DRCR Retina Network

A Pilot Study Evaluating Photobiomodulation Therapy for Diabetic Macular Edema (Protocol AE)

NCT03866473

Statistical Analysis Plan

For Protocol AE Version 4.0

VERSION NUMBER	AUTHOR	APPROVER	EFFECTIVE DATE	REVISION DESCRIPTION
1.0	Danni Liu	Michele Melia	September 20, 2019	Initial version for Protocol version 3.0.
2.0	Kristin Josic	Michele Melia	May 4, 2020	COVID-19 response Analysis window for 4-month primary outcome visit extended by 8 weeks; analysis windows for 6- and 8-month visits extended by 12 and 4 weeks, respectively. Expected dates for 6- and 8-month visits changed to correspond to 2 months and 4 months post-primary outcome, respectively, instead of 6 and 8 months from randomization.
3.0	Kristin Josic	Michele Melia	August 9, 2021	Updated analysis of visual acuity change

1.0 Introduction

This document outlines the statistical analysis plan for the DRCR Retina Network Protocol AE randomized clinical trial evaluating the effect of photobiomodulation (PBM) compared with sham on central subfield thickness in eyes with central-involved diabetic macular edema (DME) and good vision. The photobiomodulation used in this trial is irradiation by light in the far-red to near-infrared region of the spectrum (630-900 nm). The device to be evaluated is the PhotoOptx, LLC (Solon, OH, USA) Retilux Eye Patch.

There are two phases of the study. The primary objective of this study is to assess whether a treatment group difference in mean change in central subfield thickness on OCT from baseline at the end of phase 1 (primary outcome) between PBM and sham. Upon the completion of the primary outcome visit, participants originally assigned to active will end device use and participants originally assigned to sham will switch to active for additional 4 months of follow-up. Note this is not a crossover design. In addition to the 2 outcome visits, participants will also have interim visits at 1, 2, 3 and every 2 months for 4 months after the primary outcome. This pilot study is being conducted to determine whether the conduct of a pivotal trial has merit based on an anatomic outcome and provide information on outcome measures needed to design a pivotal trial. This study is not designed to definitively establish the efficacy of PBM in the treatment of DME.

Study eyes will be assigned randomly to the two treatment groups in a 1:1 ratio stratified by site and recent (within 4 months) or planned intravitreous treatment in the non-study eye, including intravitreous anti-VEGF and steroid. Participants may have only one study eye enrolled in the randomized trial.

2.0 Efficacy Analysis Plan

2.1 Primary Outcome Analysis

The primary analysis will consist of a treatment group comparison of mean change in CST from baseline to the primary outcome visit using analysis of covariance, with adjustment for baseline CST and the randomization stratification factor of recent or planned intravitreous treatment in the non-study eye. Given that OCTs can be obtained from either Spectralis or Cirrus images, and that values from these machines cannot be used interchangeably, OCT values will be converted to a common value for reporting and analyses.

The primary analysis is an intention-to-treat analysis. All randomized eyes will be included in the primary analysis irrespective of treatment received and will be analyzed according to treatment group assignment at randomization.

For the primary analysis, study participants who complete the primary outcome visit without missing data at the primary outcome visit will be considered as the **completer cohort**. Markov chain Monte Carlo (MCMC) multiple imputation with 100 imputations will be used to handle missing data for participants who do not complete the primary outcome visit, i.e., **non-completer cohort**. The imputation model will be stratified by treatment group and will include

the CST measured at baseline and at all monthly interim visits up to the primary outcome visit along with the randomization stratification factor of recent or planned intravitreous treatment in the non-study eye.

