UNITY
BIOTECHNOLOGY

STATISTICAL ANALYSIS PLAN

PROTOCOL NUMBER: UBX1325-04

A Phase 2b, Prospective, Multicenter, Randomized, Double-Masked, Active-Controlled Study to Assess the Efficacy and Safety of Repeat Intravitreal Injections of Foselutoclax (UBX1325) in Patients with Diabetic Macular Edema (Aspire)

PHASE 2B

Version 1.0
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Revision History

Version	Date	Summary of Revision
1.0	February 4, 2025	Creation of document

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List of Abbreviations

Abbreviation	Definition
AE	Adverse event
ANCOVA	Analysis of covariance
AUC	Area under the curve
ATC4	Anatomical Therapeutic Chemical level 4
BCVA	Best corrected visual acuity
CFBL	Change from baseline
CI	Confidence interval
CST	Central subfield thickness
DME	Diabetic macular edema
DRSS	Diabetic retinopathy severity scale
EDC	Electronic data capture
ET	Early termination
ETDRS	Early Treatment Diabetic Retinopathy Study
FE	Fellow eye
GLP-1	Glucagon-like peptide-1
HbA1c	Hemoglobin A1C
IOP	Intraocular pressure
IQR	Inter quartile range
IVT	Intravitreal
LoA	List of Analyses
LS	Least squares
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed model for repeated measures
NCI CTCAE v5.0	National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0
OCT	Optical coherence tomography
PT	Preferred term
SAE	Serious adverse event
SAP	Statistical analysis plan
SD-OCT	Spectral domain optical coherence tomography
SOC	System organ class
SE	Study eye
TEAE	Treatment-emergent adverse event
US	United States
VA	Visual acuity
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

2. Introduction

The statistical analysis plan (SAP) provides the detailed methodology for summary and statistical analyses of data collected in study UBX1325-04. This SAP is based on the Protocol Version 3.0 Amendment 2 dated 26 March 2024. Specifications for tables, figures, and listings will be provided in a separate document known as the List of Analyses (LoA).

1.1. Design overview

This study is a phase 2b, prospective, multicenter, randomized, double-masked, active-controlled study to assess the efficacy and safety of repeat intravitreal injections of foselutoclax (UBX1325) in patients with diabetic macular edema (Aspire). Approximately 50 patients will be enrolled and randomized 1:1 into either the foselutoclax (UBX1325) arm, 10 µg given 8 weeks apart or an anti-vascular endothelial growth factor (VEGF) control arm of aflibercept, 2 mg every 8 weeks to assess the primary objective. This is a multi-site trial with 21 sites in the US. All patients will be followed for approximately 36 weeks following a 3-month run-in period. The total duration of the trial from screening to last patient visit will be approximately 19 months. The injector was unmasked but the evaluator was masked throughout the study.

1.2. Study objectives

1.2.1 Primary objective

- Assess the efficacy of foselutoclax (UBX1325) compared to aflibercept.

1.2.2 Secondary objectives

- Assess other measures of efficacy of foselutoclax (UBX1325) compared to aflibercept.
- Assess the safety and tolerability of foselutoclax (UBX1325).

1.2.3 Exploratory objective

- Assess efficacy parameters and retinal structure improvement after treatment of foselutoclax (UBX1325) compared to anti-VEGF alone.

1.3 Study endpoints

1.3.1 Primary endpoint

- The primary objective of non-inferiority will be evaluated by comparing the difference between treatments in Best corrected visual acuity (BCVA) mean change from baseline to average of Week 20 and Week 24

1.3.2 Secondary endpoints

1. Changes in BCVA from baseline to each visit through Week 36
2. Change in BCVA from Week 24 to Week 36

3. Change in central subfield thickness (CST) from baseline to each visit through Week 36 as assessed by spectral domain optical coherence tomography (SD-OCT) and read by a Central Reading Center
4. Changes in BCVA area under the curve (AUC) from baseline to Week 36 and from Week 8 to Week 36, as well as the average of the last 2 visits
5. Changes in BCVA from baseline to last observation at or prior to Week 36
6. Proportion of participants gaining ≥15, ≥10, ≥5, or ≥0 Early Treatment Diabetic Retinopathy Study (ETDRS) letters in BCVA from baseline in the study eye (SE) to Week 24 and week 36
7. Proportion of participants who do not require rescue to week 24 and week 36
8. Measures from SD-OCT, anterior segment evaluation, posterior segment evaluation, and color fundus photography
9. Percentage of participants with at least 1 treatment-emergent adverse event (TEAE)
10. Percentage of participants with at least 1 treatment-emergent ocular adverse event in the SE or the fellow eye (FE)
11. Percentage of participants with at least 1 treatment-emergent non-ocular adverse event (AE)
12. Percentage of participants with at least 1 treatment-emergent serious adverse event

1.3.3 Exploratory endpoints

1. Change in Diabetic Retinopathy Severity Scale (DRSS) score from Baseline to Week 36
2. Change from baseline (CFBL) in BCVA and change from baseline in CST by subgroups:
    a. Baseline BCVA (≤60 letters and >60 letters)
    b. BCVA at first run-in injection pre-dose (≤60 letters and >60 letters)
    c. Baseline CST (≤400 µm and >400 µm)
    d. CST at first run-in injection (≤400 µm and >400 µm)
    e. Baseline DR severity (<47, ≥47 DRSS score)
    f. Baseline HbA1c (≤7% and >7%)
    g. Baseline duration of diabetes (by aggregate median)
3. Proportion of subjects with no macular fluid at Week 24 and 36
4. Responder analysis based on change in BCVA and CST
    a. Response is defined as change from baseline in BCVA at Week 24 ≥ 5 ETDRS letters
    b. Response is defined as change from baseline in CST at Week 24 ≤ -50 µm
5. Duration of rescue-free interval
6. Time to first rescue
7. Reason for rescue
8. Change in BCVA and CST from run-in period to randomization

NOTE: The endpoints related to Fluorescein Angiography (FA) may be analyzed if needed.

