Statistical Analysis Plan

DRCR Retina Network Protocol AH: Single-Arm Study Assessing the Effects of Pneumatic Vitreolysis on Macular Hole

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Version Number	Author	Approver	Effective Date	Revision Description
1.0	Wesley Beaulieu	Maureen Maguire	09 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	
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APPROVER	

1.0 Overview

The DRCR Retina Network Protocol AH study will evaluate the effectiveness of pneumatic vitreolysis (PVL) in treating eyes with vitreomacular traction (VMT) and full-thickness macular hole (MH). Presence of MH will be graded on optical coherence tomography (OCT) by a reading center prior to enrollment and during follow-up. The primary outcome is at 8 weeks and follow-up continues through 24 weeks.

1.1 Statistical Hypotheses

There are no formal statistical hypotheses that will be evaluated because this is a single-arm study.

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 MH closure of the inner retinal layers* without rescue treatment at 8 weeks.
    - For purposes of description only, the distribution of eyes by the following categories at 8 weeks will be tabulated without statistical comparison:
        - MH closure without rescue treatment
        - MH closure with rescue treatment
        - No MH closure and no rescue treatment
        - No MH closure despite rescue treatment

1.2.2 Secondary Efficacy Outcomes:

- Proportion of eyes with MH closure of the inner retinal layers* without rescue treatment through 24 weeks (time-to-event analysis).
- Proportion of eyes with central VMT release* without rescue treatment through 24 weeks (time-to-event analysis).
    - For purposes of description only, the following will be tabulated without statistical comparison at 8 weeks and 24 weeks:
        - MH closure with central VMT release without rescue treatment
        - MH closure without central VMT release without rescue treatment
        - MH closure with central VMT release with rescue treatment
        - MH closure without central VMT release with rescue treatment
- Mean change in visual acuity letter score from baseline at 8 and 24 weeks.

- Proportion of eyes with at least 10-letter gain (increase) in visual acuity from baseline at 8 and 24 weeks.
- Proportion of eyes with at least 10-letter loss (decrease) in visual acuity from baseline at 8 and 24 weeks.
- Proportion of eyes receiving rescue treatment before the 8-week visit.
- Proportion of eyes receiving rescue treatment before the 24-week visit.
    - For purposes of description only, the following will be tabulated 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.

1.2.3 Exploratory Outcomes:

- Proportion of eyes with MH closure of the inner retinal layers with outer retinal lucency* without rescue treatment at 8 and 24 weeks.
- Proportion of eyes with ellipsoid zone* integrity at 8 and 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 enrolled participants irrespective of treatment received.
- Safety Analysis Cohort: all enrolled participants irrespective of treatment received.
- Per-Protocol Analysis Cohort: only participants who complete the initial treatment (PVL) 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 and include all enrolled participants.

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 at least 10% of enrolled participants would be excluded by these criteria (e.g., 5 or more participants if exactly 50 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 difference.

1.4 Analysis of the Primary Efficacy Outcome

The primary outcome of MH closure of the inner retinal layers without rescue treatment through 8 weeks is a binary variable that is graded by the central reading center. The proportion of eyes meeting the primary outcome will be determined and the 95% Wilson (score) confidence interval will be calculated.[1-3]

Since the chance of re-opening after closure before 8 or 24 weeks is highly unlikely, an eye with MH closure of the inner retinal layers without rescue treatment at any time 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 MH closure or rescue treatment documented. The imputation model will include MH status at 1, 4, 8, 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 Outcomes

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

1.5.1 Secondary Efficacy Outcomes

There are two time-to-event outcomes to be evaluated through 24 weeks: (1) macular hole closure of the inner retinal layers without rescue treatment and (2) central VMT release without rescue treatment. Each of these outcomes will be graded by the central reading center. For each outcome, a Kaplan-Meier curve will be constructed and the cumulative probability of the outcome with 95% confidence interval will be estimated for the final time point. Data from eyes not observed to meet the outcome or that receive rescue treatment will be censored on the date of their final visit (not the date of rescue treatment). The analysis of central VMT release without rescue treatment will be limited to eyes with central VMT at baseline. Because the chance of re-attachment after release before 8 or 24 weeks is highly unlikely, an eye with release without rescue treatment at any time 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.

Change in visual acuity letter score from baseline at 8 and 24 weeks is a continuous outcome. The mean and 95% confidence interval will be calculated for each time point. Missing data will be imputed with multiple imputation. The imputation model will baseline visual acuity, visual acuity at 1, 4, 8, and 24 weeks, and MH status at 1, 4, 8, and 24 weeks.