For eyes that receive alternative treatment for DME (detailed in Protocol Section 3.6) in Phase 1, data measured after the initiation of the alternative treatment will be considered to be missing before entering the multiple imputation (MI) process, regardless of whether the treatment initiation is per-protocol, and whether the participant completes the primary outcome visit. Then after multiple imputation, the last OCT measurement prior to the initial alternative treatment (LOCF data) will overwrite the MCMC imputed values for all post-treatment visits and will be used for the primary analysis. It is recognized that LOCF does not reflect the uncertainty in outcomes (i.e., underestimates the variances) and is likely to introduce bias (of unknown magnitude) into the primary analysis that favors the PBM group under the alternative hypothesis, as participants may receive alternative treatment for falsely low visual acuity values, and some eyes meeting criteria for and receiving alternative treatment might have recovered if they'd been left untreated. However, the bias is not being increased by the MI since LOCF is applied after MI. Also, in a recent study for a similar cohort, only 2 (<1%) out of 236 eyes in the observation group initiated treatment without meeting pre-specified criteria during 2 years of follow-up, therefore we expect very few eyes (if any) in this study will receive alternative DME treatment against protocol.

To limit the influence of extreme data points, change in CST will be truncated at ±3 SD after imputation and LOCF to improve robustness of the treatment comparison. The cutoff values for data truncation will be calculated using the primary outcome visit data within the completer cohort, combining treatment groups, i.e. the "**completers' data**", which will consist of the observed data for completers without alternative DME treatment, and the LOCF data for completers who receive an alternative DME treatment.

Therefore, the **primary analysis dataset** will include:

- Data from completers without alternative DME treatment,
- MCMC imputed data for non-completers without alternative DME treatment,
- LOCF data for participants who receive an alternative DME treatment.

The *P* value, adjusted treatment group difference, and associated 95% confidence interval will be reported for the treatment group effect with robust variance estimation using the primary analysis dataset. If the *P* value for the test of the treatment effect is less than or equal to .05, then it will be concluded that there is a significant difference for change in CST at the primary outcome visits between the groups. In other words, if $P \leq .05$, the null hypothesis of no treatment effect will be rejected.

Multiple imputation assumes that data are missing at random (MAR). In the present study, this would mean that whether follow-up CST measurements are missing or observed may be a function of observed baseline and follow-up characteristics included in the imputation model

(baseline CST, follow-up CST, treatment group, recent or planned intravitreous treatment in the non-study eye), but not a function of the unobserved follow-up CST measurements that are being imputed. This assumption cannot be tested since these data are unknown. However, a tipping point analysis will be conducted which will adjust the imputed values for eyes without an alternative DME treatment using a shift parameter and thereby determine how severe the departure from MAR must be in order to change outcome of the primary analysis with respect to rejecting or failing to reject the null hypothesis. A shift parameter will be applied to the imputed values in the PBM group to determine the tipping point at which the results of the primary analysis are nullified. That is, if one group is found to be superior ($P \leq .05$), the tipping point will identify the shift parameter necessary to yield $P > .05$. Conversely, if the null hypothesis is not rejected ($P > .05$), two tipping points will be identified – one that would make PBM superior and one that would make sham superior. In either case, this tipping point(s) will be evaluated to determine if it is plausible. If not, the MAR assumption is reasonable. For example, if the tipping point were 500 microns, this would be evidence that the MAR assumption is reasonable for this analysis.

The assumptions of linearity, normality, and homoscedasticity will be verified using graphical methods. Serious violations may be addressed by transformation of dependent and/or independent variables, non-parametric transformation, categorizing continuous covariates, and/or excluding covariates. Transformation of the dependent variable (mean change in CST from baseline) will be used to obtain valid *P* values while ensuring statistical model assumptions are met. However, mean treatment group differences, rather than results based on transformed outcomes will be reported for clinical interpretation.

A plot showing the mean converted level of CST values on OCT by treatment group over time will be constructed using completers' data as defined above. In general, summary statistics (e.g., within-group means and standard deviations), will be based on completers' data while numbers from statistical models (e.g., treatment group differences, confidence intervals, and *P* values) will be based on primary analysis data as noted above.

2.1.1 Sensitivity Analyses

Sensitivity analyses for the primary outcome are listed in Table 1. In general, if the sensitivity analysis results differ substantially from the primary analysis results, exploratory analyses will be performed to evaluate factors that may have contributed to the differences.

