Statistical Analysis Plan

DRCR Retina Network Protocol AG: Randomized Clinical Trial Assessing the Effects of Pneumatic Vitreolysis on Vitreomacular Traction

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Version Number	Author	Approver	Effective Date	Revision Description
1.0	Wesley Beaulieu	Maureen Maguire	08 Oct 2019	Initial SAP for Protocol version 2.0
2.0	Wesley Beaulieu	Maureen Maguire	19 Jan 2021	Revisions were made to accommodate a smaller than anticipated sample size after early stopping for safety concerns. Changes were made following review of study data. Details are provided in subsection 1.17. Applies to Protocol version 3.0.

SIGNATURES	
AUTHOR	
APPROVER	

1.0 Overview

The DRCR Retina Network Protocol AG randomized clinical trial will evaluate the effectiveness of pneumatic vitreolysis (PVL) versus sham in treating eyes with idiopathic symptomatic vitreomacular traction (VMT) without macular hole. Presence of VMT will be graded by a central reading center on optical coherence tomography (OCT) prior to randomization and during follow-up. The primary outcome and final visit are at 24 weeks. Randomization will be stratified by clinical site and presence of epiretinal membrane (ERM) within 1 mm of the center of the macula. Previous reports have suggested that the proportions of eyes with VMT release differ depending on the presence of ERM.[1-3]

1.1 Statistical Hypotheses

A test of superiority will be used in evaluating the following hypotheses for the primary outcome:

Null Hypothesis (H_0): There is no difference in the proportion of eyes with central VMT release without rescue treatment between the PVL and observation groups at 24 weeks.

Alternative Hypothesis (H_a): There is a difference in the proportion of eyes with central VMT release without rescue treatment between the PVL and observation groups at 24 weeks.

Similar hypothesis tests will be conducted for all secondary, exploratory, and safety outcomes.

1.2 Outcome Measures

For the outcomes below, rescue treatment includes vitrectomy, ocriplasmin, or additional pneumatic vitreolysis during the course of the study.

1.2.1 Primary Efficacy Outcome:

- Proportion of eyes with central VMT release* without rescue treatment at 24 weeks.
    - For purposes of description only, the distribution of eyes within treatment group by the following categories at 24 weeks will be tabulated without statistical comparison:
        - Central VMT release without rescue treatment
        - Central VMT release with rescue treatment
        - No central VMT release and no rescue treatment
        - No central VMT release despite rescue treatment

*Determined by masked grader at the central reading center.

1.2.2 Secondary Efficacy Outcomes:

- Proportion of eyes with central VMT release* without rescue treatment through 24 weeks (time-to-event analysis).
- Mean change in visual acuity letter score from baseline at 24 weeks.

- Proportion of eyes with at least 10-letter gain (increase) in visual acuity from baseline at 24 weeks.
- Proportion of eyes with at least 10-letter loss (decrease) in visual acuity from baseline at 24 weeks.
- Proportion of eyes receiving rescue treatment before the 24-week visit.
    - For purposes of description only, the following will be tabulated within treatment group without statistical comparison:
        - Proportion of eyes receiving rescue treatment before the 24-week visit or for which rescue treatment is planned at the 24-week visit and medical records confirm rescue treatment occurred within the subsequent 12 weeks.
        - Type of rescue treatment.

*Determined by masked grader at the central reading center.

1.2.3 Exploratory Efficacy Outcomes:

- Mean change in shape discrimination hyperacuity (SDH) from baseline at 24 weeks.
- Proportion of eyes with ellipsoid zone* integrity at 24 weeks.
    - Both ellipsoid zone integrity within 1 mm of the center of the macula and at the foveal center will be analyzed

*Determined by masked grader at the central reading center.

1.3 Analysis Cohorts

- Intention-To-Treat (ITT) Analysis Cohort: all randomized participants irrespective of treatment received and analyzed according to treatment assignment.
- Safety Analysis Cohort: all randomized participants irrespective of treatment received and analyzed according to treatment assignment.
- Per-Protocol Analysis Cohort: only participants who complete the initial treatment (PVL or sham injection) and do not receive any non-protocol treatments during follow-up. Vitrectomy performed according to the criteria in section 4.2.1 of the protocol is considered per-protocol and eyes receiving this procedure will be included in the per-protocol cohort.

The primary analysis will follow the ITT principle. It will include all randomized participants. The data from the ITT cohort will be analyzed according to the group to which the participants were assigned through randomization, regardless of treatment actually received.

A per-protocol analysis will be performed to provide additional information regarding the magnitude of the treatment effect. The per-protocol analysis will only be performed if more than 10% of randomized participants would be excluded by these criteria (e.g., 13 or more participants if exactly 124 are enrolled).

The ITT analysis is considered the primary analysis. If the results of the per-protocol and ITT analyses give inconsistent results, then the per-protocol analysis will be interpreted with caution. In this scenario, exploratory analyses will be performed to evaluate possible factors contributing to the differences.