3. Study Methods

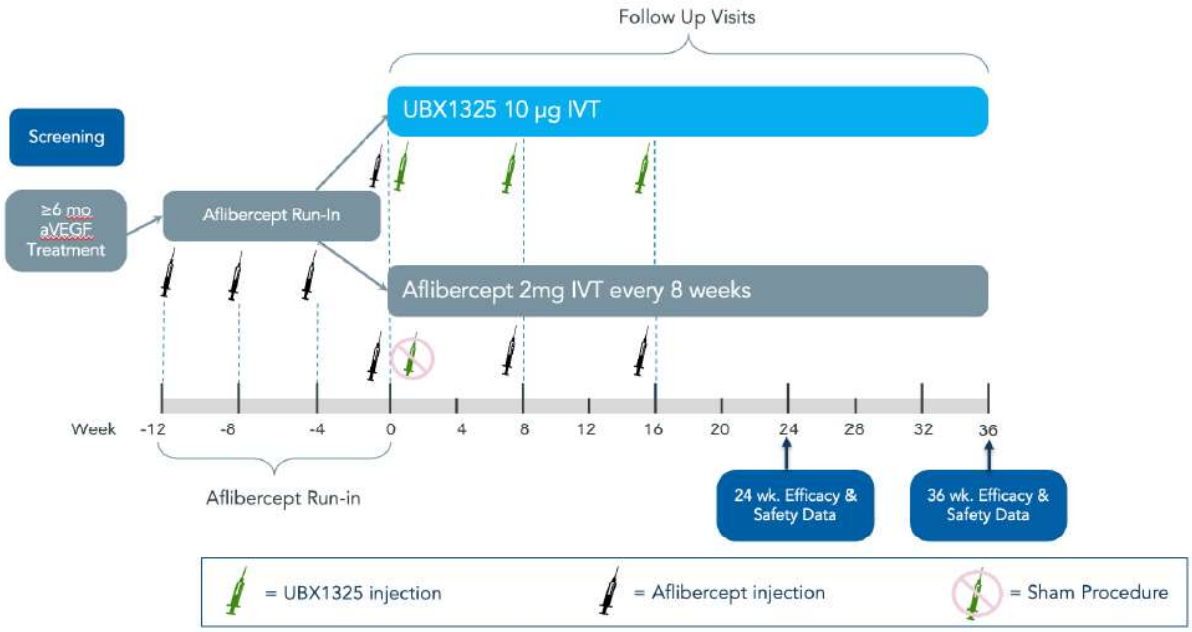
3.1 Trial design

The study is a Phase 2b, double masked, randomized, parallel group, multicenter study of foselutoclax (UBX1325) in patients with Diabetic macular edema (DME). The study will enroll approximately 50 patients, who will be randomized in a 1:1 ratio to receive foselutoclax (UBX1325) 10 µg given 8 weeks apart or an anti-VEGF control arm of aflibercept 2 mg every 8 weeks to assess the primary objective. All patients will be followed for approximately 36 weeks. Prior to randomization, participants will receive 3 IVT injections of aflibercept approximately 4 weeks apart. After this run-in period, participants will be randomly assigned to 1 of 2 treatment arms:

- Foselutoclax (UBX1325) arm: Participants will receive 10 µg foselutoclax (UBX1325) (50 µl of 0.2 µg/µl solution) IVT on Day 1 and Weeks 8 and 16. 2 mg aflibercept (50 µl of 40 µg/µl solution) will also be administered on Day 1.
- Anti-VEGF control arm: Participants will receive 2 mg aflibercept (50 µl of 40 µg/µl solution) IVT on Day 1 and Weeks 8 and 16. A sham procedure will also be administered on Day 1.

The injector will be unmasked but the evaluator will remain masked throughout the study. A schematic representation of the study design is presented below (Figure 1 from the study protocol). Schedule of visits and assessments is presented in Appendix A.

Figure 1 Schematic of Phase 2b Study



3.2 Study population

This study will enroll patients who are at least 18 years old and have been diagnosed as having DME (as defined by the AAO PPP DME Guidelines 2019) with BCVA in the study eye of 70 to 30 (inclusive) ETDRS letters (equivalent to 20/40 to 20/250 on the Snellen chart, respectively) at all Screening run-in visits and Day 1.

3.3 Randomization and masking

Randomization personnel of the Sponsor, or designee generated the randomization schedule prior to the start of the study. An interactive web response system will be used for patient randomization. All randomization information will be stored in a secured area, accessible only by authorized personnel.

This study is double-masked. Patients, Investigators, VA technicians, photographers, some Clinical Research Coordinators, Central Reading Center personnel, and the Sponsor are masked. Sites have a qualified injector who is unmasked in order to perform the IVT injection(s) [foselutoclax (UBX1325) and/or aflibercept], and/or sham procedure, as well as certain post-injection assessments on Visits 1 (Day 1), 3 (Week 8), and 5 (Week 16).
Specifically, the unmasked injector will do the anterior segment exam, central retinal artery perfusion exam, Intraocular pressure (IOP) (IOP could be done by a qualified unmasked designee), and AE assessment. Other supporting activities, such as Study Drug preparation and Study Drug accountability, will be performed by designated unmasked personnel. The Screening visits through Visit 1 (Day 1) randomization will be conducted by unmasked or masked personnel as well. However, after randomization is completed, unmasked personnel will not be involved in any other study procedures outside of visits when an IVT injection is required, including Visits where a rescue injection was necessitated. In addition to conducting the Screening visits through Visit 1 (Day 1) randomization, the unmasked personnel may likewise complete the corresponding data entry but will be restricted thereafter to view-only access in electronic data capture (EDC). The randomization code will not be revealed to anyone to maintain the integrity of the double-masking.