Table 1. Pre-Planned Sensitivity Analyses for the Primary Outcome of Mean Change in CST From Baseline at 4 Months

Eyes that receive an alternative DME treatment	Eyes that have missing data at primary outcome visit	Additional details	Rationale
Primary Analysis (MI, LOCF, then data truncation)			
• OCT data after alternative DME treatment will be considered missing for purposes of MI, hence will be imputed in the MI procedure • Last OCT measurement prior to alternative DME treatment will then overwrite MI values and will be used for analysis (LOCF).	• MCMC multiple imputation for eyes with missing primary outcome data (MI) • LOCF values will replace the imputed values for eyes that receive alternative DME treatment	Data truncation: changes in OCT from baseline will be truncated at ±3 SD after multiple imputation based on cutoff values calculated from completers' data	There is no ideal way to handle eyes receiving alternative DME treatment in analysis. For the primary analysis, LOCF will be used for these eyes. It is recognized that LOCF will tend to bias results towards a larger treatment effect, assuming a treatment effect exists. Sensitivity analyses will be performed to evaluate possible impact of LOCF on study conclusions (#2, #4, #5 below).
Sensitivity Analysis #1 (Complete-case analysis with LOCF)			
LOCF for eyes receiving alternative DME treatment	Complete–case analysis, i.e., only eyes completing primary outcome visit will be included	Same rule for data truncation with primary analysis	To compare primary results including imputed data to results using observed data only.
Sensitivity Analysis #2 (MI for ALL eyes missing 4-month visit)			
OCT measurement after the alternative DME treatment will be considered missing and will be imputed using multiple imputation	MCMC multiple imputation for all eyes missing the primary outcome visit; imputation model will include an additional covariate indicating whether an eye receives an alternative DME treatment	Same rule for data truncation with primary analysis	To compare if LOCF versus MI for handling eyes receiving alternative DME treatment will produce substantially different results when comparing treatment groups
Sensitivity Analysis #3 (No truncation)			
LOCF (same with primary analysis)	MI, then LOCF for eyes receive alternative DME treatment (same with primary analysis)	No data truncation	To explore if extreme outliers significantly impact the primary analysis results
Sensitivity Analysis #4 (Complete-case analysis with transformation in place of truncation)			

Eyes that receive an alternative DME treatment	Eyes that have missing data at primary outcome visit	Additional details	Rationale
LOCF for eyes receiving alternative DME treatment	Complete–case analysis, i.e., only eyes completing primary outcome visit will be included	Values will be converted to Van der Waerden (Normal) scores for analysis	To examine whether the primary analysis results are robust to: (1) normality assumption; (2) imputation / LOCF of data for those missing the primary outcome visit.
Sensitivity Analysis #5 (Complete case analysis without LOCF)			
Will be excluded from the analysis	Complete–case analysis, i.e., only eyes completing primary outcome visit without alternative DME treatment will be included	• Same rule for data truncation with primary analysis • The analysis will only be performed if 6 (10%) or more eyes receive alternative DME treatment in either group	By excluding eyes meeting criteria for alternative treatment, this analysis is biased towards reduction of treatment effect (assuming a treatment effect exists). Hence, a significant treatment effect in both primary analysis and this analysis would support presence of a true treatment effect. However, a non-significant treatment effect in this analysis cannot be interpreted as evidence for or against a true treatment effect.

2.1.2 Per-Protocol Analysis

A per-protocol analysis will be conducted to estimate the treatment effect for each treatment among those who complied with the treatment. This analysis will include observed data (no imputation) from all randomized eyes that complete the primary outcome visit and 70% or more of prescribed sessions of treatment, except those that receive an alternative treatment for DME prior to the primary outcome visit. The intention-to-treat analysis is considered the primary analysis. If the results of the primary and per-protocol analyses differ substantially, then exploratory analyses will be performed to evaluate the factors that may have contributed to the differences. The per-protocol analysis will only be performed if more than 10% of randomized participants would be excluded by these criteria.