1.4 Analysis of the Primary Efficacy Outcome

The primary outcome of central VMT release without rescue treatment at 24 weeks is a binary variable that is graded by the central reading center. Logistic regression will be used to test the hypothesis of superiority. The risk difference for the treatment group effect (estimated with conditional standardization)[4], along with the 95% confidence interval (estimated with the delta method)[4] and *P* value will be used to compare treatment groups. To aid in interpretation of the risk difference, observed outcome proportions will be reported for each treatment group.

Since the chance of re-attachment after release before 24 weeks is highly unlikely, an eye with central VMT release without rescue treatment prior to 24 weeks will be considered to have met the outcome through 24 weeks if the participant is lost to follow-up. Similarly, any eye receiving rescue treatment prior to 24 weeks will be considered not to have met the outcome through 24 weeks.

Multiple imputation will be used to impute missing data for eyes lost to follow-up that did not have prior release or rescue treatment documented. The imputation model will treatment group, and VMT status at 1, 4, 12, and 24 weeks.

A sensitivity analysis will be conducted using the same approach as above, but without multiple imputation (i.e., complete-case analysis).

1.5 Analysis of the Secondary and Exploratory Efficacy Outcomes

The ITT analysis cohort will be used for all secondary and exploratory outcomes.

1.5.1 Secondary Efficacy Outcomes

Development of central VMT release without rescue treatment through 24 weeks is a time-to-event outcome graded by the central reading center that will be modeled with Cox proportional hazards regression and robust variance estimation. The hazard ratio along with the 95% confidence interval and *P* value will be used to compare treatment groups. To aid in interpretation, a Kaplan-Meier plot will be constructed and the cumulative probability of the outcome will be estimated at the final time point for each group. Data from eyes not observed to have release or that receive rescue treatment will be censored on the date of their final visit (not the date of rescue treatment).

Change in visual acuity letter score from baseline to 24 weeks is a continuous variable that will be analyzed using a general linear model with robust variance estimation. Baseline visual acuity will be included as a covariate. The adjusted treatment group difference, 95% confidence interval, and *P* value will be presented. To aid in interpretation, least squares means and associated 95% confidence intervals will be reported for each treatment group. Missing data will be imputed with multiple imputation. The imputation model will include treatment group, baseline visual acuity, visual acuity at 1, 4, 12, and 24 weeks, and VMT status at 1, 4, 12, and 24 weeks.

The proportions of eyes with at least 10-letter gain (increase) and at least 10-letter loss (decrease) in visual acuity from baseline are binary variables that will be analyzed with logistic regression utilizing the imputed data sets from the analysis of mean change in visual acuity from baseline. Baseline visual acuity will be included as a covariate.

The proportion of eyes receiving rescue treatment before the 24-week visit is a binary variable that will be analyzed with logistic regression. Complete-case analysis (no imputation of missing data) will be used for this outcome.

1.5.2 Exploratory Efficacy Outcomes

Change in SDH is a continuous variable that will be analyzed similarly to change in visual acuity but substituting baseline and follow-up SDH for visual acuity. Complete-case analysis (no imputation of missing data) will be used for this outcome. Shape discrimination hyperacuity ranges from -1 to +1. On the myVisionTrack test being used in this study, normal SDH is -0.60 or less.

The proportion of eyes with ellipsoid zone integrity at 24 weeks is a binary variable graded by the central reading center (loss of integrity and no loss of integrity). Both ellipsoid zone integrity in the central subfield and at the foveal center will be analyzed. Logistic regression will be used to compare treatment groups. Ellipsoid zone status at baseline will be included as a covariate. The risk difference for the treatment group effect, 95% confidence interval, and *P* value will used to compare treatment groups. To aid in interpretation of the risk difference, observed outcome proportions will be reported for each treatment group. Complete-case analysis (no imputation of missing data) will be used for this outcome.

1.6 Safety Analyses

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. Any events occurring between randomization and study treatment will be counted. For each treatment group, the number of adverse events (ocular or systemic) considered related to treatment will be tabulated.

1.6.1 Ocular Adverse Events

The frequency of each ocular adverse event occurring at least once per eye will be calculated. The proportion of eyes experiencing each outcome will be compared between treatment groups with Barnard's unconditional exact test. The following ocular adverse events are of primary interest:

- Retinal detachment
- Retinal tear
- Macular hole development
- Cataract extraction in eyes phakic at baseline
- Vitreous hemorrhage
- Adverse intraocular pressure (IOP) events (composite outcome)

- Increase in IOP ≥ 10 mmHg from baseline (at a follow-up visit)
- IOP ≥ 30 mmHg (at a follow-up visit)
- Initiation of medication to lower IOP that was not in use at baseline
- Glaucoma procedure

The number of eyes with endophthalmitis and traumatic cataract will be tabulated without statistical comparison.

1.6.2 Systemic Adverse Events

The frequency of each systemic adverse event occurring at least once per participant will be calculated. The proportion of participants experiencing each outcome will be compared with Barnard's unconditional exact test. The following systemic adverse events are of primary interest:

- Death
- Serious adverse event (at least one)

The following systemic adverse events are of secondary interest and will be tabulated without statistical comparison:

- For each MedDRA System Organ Class, proportion of participants with at least one serious event

1.7 Intervention Adherence

Adherence will be defined as completion of the treatment assigned at randomization: either PVL or sham injection.

