A Phase 2 Study Evaluating Short-Term Efficacy of Tonabersat (Xiflam) for Diabetic Macular Edema

(Protocol AN)

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DRCR Retina Network

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Statistical Analysis Plan

VERSION	AUTHOR	APPROVER	EFFECTIVE DATE	REVISION DESCRIPTION
1.0	Kristin Josic	Michele Melia	12-02-22	Initial version for Protocol version 2.0
1.1	Kristin Josic	Michele Melia	09-06-23	Revisions for protocol amendment #1 (dosing instructions) – updated safety cohort definition
1.2	Thu Vu	Michele Melia	05-01-25	Added sensitivity analysis using multiple imputation instead of LOCF for the primary analysis

SIGNATURES	
AUTHOR	
APPROVER	

1. Introduction

This document outlines the statistical analysis plan for the DRCR Retina Network Protocol AN randomized clinical trial comparing the effect of Tonabersat (Xiflam) versus placebo on central subfield thickness (CST) in eyes with central-involved diabetic macular edema (CI-DME). Randomization will be stratified by categories of baseline CST and assign participants 1:1 to 40 mg tonabersat or placebo. Randomization is at the participant level but participants can have one or two study eyes. The study duration is 6 months. Study procedures and sample size calculations are outlined in the Study Protocol.

2. Statistical Hypotheses

All hypotheses are two-sided tests of superiority. For the primary outcome, Type 1 error rate of $\alpha = .05$ will be used as the basis for rejecting the null hypothesis in favor of the alternative hypothesis. For the secondary outcomes, a false discovery rate of $q = .05$ will be controlled using the Benjamini-Hochberg adaptive procedure.[3] The rejection of a null hypothesis for a secondary outcome indicates the outcome is of interest and warrants further investigation. Hypothesis tests will include mean differences ($\mu_{diff} = \mu_{tonabersat} - \mu_{placebo}$) compared to a null value of zero, and odds ratios ($OR = Odds_{tonabersat} / Odds_{placebo}$) compared to a null value of one. To aid in interpreting odds ratios, we will estimate an associated risk difference ($RD = Probability_{tonabersat} - Probability_{placebo}$) and 95% CI. Estimates for mean differences, odds ratios and risk differences are described in the following sections.

Primary Outcome

- Null Hypothesis (H_0): $\mu_{diff} = 0$. There is no difference in the mean change in CST from baseline to 6 months between the tonabersat and placebo groups.
- Alternative Hypothesis (H_A): $\mu_{diff} \neq 0$. There is a difference in the mean change in CST from baseline to 6 months between the tonabersat and placebo groups.

Secondary Continuous Outcomes

- H_0: $\mu_{diff} = 0$. There is no difference in the mean of the outcome between treatment groups.
- H_A: $\mu_{diff} \neq 0$. There is a difference in the mean of the outcome between treatment groups.

Secondary Binary Outcomes

- H_0: $OR = 1$. There is no difference in the odds of the outcome between treatment groups.
- H_A: $OR \neq 1$. There is a difference in the odds of the outcome between treatment groups.

The above hypothesis is equivalent to

- H_0: $RD = 0$. There is no difference in the risk of the outcome between treatment groups.
- H_A: $RD \neq 0$. There is a difference in the risk of the outcome between treatment groups.

3. Outcome Measures

Outcomes marked with an asterisk (*) will include descriptive statistics by treatment group only. Outcomes without an asterisk will include a point estimate and 95% confidence interval of the treatment effect (mean difference, odds ratio, or risk difference) but only the primary and secondary outcomes may include p-values (see analysis sections 5 and 6). P-values will not be reported for exploratory outcomes.

Primary Outcome

- Change in CST from baseline to 6 months

Secondary Outcomes

- Change in retinal volume from baseline to 6 months

- CST below the OCT machine- and gender-specific threshold for CI-DME at 6 months and a 10% or more decrease in CST from baseline to 6 months (composite outcome)
- Change in ETDRS BCVA from baseline to 6 months
- A decrease in ETDRS BCVA of 10 or more letters from baseline to 6 months, or 5 or more letters from baseline to 6 months and the previous study visit.
- Receiving other treatment for DME prior to the 6-month visit*