2.1.3 Confounding

Imbalances between groups in important covariates are not expected to be of sufficient magnitude to produce confounding in the primary analysis. However, the presence of confounding in the primary analysis will be evaluated in additional regression models using completers' data (defined above for the primary outcome) by including baseline participant and study eye covariates including but not limited to the following: duration of diabetes, hemoglobin A1c, prior anti-VEGF treatment, visual acuity, DR severity on clinical exam, and fellow eye DME status.

Additional variables associated with the outcome will be included in regression models if there is an imbalance in the variables between treatment groups. Imbalance by treatment group will not

be judged using statistical testing. Instead, imbalance will be judged by whether the size of the imbalance is clinically important, i.e., whether the imbalance is large enough to have a clinically important effect on the primary outcome.

2.1.4 Subgroup Analyses

Subgroup analyses/assessments of effect modification (interaction) will be conducted for the primary outcome. The pre-planned subgroup analyses will repeat the primary analysis while including an interaction term for the baseline subgroup factor by treatment. Only completers' data will be used for these analyses, i.e., the subgroup analyses will only include non-missing data from participants who complete the primary outcome visit without alternative DME treatment and the LOCF data from those who complete the primary outcome visit but receive an alternative DME treatment. It is recognized that analyzing only observed data may be biased, but unlike the imputed analysis, it is not automatically biased in the presence of interaction.

Since there is no strong prior rationale for potential subgroup effects, these analyses will be considered exploratory / hypothesis generating, rather than definitive. A forest plot will be created to present the estimated treatment group effect and 95% confidence interval within each level of the subgroup factors, and a test for interaction with treatment will be performed for each subgroup factor. A significant ($P \leq .05$) type III test of the interaction term will be taken as an indication that subgroup effects need to be explored for full interpretation of the trial results. It is recognized that the study is not powered to detect subgroup effects and that lack of significance is not necessarily an indication that subgroup effects do not exist.

The following baseline subgroup factors will be evaluated in exploratory analyses:

- Prior DME treatment: yes vs. no
- Intravitreous treatment in non-study eye: recent (within 4 months) or planned
- Lens status: phakic vs. pseudophakic
- Baseline CST: continuous and categorical (dichotomized based on a clinically relevant cut point or an approximate median value)
- Hemoglobin A1c: continuous and <7.5% vs. ≥7.5%
- Iris color: blue, brown, or other
- Sex: female vs. male
- Race/Ethnicity: White vs. Black/African American vs. Hispanic (exclude all other groups due to anticipated small sample size) and white vs. non-White

Interaction *P* values will be calculated using the continuous and ordinal variables, where possible, in addition to the categorizations described above. The finding of a significant subgroup effect for any of these factors will be interpreted as hypothesis generating only and in need of confirmation from further studies. To increase statistical precision, subgroups will only

be analyzed if there are at least 20 eyes in each treatment group for each subgroup. Cutoffs of continuous and ordinal outcomes may be modified to achieve a reasonable number of eyes in each group.

2.1.5 Center Effects

The number of study participants per center is expected to be small for most centers. Therefore, center effects will not be included in the statistical model.

2.1.6 Planned Interim Analyses

There is no formal interim analysis planned for this study. The Data and Safety Monitory Committee (DSMC) will review tabulated safety and outcome data approximately every 6 months while the study is ongoing.

2.2 Secondary Outcome Analyses

Secondary outcome analyses for Phase 1 are summarized in Table 2. The ITT analysis cohort will be used for all secondary outcomes unless otherwise specified. Similar to the primary analysis, eyes that receive alternative treatment for DME (see Protocol Section 3.6) will be considered missing before entering the multiple imputation, and will have the last measurement prior to treatment overwritten the imputed values and will be used for the secondary analysis unless otherwise specified ("MI then LOCF"). Unless otherwise specified, missing data will be imputed with multiple imputation. The imputation model for handling missing data will be stratified by treatment group and include the baseline value of the outcome, the randomization stratification factor, and change in the outcome for the available time points.