**This section is taken from study protocol version 3. Detailed information about randomization and masking can be found in protocol version 3 document.*

3.4 Determination of sample size

With 20 patients per arm, 10% early study discontinuation, a non-inferiority margin of -4.5 letters, a standard deviation in BCVA of 7, a correlation between repeated measures of 0.5, and $\alpha = 0.10$ the primary analysis has ≥ 93% power if the true difference between foselutoclax (UBX1325) and aflibercept is ≥2. If the standard deviation is 9, then the corresponding power is 78%.

A masked sample size re-estimation was conducted. The residual standard deviation from the primary mixed model for repeated measures (MMRM) analysis applied to the masked BCVA data was reviewed to assess the variability, and the sample size was increased to 25 per arm.

For the masked sample size re-estimation, the following details applied. Baseline was defined as the day 1 value of BCVA (i.e., the value on day of randomization). The primary analysis was MMRM based on the treatment policy estimand in which post-rescue data is included in the analysis, with a model in which BCVA change from baseline (in the study eye) is the dependent variable and the independent variables are baseline BCVA (in study eye), study week (categorical), and baseline BCVA-by-week interaction. Within-patient errors were modeled using a heterogeneous compound symmetric (type = CSH) structure. All BCVA measurements (in study eye) available on day 1 and later were included in the analysis.

4. General Methods

4.1 General considerations

All study parameters will be listed, and a selected list of parameters will be summarized descriptively by treatment arm and overall. The descriptive statistics will include number of patients, mean, standard deviation, median, minimum, and maximum for continuous parameters and frequency and percent for categorical parameters.

The following reporting conventions will be implemented:

- All statistical tests will be conducted as 2-sided tests unless otherwise specified.
- Confidence intervals (CIs) will be presented as 2-sided 95% and 90% CIs unless otherwise specified. For safety outcomes, a 95% CI will be used.
- All mean and median values will be formatted to one more decimal place than the measured value. Standard deviation values will be formatted to two more decimal places than the measured value.
- All percentages will be rounded to one decimal place. The number and percentage of responses will be presented in the form xx (xx.x), where the percentage is in the parentheses. The percentage will be omitted when the numerator is zero. In the case the numerator is equal to the denominator, the percentage should be presented as (100) instead of (100.0).
- All analysis and summary tables will have the analysis population sample size (*i.e.*, number of participants) wherever applicable.

4.2 Study Day and Baseline

Study Day 1 is defined as the day of injection after randomization. Subsequent days are numbered consecutively (*i.e.,* Day 2, Day 3) and are calculated as: visit date - dosing date + 1.

Before Day 1, study days are numbered sequentially with negative values (*i.e.,* Day -1, Day -2) and are calculated as: visit date - dosing date. There is no Day 0.

Baseline value will be the last measurement taken on or before Day 1. It will be taken from the pre-dose assessment on Day 1 unless otherwise noted.

4.3 Definition of visit window

Unless otherwise specified, data from the actual planned post-baseline visit will be used in the analysis (Appendix A). Post-baseline unscheduled visits and early termination visits will be mapped to a planned visit and will only be used in the analysis if the actual planned visit result is missing. The rules are described in appendix B. The assignment of the baseline visit is handled by a separate algorithm for this study, not by the visit windowing rules. See Section 4.2 above.

4.4 Timing of analyses

Analyses to evaluate safety, tolerability, efficacy, and exploratory outcomes will utilize data from screening through Week 36 of follow-up. Separate data locks and analyses will occur for data collected through Week 24 and Week 36.

4.5 Early termination

Patients who drop out of the study prematurely will have study data collected at the Early Termination Visit (see Appendix A). The numbers and proportions of participants who terminate study participation prematurely, by arm and overall, will be tabulated (see section 6).

4.6 Interim analysis

A masked sample size re-estimation was performed as described above in the section on sample size determination. This will have no impact on the type I error for later analyses.

Separate data locks and analyses will be conducted for data collected through Week 24 and 36. The analysis of the primary endpoint at Week 24 data lock will be considered the primary analysis for assessing the effect of treatment, with all type I error spent at this analysis.
At the Week 24 data lock, analyses from baseline through Week 24 will be conducted.
At the Week 36 data lock, analyses will be conducted from baseline through Week 24, baseline through Week 36, and Week 24 through Week 36 (to assess maintenance of treatment effect).

4.7 Multiple testing and error control

No formal adjustment for multiple comparisons is planned.

5. Analysis Sets

The following analysis sets are defined: Randomized Set, Full Analysis Set, Safety Analysis Set for the Run-in Period, Safety Analysis Set after Randomization, and Per Protocol Analysis Set.

Randomized Set

Randomized set includes all subjects who were randomized to study treatment arms for the study.

Full Analysis Set

The Full Analysis Set will include all randomized subjects who received study treatment, have a baseline BCVA assessment, and at least one post-baseline BCVA assessment. The Full Analysis Set will be the primary population for evaluating all efficacy variables. For analysis, subjects will be grouped by randomized treatment arm.

Safety Analysis Set for the Run-in Period

The Safety Analysis Set for the Run-in Period is an analysis set that will be used only for evaluating safety variables during the run-in period. This analysis set will include patients who were screened and received at least one dose of aflibercept during the run-in period. For analysis, subjects will be grouped by actual treatment received or screen failure. The screen failures will be defined as the subjects who failed the screening after run-in 1 aflibercept.