Exploratory Outcomes

- Changes in DR on fundus photos from baseline to 6 months of
    - 1-step or more worsening*
    - 2-steps or more worsening
    - 1-step or more improvement*
    - 2-steps or more improvement
- Changes on OCTA from baseline to 6 months in
    - Vessel density
    - Nonperfusion area
- Treatment compliance from baseline to 6 months (percentage of prescribed study pills taken)*
- Changes in blood and urine samples from baseline to 6 months of
    - eGFR
    - CRP
    - HbA1c
    - Cystatin C
    - TSH
    - Galectin 3
    - IL-1 β
    - IL-6
    - TNFα
    - Urine creatinine
    - Urine albumin
    - Urine MCP1
    - Urine osteopontin
    - Urine IL-18

4. Analysis Datasets and Sensitivity Analysis

The analyses of the primary, secondary, exploratory, and safety outcomes will follow the intention-to-treat (ITT) principle, analyzing all randomized participants according to their random group assignment, regardless of treatment received. A sensitivity analysis for the primary outcome will provide additional information on the magnitude of the treatment effect using a per-protocol analysis (PPA) cohort if the PPA criteria would exclude more than 10% of randomized participants. The ITT analysis is considered primary; if the ITT and PPA analysis are inconsistent, an exploratory analysis will evaluate possible differences.

- ITT Analysis Cohort: all randomized participants irrespective of treatment received, analyzed according to treatment assignment.
- Safety Analysis Cohort: all randomized participants that received at least one dose of randomized treatment. Receipt of treatment will be determined whether the participant took the first pill in the office on the day of randomization (for participants enrolled before amendment #1), or by a question on the one-week phone call or the pill count of a returned bottle at the first available follow-up visit (for participants enrolled after amendment # 1) .
- PPA Analysis Cohort: participants who complete the 6-month visit, achieve at least 75% compliance with the assigned Study Drug when required to be taken (prior to protocol-allowed discontinuation), and do not receive any other treatment for DME.

5. Analysis of the Primary Outcome

The primary outcome of change in CST from baseline to 6 months is a continuous variable. The mean difference between treatment groups will be estimated using a linear mixed model with adjustment for baseline CST. Participant-level random intercept effects will model the potential correlation within participants having two study eyes. The assumptions of linearity, normality, and homoscedasticity will be verified using graphical methods (histograms, Q-Q plots, and scatter plots of residuals). If serious violations occur, a generalized linear model (such as gamma or lognormal distributions for skewed data) or non-parametric method may be considered to estimate the mean treatment group difference. Missing data for eyes not treated with another DME treatment will be imputed using Markov chain Monte Carlo (MCMC) multiple imputation (MI). The imputation model will stratify by treatment group and include baseline CST and changes in CST from baseline to all monthly visits. For eyes that receive treatment for DME (see section 3.6) prior to the 6-month visit, OCT measurements after treatment will be considered missing for imputation, and the primary analysis will use change from baseline to the last OCT measurement prior to treatment (last observation carried forward, LOCF). Change in CST from baseline to 6 months/LOCF will be truncated at the overall mean ±3 SD of the observed values so that statistical outliers do not disproportionately impact the analysis (imputed values not used to truncate and truncated values not used to impute). Descriptive statistics for each treatment group will include the mean, standard deviation, median and interquartile range using the truncated data from eyes that completed the 6-month visit or received DME treatment prior to the 6-month visit (no imputed values). The estimated treatment effect will include the adjusted mean difference, 2-sided 95% confidence interval, and p-value using all MI/LOCF data. A p-value < .05 rejects the null hypothesis.

CST values can be obtained from either Spectralis or Cirrus OCT images, but the CST values from these machines are not interchangeable. If the machine type at a follow-up visit differs from the baseline visit, the follow-up CST measurement must be converted into the machine equivalent of baseline CST to calculate change. The rational for converting CST at the follow-up visit instead of at the baseline visit is because the outcome model adjusts for baseline CST, and the imputation model includes baseline CST and change from baseline at all monthly visits. The following equations will convert CST between Cirrus and Spectralis machines[1].

- Spectralis CST = 40.78 + 0.95 × Cirrus CST
- Cirrus CST = 1.82 + 0.94 × Spectralis CST

If the machine type is the same at baseline and follow-up, the observed values can be used to calculate change (no need for conversion). The primary outcome and imputation model will use CST values converted to the machine used for the majority of measurements.