To ensure that statistical outliers do not have undue impact on analyses of continuous outcomes, change in continuous outcomes from baseline will be truncated to ± 3 standard deviations based on the overall mean and standard deviation at the primary outcome visit from both treatment groups combined. Similar to the primary analysis, the truncation will be applied after multiple imputation and LOCF, and the cutoff values will be calculated from the completers' data. Binary outcomes will be created from the corresponding continuous outcome measurements, after multiple imputation, LOCF and data truncation.

Table 2. Secondary Outcome Analyses Phase 1 (Baseline to 4 Months).

Outcome	Analysis Technique
Mean change in retinal volume from baseline	Analysis of Covariance
Percentage of eyes with CST below OCT machine and gender-specific threshold for DME	Logistic regression
Percentage of eyes receiving alternative treatment for DME	Descriptive statistics only
Percentage of eyes with a 5-letter loss in visual acuity from baseline	Logistic regression
Mean change in visual acuity from baseline	Analysis of Covariance
Patient compliance	Descriptive statistics only

Change in retinal volume from baseline is a continuous variable and will be analyzed using analysis of covariance. The analysis will include adjustment for baseline CST, baseline retinal volume, and the randomization stratification factor. The estimated treatment-group difference, 95% confidence interval and 2-sided *P* value will be presented. The assumptions of linearity, normality, and homoscedasticity will be verified using graphical methods. Serious violations may be addressed by transformation of dependent and/or independent variables, non-parametric methods, categorizing continuous covariates, and/or excluding covariates.

The percentage of eyes with CST below OCT machine and gender-specific threshold for DME at the primary outcome visit is a binary variable that will be analyzed with logistic regression with robust variance estimation. LOCF will be used for eyes receiving alternative DME treatment, but multiple imputation will not be performed for missing data given the thresholds are machine specific. Baseline CST and the randomization stratification factor will be included as covariates. The odds ratio for the treatment group effect, 95% confidence interval, and *P* value will be presented. In addition, the treatment-group risk difference will be computed as the marginal probabilities from a counterfactual model, and the 95% confidence interval will be estimated using bootstrap resampling.

The percentage of eyes receiving an alternative treatment for DME before the primary outcome visit will be reported. Only participants receiving alternative treatment or completing the primary outcome visit without receiving alternative treatment will be included, although it is recognized that the percentage with alternative treatment will likely be overestimated with this procedure. Statistical comparison between treatment groups will not be performed.

The percentages of eyes with ≥5-letter decrease from randomization at the primary outcome visit is a binary variable that will be calculated from the continuous visual acuity letter scores and will be compared between treatment groups using logistic regression with robust variance estimation. Baseline visual acuity and randomization stratification factor will be included as covariates. The odds ratio for the treatment group effect, 95% confidence interval, and *P* value will be presented. Note the mean change in visual acuity will be imputed and LOCF values will be applied to eyes with alternative DME treatment, similar to the primary CST outcome. Statistical comparison

between treatment groups for the mean change will be performed using analysis of covariance with adjustment for baseline visual acuity and the randomization stratification factor. In addition, the treatment-group risk difference will be computed with the marginal probabilities from a counterfactual model, and the 95% confidence interval will be estimated using bootstrap resampling using a complete case analysis with LOCF applied to eyes with alternative DME treatment (no imputed values).

Patient compliance will be reported separately for completers and non-completers, which is defined as the proportion of prescribed treatment sessions completed. For completers, the denominator will be the total prescribed treatment sessions prior to the primary outcome visit; and for non-completers, the denominator will be the total prescribed treatment sessions up to the time when the study device is returned. Note that for both completers and non-completers, if alternative DME treatment is initiated, the denominator will be the total number of sessions prescribed up to the initiation of the alternative treatment. Statistical comparison between treatment groups will not be performed.

3.0 Outcomes Measures Phase 2 (4 Months Post-Outcome)

Upon completion of Phase 1, only eyes that still meet the original protocol major eligibility criteria for VA and OCT at the primary outcome visit will be included in the analysis for Phase 2. A separate table for baseline characteristics will be constructed for Phase 2 participants. Within each treatment group, the following outcomes for evaluating post-switch effects on DME from primary outcome to 4 months post outcome will be reported separately. There will be no formal statistical comparisons of treatment groups. Participants originally assigned to the active group will end device use and participants originally assigned to sham will switch to active. Participants who are not using a device during the post-outcome phase but have not received alternative treatment will be given the option to continue study visits or end study participation early.