The proportions of eyes with at least 10-letter gain (increase) and least 10-letter loss (decrease) in visual acuity from baseline at 8 and 24 weeks are binary variables. Wilson (score) 95% confidence intervals will be calculated for each outcome at both time points. The imputed data sets described above for the mean change in visual acuity from baseline will be utilized.[3]

The proportion of eyes receiving rescue treatment before 8 and 24 weeks is a binary variable. Wilson (score) 95% confidence intervals will be calculated for each time point. Complete-case analysis (no imputation of missing data) will be used for this outcome.

1.5.2 Exploratory Efficacy Outcomes

The proportion of eyes with MH closure of the inner retinal layers with outer retinal lucency at 8 and 24 weeks is a binary variable graded by the central reading center. Wilson (score) 95% confidence intervals will be calculated for each time point. Complete-case analysis (no imputation of missing data) will be used for this outcome.

The proportion of eyes with ellipsoid zone integrity at 8 and 24 weeks is a binary variable graded by the central reading center (loss of integrity and no loss of integrity). Both ellipsoid zone integrity within 1 mm of the center of the macula and in the foveal center will be analyzed. Wilson (score) 95% confidence intervals will be calculated for each time point. 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 in a listing of each reported Medical Dictionary for Regulatory Activities (MedDRA) term and summarized over each MedDRA System Organ Class. All enrolled participants will be included in safety analyses. Any events occurring between enrollment and study treatment will be counted. 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 calculated along with 95% Wilson (score) confidence intervals. The following ocular adverse events are of primary interest:

- Endophthalmitis
- Retinal detachment
- Retinal tear
- Traumatic cataract
- Cataract extraction in eyes phakic at baseline
- Vitreous hemorrhage
- Adverse intraocular pressure (IOP) events (composite outcome)
    - Increase in IOP $\geq$ 10 mmHg from baseline (at a follow-up visit)
    - IOP $\geq$ 30 mmHg (at a follow-up visit)
    - Initiation of medication to lower IOP that was not in use at baseline
    - Glaucoma procedure

1.6.2 Systemic Adverse Events

The frequency of each systemic adverse event occurring at least once per participant will be calculated. 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:

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

1.7 Intervention Adherence

Adherence will be defined as completion of PVL.

1.8 Protocol Adherence and Retention

Protocol deviations and visit completion rates (excluding deaths) will be tabulated.

1.9 Baseline Descriptive Statistics

Baseline characteristics will be tabulated 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, will be conducted to detect factors associated with the primary outcome. These analyses will be considered exploratory.

Subgroup analyses will be conducted using logistic regression. The risk difference for the subgroup factor (estimated with conditional standardization),[4] 95% confidence interval (estimated with the delta method)[4], and *P* value will be presented. To aid in interpretation of the risk difference, observed outcome proportions will be reported for each subgroup. Subgroup analyses will use data from eyes that complete the 8-week visit or have MH closure or rescue treatment prior to 8 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.

Secondary subgroup analyses will include ERM presence at the site of vitreous adhesion, lens status (phakic or pseudophakic), retinoschisis, subretinal fluid within 1 mm of the center of the macula, 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 success 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 10 eyes in each subgroup. The primary subgroup analysis will be conducted regardless of sample size.

1.12 Multiple Testing

There will be no adjustments made for multiple testing because this is a single-arm study without treatment group comparisons. 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: 8 weeks, 24 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 – 6 weeks (14 – 42 days)
8 (± 2) weeks	56 days	6 – 18 weeks (42 – 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 will be included in analyses.

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 at 8 weeks. Truncation will occur after imputation, where applicable.

1.16 Model Assumptions.

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.

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.

In addition, subgroup effects will still be analyzed with logistic regression, but the subgroup effect will be summarized with a risk difference instead of a relative risk.

References

1. Brown LD, Cai TT, DasGupta A. Interval estimation for a binomial proportion. *Statistical Science.* 2001;16(2):101-117.

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3. Lott A, Reiter JP. Wilson Confidence Intervals for Binomial Proportions With Multiple Imputation for Missing Data. *The American Statistician.* 2020;74(2):109-115.

4. Localio AR, Margolis DJ, Berlin JA. Relative risks and confidence intervals were easily computed indirectly from multivariable logistic regression. *J Clin Epidemiol.* 2007;60(9):874-882.