomized eyes. The major goal of this study is to determine the efficacy of using a collagen cross-linked cornea as a carrier for the Boston Keratoprosthesis in preventing corneal melts and increasing the retention of the keratoprosthesis.

The primary endpoint of the efficacy analysis is time from surgery to device loss or replacement. Kaplan-Meier event free survival by treatment groups will be determined by the product-limit method and compared by the log-rank test to examine if survival curves are significantly different between groups. In addition, we will use a Cox proportional hazards regression model to test the effectiveness of using a collagen cross-linked cornea as a carrier for the Boston Keratoprosthesis in preventing corneal melts and increasing the retention of the keratoprosthesis.[1] We will obtain an estimate of the hazard ratio of device loss comparing treatment with control with 95% confidence interval (95% CI). This framework allows for adjustment of baseline covariates, which may be unbalanced by chance despite the randomization of treatment assignment. The proportional hazards assumption will be assessed by examining scaled Schoenfeld residuals and log-log plots, with testing done using the "stphtest" and "stphplot" functions in Stata (version 16.0; StataCorp LLC, College Station, Texas).[2]

If Kaplan-Meier plots of event free survival by study time, or related plots of log (-log) (survival) indicate violations of the proportional hazards assumption, then a weighted log-rank test will be used according to strategies described by Peckova and Fleming.

We will further characterize the effectiveness of using a collagen cross-linked cornea as a carrier for the Boston Keratoprothesis by creating a composite endpoint that captures survival time until KPro removal or ocular surgery to address a melt. We will calculate the survival time of the latter by choosing the date of whichever of these two happened first as the endpoint. In doing so, we will better capture the effectiveness of using the collagen cross-linked corneas as carriers for the KPro in preventing corneal melts even for those subjects in which the melt did not warrant KPro replacement.

Secondary Analysis

We plan to conduct a number of pre-specified analyses of secondary study endpoints. Analyses of secondary "time-to-event" type endpoints (time to retroprosthetic membrane treatment and time to

occurrence of vitritis) will use the Kaplan-Meier approach with log-rank test and Cox proportional hazards regression, similar to the primary analysis for time to device loss.

Comparison of cornea thickness metrics at week 1, 4, 16, 24, and 52 for treatment versus control group will be performed using the Mann-Whitney Wilcoxon test at each time point. Furthermore, a mixed model regression analysis using generalized estimating equations (GEE) with compound symmetry covariance structure for repeated measures will be used to examine the effect of time, treatment and the interaction between them on cornea thickness to evaluate the change over time between and within the treatment groups. Comparison of twelve-month retention rate and incidence of delayed epithelial healing at Day 30 (week 4 visit) between groups will be performed using Chi-square test, or Fisher's exact test as appropriate.

In addition, the occurrence of any adverse events will be described and compared between treatment arms of the study.

We will run multivariable analysis with relevant information from the subject's ocular history to determine if certain characteristics inform or predict the loss of the study KPro or the survival time between insertion and removal. Among these, we will include age at study surgery, sex, race and whether patients- had previous KPros in the study eye and, if so, how many. For the subset of patients who did have previous Kpros in the study eye, we will look at the survival time of the one immediately before the study eye and compare it to the survival time of the study one. We will compare mean duration for these and analyze if there is a statistically significant difference among those who received a collagen cross-linked carrier cornea versus those who did not.

References:

1. Cox DR. Regression models and life-tables. J R Stat Soc Series B Stat Methodol 1972;34(2):187–220
2. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. Biometrika 1994;81(3):515–526.