- Effect on DME after active treatment is stopped (for the initial treated group only)
    - Mean change in CST between the primary outcome and 4 months post outcome visit
    - Mean change in retinal volume between the primary outcome and 4 months post outcome visit
    - Percentage of eyes with CST below OCT machine and gender-specific threshold for DME at 4 months post outcome
- Effect on DME in eyes previously receiving sham (for the initial sham group only)
    - Mean change in CST between the primary outcome and 4 months post outcome visit
    - Mean change in retinal volume between the primary outcome and 4 months post outcome visit
    - Percentage of eyes with CST below OCT machine and gender-specific threshold for DME at 4 months post outcome

- Patient compliance (for the initial sham group only)
    - Proportion of prescribed treatment sessions completed between the primary outcome and 4 months post outcome visit

For continuous outcomes, median and interquartile ranges and/or means and standard deviations will be reported to describe the data. For the assessment of CST and retinal volume outcomes, the analysis will include 4-month post-outcome completers without missing data at both the primary outcome and 4-month post-outcome visit, as well as 4-month post-outcome non-completers who receive alternative DME treatment in Phase 2. Missing data will not be imputed for 4-month post-outcome non-completers who do not receive alternative DME treatment. It is recognized there is bias in handling of the analysis cohort by not including non-completers who do not meet the failure criteria for alternative DME treatment. Exploratory analysis will be conducted within each group to test whether the post-switch change is significantly different from zero. Similar to Phase 1, patient compliance will be reported separately for 4-month post-outcome completers and non-completers.

4.0 Intervention Adherence

For the primary analyses at the end of Phase 1, adherence will be defined as compliance with device use during Phase 1. An exploratory dose-response analysis will be performed to evaluate whether there appears to be an association of compliance (defined as the proportion of total prescribed sessions of the study device use completed during Phase 1) versus magnitude of treatment effect (defined as the change in OCT CST from baseline at the primary outcome visit). The analysis will include all randomized eyes. Eyes that are lost to follow-up or receive an alternative DME treatment will be considered missing for change in OCT CST and compliance, from the time of dropout or initiation of alternative DME treatment. Markov chain Monte Carlo (MCMC) multiple imputation will be used to handle missing data for change in CST and compliance. For eyes receiving alternative DME treatment, after the multiple imputation is performed, the imputed change in CST will be replaced with the last observed CST prior to receiving treatment (i.e. LOCF will be used). The imputation model will be stratified by treatment group and will include the CST measured at baseline and at all monthly interim visits through Phase 1, cumulative compliance (as defined above) through Phase 1, number of days from randomization to the last completed visit (through Phase 1), along with the randomization stratification factor of recent or planned intravitreous treatment in the non-study eye. For eyes that complete the primary outcome visit, the actual visit date will be used for calculating the number of days since randomization, regardless of alternative DME treatment; for eyes that are lost to follow-up, the target visit date will be used. The distribution of the total number of sessions through the primary outcome visit will be described using summary statistics and graphical methods. A scatter plot with a regression line will be constructed to examine for evidence of dose-response, separately for each treatment group.

As a dose-response effect is expected only in the active treatment group, and the study is not powered to detect a significant interaction between treatment and compliance, a stratified analysis will be performed using the imputed dataset at the primary outcome visit to evaluate the

potential association between compliance and treatment effect separately within each treatment group. The stratified analysis will include adjustment for baseline CST, randomization stratification factor, and number of days from randomization to the primary outcome visit. Model assumptions will be checked, and transformation or categorization of compliance will be used if there is evidence of non-linearity in the dose response. It is recognized that the study may not be adequately powered to detect a definitive dose-response association existing only in the active treatment group, and lack of significance is not necessarily an indication that the association does not exist. It is also recognized that compliance may be affected by both measured and unmeasured post-randomization factors, including efficacy of masking and perceived effects of the treatment; hence, the observed dose-response associations may be biased.