Safety Analysis Set after Randomization

The Safety Analysis Set after Randomization will include all patients who received study treatment. The Safety Analysis Set will be the primary population for evaluating all safety variables and patient characteristics. For analysis, subjects will be grouped by actual treatment received.

Per Protocol Analysis Set

The Per Protocol Analysis Set will include all subjects from the Full Analysis Set that have no protocol deviations that compromise the validity of subjects' study data. A list of such deviation categories will be defined prior to the unmasking of the treatment assignment data and will be used to identify the Per Protocol Analysis Set; any subject with a deviation category on the list will be excluded from the Per Protocol Analysis Set. The Per Protocol Analysis Set will be the primary population for conducting the sensitivity analysis of efficacy due to protocol deviations. For analysis, subjects will be grouped by randomized treatment arm.

6. Subject Disposition

The number of patients screened, screen failures (overall, at run-in 1, run-in 2, run-in 3 and Day 1), randomized, completed the study, analysis population will be summarized for the study. The following data will be summarized for each treatment arm and overall, for all screened subjects:

- Randomized
- Completed study
- Did not complete study
- Patients in each analysis population: (Full, randomized, per protocol, and Safety sets)

A protocol deviation listing will be provided that includes the start/end date of the deviation and the deviation description. A listing of subject disposition data will also be provided. A listing for the reasons for the patients that did not qualify for this study will also be provided.

7. Demographics and Baseline Characteristics

Demographics and baseline characteristics, including medical history, prior and concomitant medications, will be summarized using the Safety Analysis Set after Randomization. These data will be listed and summarized for the study overall, as well as for each treatment arm.

7.1 Demographics and baseline characteristics

Demographic and baseline characteristics include age, sex, race, ethnicity, weight, height, and other parameters as appropriate, e.g., anti-VEGF medication used immediately prior to Screening Run-In #1. These variables will be listed and summarized using descriptive statistics, based on the Safety Analysis Set after Randomization. Any variables that are found to differ by treatment arm may be considered for inclusion in statistical models as covariates.

7.2 Medical history

Medical history will be coded using the latest version of Medical Dictionary for Regulatory Activities (MedDRA) and summarized by system organ class (SOC) and preferred term (PT) by treatment arm and total. A patient will be counted only once for multiple events within each SOC/PT. Medical History, including a detailed ophthalmic history reported prior to dosing, will be listed by patient.

7.3 Prior and concomitant medications and procedures

Prior medications and procedures are the medications/procedures that the patient stopped prior to the first screening visit. Concomitant medications/procedures are medications/procedures that the patient continued after the first screening visit or started after the first screening visit through end of study. Prior and concomitant medications will be coded using the latest version of World Health Organization (WHO) Drug Dictionary and summarized by Anatomical Therapeutic Chemical level 4 (ATC4) and PT by study arm and total. Prior and concomitant procedures will be

coded using MedDRA terms (as for medical history). A patient will be counted only once for multiple events within each higher-level category (ATC4, SOC) and PT.

Prior and concomitant medications and procedures will be summarized in table form. Listings of prior and concomitant medications and procedures will also be provided.

8. Safety Analysis

8.1 Adverse events

The adverse events are summarized separately for run-in period only and for period after randomization.

Adverse events after randomization

Ocular and systemic safety and tolerability of a repeat Intravitreal (IVT) injection of UBX1325 will be evaluated by treatment emergent adverse events (TEAEs). A TEAE is an adverse event that first occurs or worsens after study drug initiation or is a serious adverse event (SAE). Summary tables will include only TEAEs, and listings will include all adverse events reported in the clinical database. The Safety Analysis Set after Randomization will be used as the analysis population for the below summaries.

The following summaries of TEAE data will be tabulated by study arm and overall:

- Percentage of participants with at least one TEAE
- Percentage of participants with at least one treatment-emergent ocular AE in the SE or FE
- Percentage of participants with at least one treatment-emergent non-ocular AE
- Percentage of participants with at least one treatment-emergent serious adverse event
- Percentage of participants with at least one TEAE related to the investigational product (study drug, active control, or sham)
- Percentage of participants with at least one TEAE related to a post-randomization injection procedure

An overall summary table of TEAEs will include the above-mentioned categories as well as the number and percentage of patients experiencing at least one grade >=3 TEAE, serious TEAE, or TEAE leading to death. Adverse events are graded according to NCI CTCAE v.4.03 in which a severe adverse event is classified as grade 3. Relatedness to study drug will be graded as “related”, “probably related”, “possibly related”, “unlikely related”, or “not related” based on an investigation of the temporal sequence, the patient’s clinical state, and other modes of therapy administered to the patient. TEAEs will be summarized for Baseline through Week 24 and Baseline through Week 36.

For summaries by SOC and PT, a patient will only be counted once for each PT and once for the overall count for a SOC. However, patients may be included in more than one PT category within a SOC. For summaries which also include severity, a patient who experiences the same event more than once will have the event with the worst severity counted in the summary. Summaries by SOC and PT will be presented by SOC alphabetically then by descending frequency of PT within SOC based on the overall column.

A table summarizing the count and the percentage of the non-serious TEAE that occurred in ≥5% of subjects, grouped by SOC, PT, and severity will be presented. The results will be presented for both treatment arms and overall.

Adverse events during run-in period

Ocular and systemic safety of run-in aflibercept injections will be evaluated by AE during run-in period. An AE during the run-in period is an adverse event that first occurs or worsens after the first run-in dose of aflibercept or is a serious adverse event (SAE). The Safety Analysis Set for the Run-in Period will be used as the analysis population.

Summary tables will include only AE during run-in period and prior to randomization.