A sensitivity analysis will be conducted using an alternative method to LOCF. In this analysis, eyes receiving treatment for DME prior to the 6-month visit will have missing OCT measurements imputed using control group data. Specifically, missing values will be imputed based on a multiple imputation model that incorporates baseline CST and changes in CST from baseline to all monthly visits, using only data from the control group. This approach provides an alternative method for handling missing data, assessing the robustness of the imputation method. Additionally, if more than 5 eyes received intravitreal anti-VEGF or steroid injections for conditions other than DME before the 6-month visit, post-treatment OCT measurements will be imputed using the same approach.

6. Analysis of Secondary and Exploratory Outcomes

The analysis of all secondary and exploratory outcomes will use the ITT analysis cohort. Unless otherwise specified, eyes that receive treatment for DME will use LOCF values for analysis. Missing data for eyes that did not receive treatment for DME will be imputed with multiple imputation for the secondary outcomes but not for the exploratory outcomes. The imputation models will be stratified by treatment group and include baseline CST (the randomization stratification factor), the baseline value of the outcome and changes in the outcome from baseline to all monthly visits. All values after DME treatment will be considered missing for the imputation and then overwritten with LOCF values. Continuous outcomes will be truncated at the overall mean ±3 SD so that statistical outliers do not disproportionately impact the analysis. Truncation will be performed after imputation (where applicable) and truncation limits will be determined from the observed values (imputed values not used to truncate and truncated values not used to impute).

All secondary outcomes will be summarized with descriptive statistics by treatment group, but only some will have inferential statistics on treatment effect. Descriptive statistics will be computed using observed 6-month/LOCF data only (no imputed values). For continuous outcomes, means with standard deviations, and/or medians with interquartile ranges of the truncated data will be reported. For binary outcomes, frequencies with percentages will be reported. Treatment effects will include mean differences and odds ratios that adjust for baseline levels of the outcome and baseline CST (the randomization stratification factor). Treatment effects will be estimated using linear or generalized linear models with multiple imputation for missing data (where applicable). For eye-level outcomes, random intercepts will model the potential correlation among participants having two study eyes. For participant-level outcomes, a participant-level model will be used.

For each continuous outcome with a mean difference estimated from a linear model, the assumptions of linearity, normality, and homoscedasticity will be verified using graphical methods (histograms, Q-Q plots and scatter plots of residuals). If serious violations occur, a generalized linear mixed model or non-parametric method may be considered to estimate the mean difference. The adjusted mean difference between treatment groups and two-sided 95% CI will be reported.

For each binary outcome with an odds ratio estimated from a generalized linear model with a logistic link function, the adjusted odds ratio between treatment groups, and two-sided 95% CI will be reported. Additionally, the risk difference between treatment groups and two-sided 95% CI will be estimated at the mean levels of other covariates in the model, with the variance estimated using the delta method.[2]

P-values will be reported for two-sided tests of superiority on the pre-specified secondary outcomes only. P-values will not be reported for exploratory outcomes. The false discovery rate (FDR) of secondary

outcomes will be controlled at q = .05 with the Benjamini-Hochberg adaptive FDR procedure.[3] The steps to determine if a null hypothesis for a secondary outcome is rejected or not using the adaptive FDR procedure are summarized as follows.[3]

1. Order the p-values $p_{(1)} \leq \cdots \leq p_{(i)} \leq \cdots \leq p_{(m)}$ that correspond to each tested null hypothesis $H_{(1)} \leq \cdots \leq H_{(i)} \leq \cdots \leq H_{(m)}$
2. Compare each $p_{(i)}\ to \frac{i}{m}q$
    a. If no $p_{(i)} < \frac{i}{m}q$ then do not reject any hypothesis and stop
3. Calculate the slopes $S_i = \frac{1-p_{(i)}}{m+1-i}$
    a. Start with $j = 1$ and find the smallest j that satisfies $S_j < S_{j-1}$
    b. Set $\widehat{m}_0 = \min\left(\left[\frac{1}{S_j} + 1\right], m\right)$
4. Compare each $p_{(i)}\ to \frac{i}{\widehat{m}_0}q$
    a. Start with $k = m$ and find the largest k that satisfies $p_{(k)} \leq \frac{k}{\widehat{m}_0}q$
    b. Reject all $H_{(i)}, i = 1, \ldots, k$

Figures will show descriptive statistics by treatment group over time. Continuous outcomes will use line graphs of the mean and 95% standard error bars and binary outcomes will use bar graphs of percentages.