If compliance lessens over time during Phase 1, an exploratory analysis will investigate whether there appears to be any association of compliance versus magnitude of treatment effect over those months. A 4-category variable for compliance will be created from the imputed dataset, based on the compliance in the first 2 months and the remainder of Phase 1, using a cutoff at 80% compliance for each period. Thus the overall primary outcome compliance will be categorized into: 1) ≥80% compliance in both periods, 2) ≥80% compliance in the first 2-month period but not the second period, 3) ≥80% compliance in the second period but not the first period, and 4) <80% compliance in both periods. A box-plot for change in CST at the primary outcome visit by compliance category will be created, and global test will be performed to test the association between compliance and change in CST using ANCOVA adjusting for baseline CST level, number of days from randomization to the primary outcome visit, and randomization stratification factor. Any compliance category with fewer than 20 eyes will be excluded from the ANCOVA analysis. If a significant association is found by the global test, the contrast between categories 1) and 2) will be tested.

For Phase 2, adherence will be evaluated in a similar fashion to assess whether compliance affects the post-switch effect within the initial sham group, specifically:

- For the initial sham group, whether there is an association between compliance during phase 2 and treatment effect in phase 2, which is defined as the change in CST from the primary outcome to 4 months post outcome visit.

Adherence in Phase 2 will be evaluated only among eyes participating in Phase 2. The protocol specifies that if alternative treatment is given during Phase 2, participation in the study will be discontinued following next study visit. Missing data will be handled similarly to the Phase 1 analysis.

In addition, for each phase the effect of text message reminders on compliance will be evaluated using the imputed dataset. Compliance will be defined as the total number of sessions of the study device use during each phase divided by the total number of possible treatment sessions based on visit completion status and alternative DME treatment as noted above; and only participants who have been randomly assigned to receive or not receive text messages will be included in this analysis. This analysis will consist of a comparison of mean compliance between

those receiving texts and those not receiving texts using analysis of covariance, and will include treatment, number of days from randomization to the primary outcome visit for Phase 1 (and number of days from the primary outcome to 4 months post outcome visit for Phase 2), and an interaction between treatment group and text message reminders, given it is possible that the participants who are assigned to the control group are less compliant and more likely to ignore text messages. However, it is also recognized that the power for testing the interaction is low in this study due to the limited sample size. If a significant interaction is not present, the analysis will be conducted combining the two treatment groups. If a significant interaction between treatment and text message reminders is present, a stratified analysis will be performed.

5.0 Safety Analysis

All reportable adverse events will be categorized as study eye or systemic. All events will be tabulated by treatment group in a listing of each reported Medical Dictionary for Regulatory Activities (MedDRA) term and summarized over each MedDRA System Organ Class. All randomized participants will be included in safety analyses.

Since there are no known risks of the device, there are no pre-specified safety outcomes of interest. However, the frequency of each ocular adverse event occurring at least once per eye and each systemic event occurring at least once per participant will be calculated.

In addition, the following will be tabulated by treatment group:

- For each MedDRA System Organ Class, percentage of participants with at least one serious event
- Number of adverse events thought by investigator to be related to treatment

No formal statistical comparisons will be performed for reported adverse events.

6.0 Additional Tabulations and Analyses

The following will be tabulated according to treatment group:

- Baseline demographic and clinical characteristics
    - for overall cohort
    - for Phase 2 participants
- Visit completion rate for each visit (excluding death)
- Protocol deviations
- Number of reported device issues
- Treatment completion

In addition, to evaluate the potential contralateral effect, visual acuity, OCT measurements, and treatment for DME in non-study eyes will be tabulated at primary outcome visit by the treatment group assigned to the study eye. These outcomes will be analyzed and presented similarly to the

primary and secondary analyses as specified above for the study eyes, with the exception that no imputation will be performed for missing data. If the study eye receives a treatment for DR or DME, the last non-study eye measurement prior to the study eye treatment will be used for analysis.