- Overall summary of AE during run-in period
- Ocular AE by SOC, PT, and severity, by eye (SE & FE) and by study arm, screen failure, and overall
- Non-ocular AE by SOC, PT, and severity, by study arm, screen failure, and overall

8.2 Intraocular Pressure

Intraocular Pressure (IOP) will be measured using Goldmann applanation tonometry or equivalent tonometer in both eyes at all scheduled visits. Tonometry should be performed prior to pupillary dilation when possible. IOP will be summarized for each eye by visit and by dose using descriptive statistics. Change from baseline in IOP will be summarized by visit and treatment group. The Safety Analysis Set after randomization will be used as the analysis population. Intraocular pressure data will be listed for both eyes at each visit as study eye and non-study eye.

8.3 Laboratory data

Laboratory safety parameters are measures of hematology and serum chemistry. These measures will be obtained at Screening, Week 36, and Unscheduled Visits/ET (if applicable). Actual values and CFBL will be summarized for each treatment arm, and 95% confidence intervals will be calculated for the mean CFBL for each treatment arm. A shift table of the changes from baseline to Week 36 and ET will also be presented. A shift table is a table that shows the progression of values over time. The Safety Analysis Set after randomization will be used as the analysis population.

8.4 Vital signs

Vital signs will be taken at all study visits. Actual values and CFBL for each vital sign parameter will be summarized for each treatment arm at each visit, and 95% confidence intervals will be calculated for the mean CFBL for each treatment arm and visit. The Safety Analysis Set after randomization will be used as the analysis population.

8.5 Physical examinations

Complete physical examinations will be performed at Screening, Visit 10 (Week 36), or Unscheduled Visits/ET. The physical examination data will be listed. The Safety Analysis Set after randomization will be used as the analysis population.

9. Efficacy Analysis

9.1 Primary endpoint

The primary assessment of efficacy will be based on a non-inferiority test comparing UBX1325 vs Aflibercept mean change from baseline to Week 24.

The primary estimand consists of the following:

- Treatment regimen: foselutoclax (UBX1325) 10 µg fixed dose given at Baseline, Week 8, and Week 16 vs. aflibercept 2 mg given at Baseline, Week 8, and Week 16
- Population: Full Analysis Set
- Endpoint: BCVA ETDRS letters
- Summary measure: the difference between treatments in mean change from baseline to the average of Weeks 20 and 24. If the lower bound of the two-sided 90% confidence interval for the mean difference between UBX1325 and aflibercept is ≥ -4.5, UBX1325 will be declared not inferior to aflibercept. If the lower bound of the 90% confidence interval is > 0, UBX1325 will be declared superior to aflibercept.
- Intercurrent events: a treatment policy strategy will be used to account for the intercurrent event of use of rescue medication, and a hypothetical strategy will be used to account for the intercurrent event of early study discontinuation.

Change in BCVA from Baseline will be analyzed using restricted-maximum likelihood based mixed models for repeated measures (MMRM). BCVA will be assessed using the ETDRS chart starting at 4 meters at Screening, on Visits 1-10 and Unscheduled Visits/ET. The model specifications will depend on the objective.

For the primary analysis, the model will include the change from baseline to each study visit as the dependent variable, and the independent variables will include continuous baseline BCVA,

categorical study arm (treatment or active), categorical study visit, and arm-by-visit interaction, along with the BCVA baseline-by-visit interaction. Within-patient errors will be modeled using an unstructured covariance structure. If this analysis fails to converge, more parsimonious error correlation structures will be tested in the following order: heterogeneous Toeplitz, heterogeneous compound symmetric, and compound symmetric. Analysis for this objective will include data from baseline through Week 24. Least squares (LS) mean estimates of the difference between treatment arms and the within-arm values of change from baseline BCVA will be presented for each visit and for the mean of Weeks 20 and 24 with 90% confidence intervals for each estimated difference. All type I error ($\alpha = 0.10$) will be spent at the analysis of the primary endpoint at the Week 24 database lock.

A supportive analysis will be done in the same way as the primary analysis except that it will be limited to data collected prior to or on the date of first receipt of off-schedule aflibercept (hypothetical strategy).

When BCVA outliers occur, a sensitivity analysis excluding the outliers will be performed. If deemed appropriate, other sensitivity analyses may also be considered to assess the impact of outliers. An outlier is defined using the 3(IQR) Criterion: an observation is classified as an outlier if it is below Q1 – 1.5(IQR) or above Q3 + 1.5(IQR). A far outlier is an observation that is below Q1 – 3(IQR) or above Q3 + 3(IQR).

A per protocol sensitivity analysis will also be performed repeating the same primary analysis methods on the Per Protocol Analysis Set to determine the impact of protocol deviations on the results.

9.2 Secondary endpoints

For secondary endpoints (1)-(4) a similar MMRM modeling approach as described in the previous section will also be used for the analyses. These analyses will include (1) & (2) analyses of change in BCVA from Baseline over time, (3) change in CST from Baseline over time as assessed by SD-OCT and read by a Central Reading Center, and (4) BCVA AUC over time. The model specifications will be updated as per the secondary endpoints (1)-(4).

For (1), (3), and (4), separate analyses will be run from Baseline through Week 24 and Baseline through Week 36. Also, for (4), an additional analysis will be run on change from Week 8 to Week 36. For (2), an analysis will be run on change from Week 24 to Week 36. Analyses through Week 24 will also present the results for the mean of Weeks 20 and 24. Analyses through Week 36 will also present the results for the mean of Weeks 20 and 24 and the results for the mean of Weeks 32 and 36.