Secondary Outcomes

- Change in retinal volume from baseline to 6 months is a continuous variable. The mean difference between treatment groups will be estimated using a linear mixed model with adjustment for baseline retinal volume, baseline CST, and participant-level random intercepts.
    - The imputation model will be stratified by treatment and include baseline CST, baseline retinal volume and changes in retinal volume from baseline to 1, 2, 3, 4, 5, and 6 months.
    - The outcome and imputation model will use Stratus equivalents for baseline retinal volume. If the OCT machine at the baseline and follow-up visit differ, both retinal volume measurements will be converted to Stratus equivalents to calculate change because we are not aware of any equations to convert retinal volume between Cirrus and Spectralis machines. The following equations will convert Cirrus and Spectralis retinal volume measurements into Stratus equivalents[3].

        - Stratus Retinal Volume = -1.21 + 1.02 × Cirrus Retinal Volume
        - Stratus Retinal Volume = -2.05 + 1.06 × Spectralis Retinal Volume

- CST below the OCT machine- and gender-specific threshold for CI-DME at 6 months and a 10% or more decrease in CST from baseline to 6 months is a binary variable. The odds ratio between treatment groups will be estimated using a generalized linear mixed model for a binary outcome with a logit link function and adjustment for baseline CST participant-level random intercepts.
    - Missing data will not be imputed because CI-DME indicators are OCT machine and gender specific.
    - CI-DME is indicated if
        - Zeiss Cirrus CST: ≥290μm in females, and ≥305μm in males
        - Heidelberg Spectralis CST: ≥305μm in females, and ≥320μm in males

- Change in ETDRS BCVA from baseline to 6 months is a continuous variable. The mean difference between treatment groups will be estimated using a linear mixed model with adjustment for baseline ETDRS BCVA, baseline CST, and participant-level random intercepts.
    - The imputation model will be stratified by treatment and include baseline CST, baseline ETDRS BCVA, and changes in ETDRS BCVA from baseline to 1, 2, 3, 4, 5, and 6 months.

- A decrease in ETDRS BCVA of 10 or more letters from baseline to 6 months, or of 5 or more letters from baseline to 6 months and the previous study visits is a binary variable. The odds ratio between treatment groups will be estimated using a generalized linear mixed model for a binary outcome with a logit link function and adjustment for baseline ETDRS BCVA, baseline CST, and participant-level random intercepts.
    - The imputation model will be the same as the outcome of change in ETDRS BCVA from baseline to 6 months. (Missing data for this outcome will not be imputed directly as a binary outcome).
    - If the outcome is missing, the imputed/LOCF values for ETDRS BCVA change at 5 and 6 months will determine the imputed values of the outcome.
    - Descriptive statistics will use data from eyes that completed the 6-month visit and at least one other study visit between baseline and 6 months.

- Receiving any other treatment for DME prior to 6 months is a binary variable. The odds ratio between treatment groups will not be estimated with a model or tested with a hypothesis.
    - The frequencies and percentages will use data from eyes that received other treatment for DME or completed the 6-month visit without receiving other treatment for DME. It is recognized that if participants drop out prior to the 6-month visit without receiving other treatment for DME, this procedure may overestimate the true proportion receiving other treatment for DME.

Exploratory Outcomes

- A 1- step or more worsening of DR and a 1-step or more improvement of DR on fundus photos from baseline to 6 months are binary variables. The odds ratios between treatment groups will not be estimated with a model or tested with a hypothesis, but descriptive statistics will be used to summarize DR worsening and improvement in each treatment group.

- A 2-stepsor more worsening of DR and a 2-steps or more improvement of DR on fundus photos from baseline to 6 months are binary variables. The odds ratios between treatment groups will be estimated using generalized linear mixed models for a binary outcome with a logit link function and adjustment for baseline DR, baseline CST, and participant-level random intercepts.

- Change in OCTA vessel density and change in OCTA nonperfusion area from baseline to 6 months are continuous variables. The mean differences between treatment groups will be estimated using linear mixed models with adjustment for the baseline level of the variable, baseline CST, and participant-level random intercepts.