1.8 Protocol Adherence and Retention

Protocol deviations and visit completion rates (excluding deaths) will be tabulated for each treatment group.

1.9 Baseline Descriptive Statistics

Baseline characteristics will be tabulated by treatment group and summary statistics appropriate to the distribution will be reported.

1.10 Planned Interim Analyses

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

1.11 Subgroup Analyses

Subgroup analyses, i.e., assessments of effect modification (interaction), will be conducted for the primary outcome. These analyses will be considered exploratory. Additionally, interpretation of the analyses will depend on whether the primary analysis demonstrates a significant treatment

group difference; in the absence of such a difference, subgroup analyses will be interpreted with caution.

The general approach for these exploratory analyses will be to add an interaction term for the subgroup factor by treatment into the primary analysis model. In addition, within-subgroup risk differences and 95% confidence intervals will be estimated from the interaction model if the interaction *P* value is less than .05. Subgroup analyses will use data from eyes that complete the 24-week visit or have VMT release or rescue treatment prior to 24 weeks (i.e., complete case analysis as described in section 1.4).

The primary subgroup analysis will evaluate the effect of ERM presence within 1 mm of the center of the macula at baseline. In previous studies, eyes with ERM had lower release rates compared with eyes not having ERM.[1-3]

Secondary subgroup analyses will include ERM presence at the site of vitreous adhesion, lens status (phakic or pseudophakic), retinoschisis, subretinal fluid within the central 1 mm, length of adhesion on OCT (less than or equal to 1500 microns or greater than 1500 microns), and diabetes status (has diabetes or does not have diabetes). Subgroups will be defined by the value at baseline.

There are no data to suggest that the treatment effect will vary by sex or race/ethnicity. However, both of these factors will be evaluated in exploratory subgroup analyses as mandated by National Institutes of Health (NIH) guidelines.

Subgroup factors will be analyzed as categorical and continuous or ordinal variables where possible. Secondary and exploratory subgroup analyses will only be conducted if there are at least 20 eyes in each subgroup for each treatment group. The primary subgroup analysis will be conducted regardless of sample size.

1.12 Multiple Testing

There will be no formal adjustment for multiple testing. Only $P \leq .05$ will be considered of interest.

1.13 Visit Windows for Analysis

The analysis windows for visits will be defined according to Table 1. If multiple visits fall within the same window, priority will be given to the protocol visit over unspecified visits. If there is no protocol visit in the window, then the visit closest to the target date (but within the analysis window) will be designated as the analysis visit. Visit windows will be filled in the following order to handle visits occurring on the border of two windows: 24 weeks, 12 weeks, 4 weeks, 1 week.

Table 1. Analysis Windows

Visit ± Protocol Window	Target	Analysis Window
1 week (-4 days to +3 days)	7 days	1 day – 2 weeks (1 – 14 days)
4 (± 1) weeks	28 days	2 – 8 weeks (14 – 56 days)
12 (± 2) weeks	84 days	8 – 18 weeks (56 – 126 days)
24 (± 4) weeks	168 days	18 – 40 weeks (126 – 280 days)

1.14 Missing Data

The strategy for handling missing data generally is included with the description of each analysis. For analyses using multiple imputation, the Markov chain Monte Carlo (MCMC) method with 100 imputations will be used. Where otherwise not specified, only participants with non-missing data are included in analyses (i.e., complete-case analysis).

1.15 Outliers

To ensure that statistical outliers do not have an 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 from both treatment groups combined at 24 weeks. Truncation will occur after imputation, where applicable.

1.16 Model Assumptions and Nonconvergence

All model assumptions will be verified. If model assumptions are seriously violated, covariates may be categorized or excluded, and a non-parametric approach, robust method, or transformation may be considered. The proportional hazards assumption will be assessed by visual inspection of Kaplan-Meier curves. If the proportional hazards assumption is seriously violated, then an alternative approach, such as analysis of restricted mean survival time, may be undertaken.

If a logistic regression models fail to converge, then covariates will be excluded, missing data will not be imputed (where applicable), the confidence interval for the risk difference will be estimated with the Newcombe method, and the *P* value for the treatment group comparison will be calculated with Barnard's unconditional exact test.

1.17 Revisions

Owing to lower than anticipated final sample size, the following key changes were made to the analysis plan after review of study data:

- Presence of epiretinal membrane has been removed as a covariate from all imputation and regression models.
- Proportion of eyes with central VMT release and vitreopapillary traction (VPT) release without rescue treatment at 24 weeks has been removed from the list of secondary outcomes.
- A sensitivity analysis of confounding for the primary outcome has been removed.
- An alternative analysis method has been described for outcomes in which logistic regression fails to converge.

In addition, treatment and subgroup effects from all logistic regression analyses will now be summarized with a risk difference instead of a relative risk.

References

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