This pilot study is being conducted to determine whether the conduct of a pivotal trial has merit based on an anatomic outcome and to provide information on outcome measures needed to design a pivotal trial. If the results of this study support proceeding with a pivotal trial after evaluation of all the data and discussion within DRCR, information from this study will contribute to designing the pivotal trial. The standard deviation of the difference in visual acuity will be used in the sample size calculation of the pivotal trial. Patient compliance (for example, the proportion of enrolled participants that are randomized and the proportion of randomized participants who comply with the use of the study device post-randomization) will also aid in the design of the pivotal trial.

7.0 OCT Angiography Ancillary Study

At a subset of sites with OCT angiography capabilities, images will be taken at baseline and primary outcome to explore whether there are changes in any features evident on OCT angiography. The statistical analysis plan will be detailed in a separate document.

8.0 General Principles for Analysis

8.1 Analysis Cohort

Unless otherwise stated, all treatment comparison analyses will follow the intention-to-treat principle with all randomized eyes included and each eye analyzed according to the randomized treatment assignment, regardless of treatment actually received.

8.2 Visit Windows for Analysis

For common visits, the analysis windows will be defined according to Table 3. For visits falling in more than 1 window, priority will be given to the key outcome visits. Otherwise, the visit will be assigned to the earlier window (e.g., a visit on day 42 would be assigned as the 1-month visit).

Table 3. Analysis Windows for Outcome Visits

Visit (Protocol Window)	Target	Analysis Window
1 Month (±1 week)	4 weeks	14 – 42 days (2 – 6 weeks)
2 Month (±1 week)	8 weeks	42 – 70 days (6 – 10 weeks)
3 Month (±1 week)	12 weeks	70 – 98 days (10 – 14 weeks)
4 Month/Primary Outcome * (±2 week)	16 weeks	84 – 224 days (12 – 32 weeks)
~6 Month/2-Month Post Outcome (±2 week)	8 weeks after 4-Month visit	140 – 280 days (20 – 40 weeks)
~8 Month/4-Month Post Outcome* (±2 week)	16 weeks after 4-Month visit	168 – 336 days (24 – 48 weeks)

*Key visits

8.3 Missing Data

The strategy for handling missing data generally is included with the description of each individual analysis. Where not otherwise specified, only participants with non-missing data are included in the analysis.

8.4 Outliers

To help ensure that statistical outliers do not have undue impact on analyses of continuous outcomes including visual acuity, OCT central subfield thickness (primary outcome) and retinal volume, change in continuous outcomes will be truncated to ± 3 standard deviations based on the overall mean and standard deviation at the primary outcome visit for primary outcome completers' data, irrespective of treatment group. Visual acuity letter score, change in visual acuity from baseline, OCT central subfield thickness, change in CST from baseline, and change in retinal volume from baseline will be truncated. Truncation will be performed after imputation of missing data and LOCF where applicable (i.e., raw data will be used for imputation).

8.5 Model Assumptions

All model assumptions, including linearity, normality of residuals, and heteroscedasticity, will be verified. If model assumptions are not reasonably satisfied, then covariates may be categorized or excluded, and a nonparametric approach, robust estimation method, or transformation may be considered.

8.6 Type I Error Rate

There is no formal adjustment for multiplicity to compensate for the number of outcomes being compared. All comparisons are conducted at alpha level 0.05 unless otherwise noted. In particular, a number of sensitivity analyses and a non-ITT analysis are proposed along with the primary analysis. The intent of these analyses is to explore the effect of primary analysis assumptions on study conclusions, and if they are different, explain why. These analyses are not a substitute for the primary analysis, and primary conclusion will be based on the pre-specified primary analysis. Only 3 of the secondary outcomes of "primary interest" will be compared

statistically. Two of these, change in central subfield volume and percentage of participants below the threshold for DME on OCT, are expected to be correlated with the primary outcome, and are intended to help support and interpret the primary outcome findings and interpretation, not to form the basis for independent conclusions.

References

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