- Treatment compliance from baseline to 6 months is a participant-level continuous variable. The mean difference between treatment groups will not be estimated with a model or tested with a hypothesis, but descriptive statistics will be used to summarize compliance in each treatment group.
    - Treatment compliance will be calculated as the number of pills taken (= sum across completed visits of number of pills dispensed minus sum across completed visits of number of pills remaining) out of the number expected to have been taken.

- The exploratory outcomes, change in eGFR, CRP, and HbA1c Cystatin C, TSH, Galectin 3, IL-1 β, IL-6, TNFα, urine creatinine, urine albumin, urine MCP1, urine osteopontin, and urine IL-18 from baseline to 6 months are all participant-level continuous variables. The mean differences between treatment groups will be estimated using linear models with adjustment for baseline values of the outcome and baseline CST.

7. Safety Analysis

The analysis of all safety outcomes will use the safety cohort, analyzing all randomized participants who received treatment. Safety outcomes will be summarized with descriptive statistics only and will be reported for the 6-month (primary outcome visit) and 12-month follow-up safety visit (study treatment discontinued after 6 months). No formal hypothesis test comparing safety outcomes between treatment groups will be performed. All reportable adverse events (AEs) will be categorized as ocular 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. For each ocular AE, the number of events and the number of eyes that had the event will be reported separately for study and non-study eyes. For each systemic AE, the number of events and the number of participants that had the event will be reported. In addition to the listing of all AEs, the following safety outcomes will be tabulated.

Ocular Safety Outcomes (at 6 and 12 months)

- At least 1 ocular AE
- At least 1 serious ocular AE
- At least 1 serious ocular AE for each MedDRA System Organ Class reported
- At least 1 ocular AE thought by investigator to be related to study treatment
- Frequencies of ocular AEs thought by investigator to be related to study treatment

Systemic Safety Outcomes (at 6 and 12 months)

- At least 1 systemic AE
- At least 1 serious systemic AE
- At least 1 serious systemic AE for each MedDRA System Organ Class reported
- At least 1 systemic AE thought by investigator to be related to study treatment
- Frequencies of systemic AEs thought by investigator to be related to study treatment (systemic side effects)
- Frequencies of headache, drowsiness, or dizziness
- A change in renal function defined as:
    - eGFR reduced from ≥ 60 to < 60
    - eGFR reduced from ≥ 30 to < 30

    - Dialysis
    - Renal transplant
- An increase in liver function tests defined as greater than 3 times the upper limit of the normal range by sex:
    - ALT: male >123 U/L, female >99 U/L
    - AST: male >120 U/L, female >96 U/L
- A change in any blood count analyte from a normal to abnormal range
- A change in any metabolic panel analyte from a normal to abnormal range

Other Safety Outcomes at 12 months

- Change in CST from 6 to 12 months
- Change in retinal volume from 6 to 12 months
- CST below the OCT machine- and gender-specific threshold for DME at 12 months and a 10% or more decrease in CST from 6 to 12 months
- Change in ETDRS BCVA from 6 to 12 months
- Receiving other treatment for DME between the 6 and 12-month visit
- Changes in blood and urine samples from 6 to 12 months
    - eGFR
    - CRP
    - HbA1c
    - Cystatin C
    - TSH
    - Galectin 3
    - IL-1 β
    - IL-6
    - TNFα
    - Urine creatinine
    - Urine albumin
    - Urine MCP1
    - Urine osteopontin
    - Urine IL-18

8. Intervention Adherence

The clinical site will perform a compliance assessment each time Study Drug is returned (approximately 1-month intervals). Treatment compliance will be calculated as one minus the proportion of pills remaining out of the number expected to have been taken.

9. Protocol Adherence and Retention

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

10. Baseline Descriptive Statistics

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

11. Planned Interim Analyses

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

12. Sub-Group Analyses

Subgroup analyses/assessments of effect modification (interaction) will be conducted for the primary outcome. Since there is no strong prior rationale for potential subgroup effects, these analyses will be considered exploratory and hypothesis generating, rather than definitive. 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. 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. Subgroup analyses will use observed data (no imputation) from participants who complete the primary outcome visit without missing CST data. Exploratory subgroup analysis will evaluate the effects of the following baseline factors:

- Baseline CST: continuous and by the randomization stratification factor of baseline CST relative to the CI-DME CST values above the threshold (<75 μm above threshold, 75 to <175 μm above threshold, ≥175 μm above threshold)
- Hemoglobin A1c: continuous and <7.5% vs. ≥7.5%
- Sex
- Race/ethnicity

Subgroup analyses will only be conducted if there are at least 20 eyes in each subgroup for each treatment group. There are no data to suggest that the treatment effect will vary by sex or race/ethnicity. However, both these factors will be evaluated in exploratory subgroup analyses as mandated by NIH guidelines. Subgroup factors will be analyzed as continuous/quasi-continuous ordinal variables where possible.