Separate analyses of secondary endpoints (1) - (4) will be performed using two different strategies to address intercurrent events. For each of these endpoints, a hypothetical strategy analysis will be performed, limited to data collected prior to or on the date of first receipt of off-schedule aflibercept. Also, a treatment policy analysis of each endpoint will be performed for all data collected, regardless of receipt of off-schedule aflibercept. Within-patient errors will be modeled using an unstructured covariance structure. If an analysis fails to converge, more parsimonious error correlation structures will be tested in the following order: heterogeneous Toeplitz, heterogeneous compound symmetric, and compound symmetric.
For secondary endpoint (2), a linear contrast will be defined to test the difference between treatment arms in mean change from baseline between Weeks 24 and 36.

For secondary endpoint (5), the change in BCVA from baseline to last observation at or prior to Week 36, mean change from baseline to last observation (one value per patient) will be analyzed using an ANCOVA model in which change from baseline is the dependent variable, and the independent variables will include continuous baseline BCVA and categorical study arm (treatment or active). Analyses will be done using both the treatment policy strategy & hypothetical strategy (as described above). Analyses will also be conducted using Week 24 as the last observation. LS means with standard error bars will be presented as bar plots for both treatment groups.

For secondary endpoint (6), the proportion of participants gaining ≥15, ≥10, ≥5, or ≥0 ETDRS letters in BCVA from baseline in the study eye (SE) to Weeks 24 and 36 will be summarized with tables and bar plots.

For secondary endpoint (7), a frequency table of the proportion of participants who received 0, 1, or 2 or more rescues at each visit will be presented for both Weeks 24 and 36. Pie charts will also be generated to visualize the summary results for week 36, by treatment arm.

For secondary endpoint (8), listings of measures from SD-OCT, anterior segment evaluation, posterior segment evaluation, and color fundus photograph will be generated.

***Secondary endpoints (9)-(12)** analyses are described in section 8.1.*

9.3 Exploratory endpoints and analyses

This section describes the statistical analyses for the exploratory endpoints listed in section 1.3.3 and some additional exploratory analysis considerations.

For exploratory endpoint (1),
DRSS Score

- Change in Diabetic Retinopathy Severity Scale (DRSS) score from Baseline to Week 36

- A frequency table of the change from baseline to Week 36 (measured in number of steps on the DRSS scale) will display the count and percentage by treatment arm. Bar charts will present the same results graphically. The analysis will be done using both the treatment policy strategy & hypothetical strategy (as described above).

For exploratory endpoint (2), The following subgroups will be analyzed for change from baseline BCVA and change from baseline in CST.

- Baseline BCVA (≤60 letters and >60 letters)
- BCVA at first run-in injection pre-dose (≤60 letters and >60 letters)
- Baseline CST (≤400 µm and >400 µm)
- CST at first run-in injection (≤400 µm and >400 µm)
- Baseline DR severity (<47, ≥47 DRSS score)
- Baseline HbA1c (≤7% and >7%)
- Baseline duration of diabetes (by aggregate median)

The subgroup category definitions may be adjusted if there is not enough representation of a specific subpopulation or treatment group. The analysis method will be MMRM modeling where the dependent variable is change from baseline to each study visit. The independent variables will consist of the independent variables from the primary analysis and the separate models will be run for each subgroup. Within-patient errors will be modeled as for the primary endpoint analysis, with an ordered sequence of covariance structures to apply if the first structure applied does not result in model convergence. Models will be run using both treatment policy and hypothetical strategies to address intercurrent events. Models will be run for Baseline through Week 24 and Baseline through Week 36. LS mean results will be presented for all study visits and for the mean of Weeks 20 and 24 and the mean of Weeks 32 and 36. Additionally, a forest plot will display the LS mean difference for all subgroups in one graph.

Additional analysis of BCVA and CST with the subgroup as covariate may be performed if a clinically significant subgroup difference is observed in the subgroup analysis.

For exploratory endpoint (3), Absence of macular fluid is defined as CST ≤ 290µm at one or more post-baseline visits. The summary table of the proportion of the subjects with no macular fluid will be generated for both treatment arms, through week 24 and 36. The difference between the proportion of subjects with confidence interval will also be presented. The results will be visualized as bar plots.

For exploratory endpoint (4),

The responder analysis will be conducted separately based on the endpoints BCVA and CST. Response based on BCVA will be defined as BCVA change from baseline at Week 24 ≥ 5 and CST change from baseline at Week 24 ≤ -50. The summary tables will display descriptive statistics, by

response/no response and by treatment group, for the baseline variables BCVA, CST, DRSS, number of prior anti-VEGF injections (prior to randomization), HbA1c, and GLP-1 drug status. The analyses will be repeated for Week 36.

For exploratory endpoint (5)-(7),

Rescue Summaries

The following summaries will be presented for anti-VEGF rescue. For the definition of criteria and procedure to trigger anti-VEGF rescue, please refer to study protocol version 3. Rescue is defined as post-Day 1, off-cycle anti-VEGF treatments injected in the study eye.

- Duration of rescue-free interval: Frequency tables of the rescue-free interval (in weeks) will be presented as counts and percentages, by treatment arm and through week 36. The duration presented will be from prior rescue treatment (or initial study treatment) of no rescues (through earlier of the last follow-up date or the specified visit). Pie charts will also be generated to visualize the summary results, by treatment arm, and at Week 36.
- Time to first rescue: Kaplan-Meier plots by treatment arm will be presented to summarize the time to first rescue after randomization and the time to first rescue after week 16, at week 36 data lock.
- Patient-specific anti-VEGF summaries: Swimmer plots will be generated to summarize the subject-specific anti-VEGF treatments received before and after randomization for the two arms. Bar plots will also be generated to summarize the anti-VEGF burden before and after randomization.
- Reason for rescue: Summary table with the reason of anti-VEGF rescue will be presented as counts and percentage, by treatment arm, at week 24 and 36.