13. Multiple Comparison/Multiplicity

The primary analysis consists of one comparison of the primary outcome evaluated at the $\alpha = .05$ significance level. For the secondary outcomes, p-values will be reported but the decision to reject or fail to reject the null hypotheses will control for a false discovery rate of $q = .05$ using the Benjamini-Hochberg adaptive procedure.[3] A rejection of the null hypothesis for a secondary outcome indicates that the outcome is of interest and warrants further investigation. For exploratory outcomes, no p-values or hypothesis tests will be reported.

14. Confounding

Imbalances between groups that are large enough to produce confounding are not expected. If confounding is suspected, a sensitivity analysis that mimics the primary analysis will investigate adjusting for the potential confounder. Suspected confounding will not be judged by statistical tests but by the investigators' judgment whether the imbalance is large enough to have a clinically meaningful effect on the primary outcome.

15. Outliers

Continuous outcomes will be truncated at the overall mean ±3 SD of the observed values to limit the influence of statistical outliers from disproportionately impacting the analysis. Truncation will be

performed after imputation where applicable. Truncated data will not impute missing data, and imputed data will not determine truncation limits. Measurements used to determine safety outcomes will not be truncated.

16. Analysis Windows

The visits used for analysis will be defined as analysis visits. Table 1 defines the windows for analysis visits based on the number of days from randomization. The targeted visit will be used as the analysis visit if it falls within its window. Otherwise, if other visits fall within the window, visit type and timing will determine which is used as the analysis visit. The highest priority visit types are the protocol (monthly) visits, followed by treatment initiation visits and then unscheduled visits. The visit closest to the target day will have the highest priority if visit types are the same. Visits cannot be used as more than one analysis visit. For visits falling in multiple windows, the key visit (primary outcome visit) or the earlier visit will get priority (e.g., a visit on day 42 would be assigned as the 1-month analysis visit; a visit on days 140 to 154 would be assigned as the 6-month analysis visit).

Table 1. Analysis Visit Windows

Visit	Target Day/Week	Analysis Window
1 Month	28 days (4 weeks)	14 – 42 days (2 – 6 weeks)
2 Month	56 days (8 weeks)	42 – 70 days (6 – 10 weeks)
3 Month	84 days (12 weeks)	70 – 98 days (10 – 14 weeks)
4 Month	112 days (16 weeks)	98 – 126 days (14 – 18 weeks)
5 Month	140 days (20 weeks)	126 – 154 days (18 – 22 weeks)
6 month/Primary Outcome Visit*	168 days (24 weeks)	140 – 196 days (20 – 28 weeks)
12 Month/6-Month Post Outcome	364 days (52 weeks)	322 – 406 days (46 – 58 weeks)

*Key visit.

17. Primary Estimand

The target population is adults with type 1 or 2 diabetes and CI-DME with good vision in at least one eye. The Study Protocol defines specific participant and study-eye eligibility criteria. The primary outcome is change in CST from baseline to 6 months. For eyes that receive DME treatment between baseline and 6 months (an intercurrent event), change in CST from baseline to the last observation prior to treatment will be used for analysis. Missing data for eyes that did not receive DME treatment will be imputed as described in the primary analysis section. The population-level summary measure is the difference in mean change in CST from baseline to 6 months between treatment groups. A mixed linear model with adjustment for baseline CST and correlation within participants having two study eyes will be used to estimate the mean difference between treatment groups.