For exploratory endpoint (8), The below analyses for the BCVA and CST by timepoints from run-in period through randomization.

- Descriptive summaries of BCVA and CST actual values and change from screening run-in injection 1 (pre-dose) will be summarized as a table from run-in period to randomization, grouped by study arm and overall. The Full Analysis Set will be used.
- Supporting line plots to summarize the mean of actual values and mean change from screening run-in injection 1 (pre-dose) from run-in period to randomization will also be generated.

10. Quality Assurance

10.1 Software and programming specifications

All statistical programs written to generate TFLs and perform statistical analyses will use either SAS version 9.4 or higher, or R version 4.0 or higher. Every statistical program will be developed and reviewed for correctness according to the four-stage process. Stage I of the process requires

the creation of specification document describing the intent and project guidelines. Stage II comprises of program development based on approved specifications and Stage III involves the review process. Independent replication and peer review procedures are the two types of review processes. The type of review procedure will be determined based on category of program. Stage IV describes generation of reviewed outputs and documentation of adherence to project requirements.

10.2 File management and data security

Data and program files will be managed according to Cencora folder and file structure management. Electronic file security and integrity will be protected by Cencora network system and structure.

11. Changes in the Statistical Analysis Plan from the Protocol Analysis Plan

The study protocol noted that details of statistical analyses will be provided in the SAP. The only changes from the protocol to document in the statistical analysis plan are as follows.

- The protocol was inconsistent with regards to the time points included in the primary estimand (mentioning in some places the mean change from baseline to Week 24 and in other places the mean change to the average of Weeks 20 and 24). This SAP has the definitive description of the primary estimand (i.e., using the average of Weeks 20 and 24).
- Added secondary endpoints
    - Change in BCVA from Week 24 to Week 36
    - Measures from SD-OCT, anterior segment evaluation, posterior segment evaluation, and color fundus photograph
- Added exploratory analyses
    - Subgroup analyses of BCVA & CST
    - Proportion of subjects with no macular fluid
    - Responder analysis
    - Duration of rescue-free interval
    - Time to first rescue
    - Reason for rescue
    - Change in BCVA and CST from run-in period to randomization
    - Line plots to summarize the mean BCVA and mean CST from run-in period through randomization.
- Added the safety analyses for the run-in period in section 8.1

If there are any other discrepancies, the SAP takes precedence over the protocol.

12. Appendices

12.1 Appendix A – Schedule of Events (Protocol Table 1)

Test/Procedure	3 Screening Visits			Visit 1 Week 0 Day 1[b]	Visit 2 Week 4 Day 29 ± 7	Visit 3 Week 8 Day 57 ± 7	Visit 4 Week 12 Day 85 ± 7	Visit 5 Week 16 Day 113 ± 7	Visit 6 Week 20 Day 141 ± 7	Visit 7 Week 24 Day 169 ± 7	Visit 8 Week 28 Day 197 ± 7	Visit 9 Week 32 Day 225 ± 7	Visit 10 Week 36 Day 253 ± 7	Unscheduled Visit/ET[d]
	Run-in #1 (4-6 weeks post last SOC anti-VEGF)	Run-in #2 (28 days ± 5 from Run-in # 1)	Run-in #3 (28 days ± 5 from Run-in # 2) Day -42 to -28											
Informed Consent	X													
Demographics	X													
Medical/Ophthalmic & Medication History	X													
Concomitant Medications & Procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination (including ht/wt)[f]	X												X	X
Vital Signs	X			X	X	X	X	X	X	X	X	X	X	X
Labs: Hematology and Chemistry	X												X	X
Pregnancy Test	X (serum)[a]			X (urine)									X (urine)	X (urine)
BCVA OU (pre IVT)[c]	X	X (SE only)	X (SE only)	X	X	X	X	X	X	X	X	X	X	X
Hand Motion VA (post IVT)[c]	X	X	X	XX		X		X						
Anterior Segment Evaluation[c]	XX (pre/post IVT)			XXX (pre/2 post IVT)	X	XX (pre/post IVT)	X	XX (pre/post IVT)	X	X	X	X	X	X
Posterior Segment Evaluation[c]	X (pre IVT)			X (pre IVT)	X	X (pre IVT)	X	X (pre IVT)	X	X	X	X	X	X
Central Retinal Artery Perfusion Exam (post IVT)[c]	X	X	X	XX		X		X						
IOP[c]	XX (pre/post IVT)	X (post IVT)	X (post IVT)	XXX (pre/2 post IVT)	X	XX (pre/post IVT)	X	XX (pre/post IVT)	X	X	X	X	X	X

Test/Procedure	3 Screening Visits			Visit 1 Week 0 Day 1[b]	Visit 2 Week 4 Day 29 ± 7	Visit 3 Week 8 Day 57 ± 7	Visit 4 Week 12 Day 85 ± 7	Visit 5 Week 16 Day 113 ± 7	Visit 6 Week 20 Day 141 ± 7	Visit 7 Week 24 Day 169 ± 7	Visit 8 Week 28 Day 197 ± 7	Visit 9 Week 32 Day 225 ± 7	Visit 10 Week 36 Day 253 ± 7	Unscheduled Visit/ET[d]
	Run-in #1 (4-6 weeks post last SOC anti-VEGF)	Run-in #2 (28 days ± 5 from Run-in # 1)	Run-in #3 (28 days ± 5 from Run-in # 2) Day -42 to -28											
SD-OCT	X			X	X	X	X	X	X	X	X	X	X	X
FA	X			X									X	X
CFP	X			X									X	X
DRSS Score[g]				X									X	X
mfERG[h]				X		X		X		X			X	X
Eligibility Criteria[a]	X	X	X	X										
Aflibercept Run-In Administration[e]	X	X	X											
Study Drug Arm[e]				XX		X		X						
Aflibercept Control Arm[e]				XX		X		X						
AE assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X

AE = adverse event; BCVA = best corrected visual acuity; CFP = color fundus photography; DRSS = diabetic retinopathy severity scale; ET = Early Termination; FA = fluorescein angiography; IOP = intraocular pressure; IVT = intravitreal; SD-OCT = spectral domain optical coherence tomography; mfERG = multifocal electroretinography.