18. Example SAS Code

7.1 Multiple Imputation (MI)

```
proc mi data=studyEyes nimpute=100 seed=12345 out=mi;
/* stratify by treatment group */
by TrtGroup;

var cstConv0 /* baseline CST (converted to a common metric) */
```

```
      cstChg1 /* changes in CST from baseline to months 1-6 */
      cstChg2
      cstChg3
      cstChg4
      cstChg5
      cstChg6;
run;
```

7.2 Mixed Linear Model with MI Data

```
proc mixed data=mi;
/* get estimates within each imputation */
by _Imputation_;

/* set placebo group as reference to estimate the mean difference as
tonabersat - placebo */
class TrtGroup (ref = 'Placebo') PtID ;

/* Outcome model (after MI, LOCF and truncation) comparing treatment groups,
adjusted for baseline CST */
model cstChg6_locftrunc = ANTrtGroup cstConv0;

/* Participant-level random intercept to adjust for correlation in
participants with 2 study eyes */
random int/subject= PtID;

/* Adjusted mean difference between treatment groups */
lsmeans trtGroup / diff;

/* Output for miAnalyze procedure*/
ods output Diffs = trtdiffs;
run;


/* get MI estimate of Tonabersat vs. Placebo mean difference */
proc miAnalyze data = trtdiffs;
modeleffects Estimate;
stderr StdErr ;
run;
```

7.3 Mixed Generalized Linear Model for a Binary Outcome with MI Data

```
proc nlmixed data= mi;
/* get estimates within each imputation */
by _imputation_;

/* Center baseline VA by subtracting baseline VA mean */
/* Eliminates need to specify mean level in estimate statements for risk
difference (centered mean=0) */
va0Cent=va0-va0mean;

/* Starting values */
parms b0_int=0 b1_trt=0 b2_va0Cent=0 /* fixed effects */
      varU=1 /* random effect variance */;

/* Probability of the outcome = 1 */
```

```
p = logistic(b0_int + b1_trt*(TrtGroup='Placebo') + b2_va0Cent*va0Cent + u);

/* Fit model for binary outcome */
model vaChgFlg ~ binary(p);

/* Random intercept for participant */
random u ~ normal(0, varU) subject=PtID;

/* Estimate risk difference at mean baseline VA */
estimate 'Tonabersat vs. Placebo Risk Difference' logistic(b0_int + b1_trt) -
logistic(b0_int);

/* Output for miAnalyze to get OR and RD*/
ods output ParameterEstimates=pe AdditionalEstimates=ae ;
run;


/* get MI estimate of Tonabersat vs. Placebo log odds effect*/
proc mianalyze data=pe(where = (Parameter = 'b1_trt'));
modeleffects estimate;
stderr standardError;

/* Output for data step to get odds ratio */
ods output ParameterEstimates=mipe;
run;

/* get MI estimate of Tonabersat vs. Placebo Odds Ratio */
data miOR;
set miPe;

/* exponentiate the log odds to get odds ratio */
OR = exp(Estimate);
ORlcl= exp(LCLMean);
ORucl = exp(UCLMean);
ORpval = Probt;
run;
proc print data=miOR;
run;


/* get MI estimate of Tonabersat vs. Placebo Risk Difference */
proc mianalyze data=ae;
modeleffects estimate;
stderr standardError;
run;
```

19. References

1. Sun, J.K., Josic, K., Melia, M., Glassman, A.R., Bailey, C., Chalam, K.V., Chew, E.Y., Cukras, C., Grover, S., Jaffe, G.J. and Lee, R. (2021). Conversion of Central Subfield Thickness Measurements of Diabetic Macular Edema Across Cirrus and Spectralis Optical Coherence Tomography Instruments. *Translational vision science & technology*, *10*(14), pp.34-34.
2. Billingsley, P. (1986). *Probability and Measure*. 2nd ed. New York: John Wiley & Sons.

3. Benjamini, Y., & Hochberg, Y. (2000). On the adaptive control of the false discovery rate in multiple testing with independent statistics. *Journal of educational and Behavioral Statistics*, 25(1), 60-83.
4. Bressler, S.B., Edwards, A.R., Chalam, K.V., Bressler, N.M., Glassman, A.R., Jaffe, G.J., Melia, M., Saggau, D.D., Plous, O.Z. and Diabetic Retinopathy Clinical Research Network Writing Committee (2014). Reproducibility of spectral-domain optical coherence tomography retinal thickness measurements and conversion to equivalent time-domain metrics in diabetic macular edema. *JAMA Ophthalmology*, *132*(9), pp.1113-1122.