[a] Screening visit Run-in #1 requires the following assessments to be completed; however, the results related to study eligibility will not be immediately available and need to be verified prior to Screening visit Run-in #2.

- Serum pregnancy test and lab results will be available within 72 hours
- SD-OCT, FA, and CFP images should be promptly transmitted to the Central Reading Center. Eligibility reports will be issued within 2 weeks

Screening visits Run-in #2 and Run-in #3 require the following assessment to be resulted before proceeding with all other procedures required for Run-in #2 and #3.

- BCVA SE only- if the results show ≥10 letter gain in the SE, then this is a screen fail

Visit 1 (Day 1) requires the following assessments to be performed and results available before proceeding to randomization.

- Urine pregnancy test (kit included in lab supplies) for females of childbearing potential
- BCVA OU- if the results show ≥10 letter gain in the SE, then this is a screen fail
- IOP ≤23 mmHg in the SE
- Medication history (anti-VEGF taken in OU 6 months prior to Screening is needed so the randomization module can accurately stratify)

[b] Screening visit Run-in #1 requires the following assessments to be completed; however, the results related to study eligibility will not be immediately available and need to be verified prior to Screening visit Run-in #2.

- Serum pregnancy test and lab results will be available within 72 hours
- SD-OCT, FA, and CFP images should be promptly transmitted to the Central Reading Center. Eligibility reports will be issued within 2 weeks

Screening visits Run-in #2 and Run-in #3 require the following assessment to be resulted before proceeding with all other procedures required for Run-in #2 and #3.

- BCVA SE only- if the results show ≥10 letter gain in the SE, then this is a screen fail

Visit 1 (Day 1) requires the following assessments to be performed and results available before proceeding to randomization.

- Urine pregnancy test (kit included in lab supplies) for females of childbearing potential
- BCVA OU- if the results show ≥10 letter gain in the SE, then this is a screen fail
- IOP ≤23 mmHg in the SE
- Medication history (anti-VEGF taken in OU 6 months prior to Screening is needed so the randomization module can accurately stratify)

[c] On Day 1 patients will either get coadministration of aflibercept and foselutoclax (UBX1325), or aflibercept and a sham procedure. Aflibercept should be administered first, then conduct the post-injection assessments before proceeding to either foselutoclax (UBX1325) administration or sham procedure. See footnote c for timing of post-injection procedures.

[d] Procedure to be performed pre-dose in OU and post-dose in SE only. Post-dose procedures will be done by the unmasked injector or a qualified unmasked designee, 30 minutes ± 15 minutes and no more than 2 hours post dose. On Day 1, when there is either coadministration of aflibercept and foselutoclax (UBX1325), or aflibercept and a sham procedure, these assessments should be done pre and at each post IVT injection.

[e] For Unscheduled Visits, tests/procedures can be performed per Investigator discretion. For ET Visits, all tests/procedures need to be performed. FA should only be completed if it has not been performed within the preceding 30 days.

[f] After all other study procedures have been completed, optional post-injection prophylactic antibiotics may be administered. Sites should follow their standard practice and document all medication given.

[g] Complete physical examinations inclusive of height and weight will be performed at Screening as a baseline assessment and at Visit 10 (Day 253) or ET. Symptom-directed physical examinations may be conducted at all other times as clinically indicated and as part of an AE assessment.

[h] DRSS scores will be generated by the Central Reading Center, so it is important that sites promptly transmit the CFP.

[i] mfERG to be conducted as the first ocular evaluation at prequalified sites with the proper equipment available and completed prior to the first IVT injection.

12.2 Appendix B – Analysis Visit Windowing Rules for Unscheduled and Early Termination Visits

Unless otherwise specified, data from the planned post-baseline visit (Appendix A) will be used in the analysis. Post-baseline unscheduled visits and early termination visits will be mapped to a planned visit and will only be used in the analysis if the actual planned visit result is missing, Table B.1 presents the analysis visit window mapping for unscheduled and early termination visits. Note that the assignment of the baseline visit is handled by a separate algorithm for this study, not by the visit windowing rules (see Section 4.2)

Table B.1: Analysis Windows for unscheduled and early term visits

Planned Visit Day	Lower Limit	Upper Limit	Visit	Visit Number
	-300	-1	Screening	0
1	1	1	Visit 1 (Baseline)	1
29	2	43	Visit 2	2
57	44	71	Visit 3	3
85	72	99	Visit 4	4
113	100	127	Visit 5	5
141	128	155	Visit 6	6
169	156	183	Visit 7	7
197	184	211	Visit 8	8
225	212	239	Visit 9	9
253	240	267	Visit 10	10

Rules

1. If there is one visit in a window, assign that visit to be the analysis visit.
2. If there is more than one visit in a window, then calculate the absolute difference (in days) from the planned visit day to each visit.
   - If a single visit has the smallest absolute difference, then assign that visit as the analysis visit.
   - If multiple visits are tied for the smallest absolute difference, take the latest visit as the analysis visit. Note: if the latest visit includes both a pre-dose measurement and a post-dose measurement, take the pre-dose measurement and associated details (e.g., timing of pre-dose measurement) as the results of the analysis visit.