STATISTICAL ANALYSIS PLAN

AKB-9778-CI-5001

PHASE 2 DOUBLE-MASKED, PLACEBO-CONTROLLED STUDY TO ASSESS THE SAFETY AND EFFICACY OF SUBCUTANEOUSLY ADMINISTERED AKB-9778 15MG ONCE DAILY OR 15MG TWICE DAILY FOR 12 MONTHS IN PATIENTS WITH MODERATE TO SEVERE NON-PROLIFERATIVE DIABETIC RETINOPATHY

Prepared for:

Aerpio Therapeutics, Inc.

Version and Author details:

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SIGNATURES

The undersigned have approved this Statistical Analysis Plan for use in this study.

Jatinder Singh, Biostatistician, Trial Runners, LLC	Date
Steve Pakola, Chief Medical Officer, Aerpio Therapeutics, Inc.	Date
Mitchell Brigell, Vice-President of Clinical Development, Aerpio Therapeutics, Inc.	Date

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1 INTRODUCTION

This statistical analysis plan (SAP) describes the statistical methods to be used during the reporting and analyses of data collected under Aerpio Therapeutic, Inc. Protocol AKB-9778-CI-5001.

This SAP should be read in conjunction with the study protocol and case report form (CRF). This version of the plan has been developed using the protocol (amendment 5 integrated) dated 18 Sep 2017. The final approval of the SAP by Aerpio Therapeutic, Inc. and Trial Runners will occur prior to database lock.

1.1 Change from Protocol

Not applicable.

2 STUDY OBJECTIVES

The primary objective is to assess the effects of AKB-9778 15 mg once daily (QD) or 15 mg twice daily (BID) for 48 weeks on severity of diabetic retinopathy (DR) in subjects with moderate to severe non-proliferative DR (NPDR).

Secondary objectives include:

- To assess the safety and tolerability of AKB-9778 15 mg QD or 15 mg BID for 48 weeks in subjects with moderate to severe NPDR.
- To determine the AKB-9778 systemic exposure based on sparse Pharmacokinetic (PK) sampling in subjects with NPDR.

3 STUDY DESIGNS

This is a Phase 2 randomized, double-masked, placebo controlled, multi-center study, to evaluate the safety and efficacy of 48 weeks of subcutaneous AKB-9778 administered 15 mg QD or 15 mg BID in subjects with moderate to severe NPDR. Exploratory evaluations include pharmacokinetic assessments, as well as exploratory efficacy assessments (OCT-Angiography (OCT-A), Ultra Wide-Field (UWF) imaging and non-invasive Electroretinography (ERG) at a subset of sites. Blood samples will be retained for biomarker and genetic analysis.

Subjects will participate in the study for approximately 56 weeks (screening period up to 4 weeks, baseline and treatment periods for 48 weeks, and follow-up period for 4 weeks). The study will be conducted at approximately 50 investigative sites. Subjects will be randomized 1:1:1 to either AKB-9778 15mg QD, AKB-9778 15mg BID, or placebo treatment groups. Fifty (50) subjects are planned per group. The treatment groups are as follows:

- Active Group: SC AKB-9778 15 mg (QD); To maintain masking, subjects will receive BID dosing with masked study medication administered as one dose of active and one dose of matching placebo.
- Active Group: SC AKB-9778 15 mg (BID)
- Placebo Group: SC phosphate-Buffered-Saline (PBS) (BID)

Subjects will self-administer study medication as subcutaneous (SC) injections in the abdomen (preferably) around the same time every morning and evening, if possible. The investigator, all study-site personnel, all Sponsor personnel (except for an unmasked CMC representative), Central Image Reading Center personnel, and subjects will be masked to treatment assignment during the entirety of the study. Randomization will be stratified by screening Early Treatment of Diabetic Retinopathy Study (ETDRS) Diabetic Retinopathy Severity Score (DRSS) (levels 4, 5 and 6 equally distributed among the treatment groups).

Spot UACR and eGFR will be assessed at baseline, and thereafter every 12 weeks as exploratory assessments to evaluate for any potential effects on renal function. Plasma samples will be collected at specified times on Day 1 and Month 6 visit for PK assessments. These samples will be analyzed for AKB-9778 using validated bioanalytical methods.

A blood sample will be retained for exploratory genetic analysis that may provide insight into the role of the VE-PTP/Tie2 pathway in diabetic retinopathy and an individual subject's response to AKB-9778 treatment. Similarly, blood samples collected at baseline and week 48 End of Treatment (EOT) will be retained for exploratory analysis of biomarkers in plasma associated with VE-PTP inhibition and Tie2 activation. Once results of the study are known, the Sponsor will determine the value of conducting the genetic and biomarker assessments.

3.1 Sample Size Considerations

Estimates for sample size calculations are derived from published data from the analysis of the effect of ranibizumab (Lucentis®) and aflibercept (Eylea®) intravitreal administration regimens on DR severity score in the RISE/RIDE studies and VIVID/VISTA studies, respectively, in which at least 28-46% of subjects improved by ≥ 2 steps after 12 months of treatment.

With 45 evaluable subjects per treatment group, the study has >85% power (based on Fisher's Exact Test and a 2-sided alpha =0.05) to demonstrate a statistically significant improvement in percent of subjects improving by ≥ 2 steps on the ETDRS diabetic retinopathy severity scale, assuming underlying rates for active and placebo of 30% and 5%, respectively. Assuming 10% drop-out/nonevaluable rate, a sample size of 50 subjects per treatment arm (approximately 150 total) was selected.

3.2 Randomization

Approximately 150 subjects will be enrolled in the study. Subjects meeting all eligibility criteria will be randomized 1:1:1 to either AKB-9778 15 mg QD, AKB-9778 15 mg BID, or placebo treatment groups. Fifty (50) subjects are planned per group. The treatment groups are as follows:

- Active Group: SC AKB-9778 15 mg (QD)
- Active Group: SC AKB-9778 15 mg (BID)
- Placebo Group: SC PBS (BID)

Using a central IWRS randomization system, eligible subjects will be assigned in a double-masked fashion 1:1:1 to one of the 3 treatment groups. To maintain balance between treatment groups with respect to DR severity, randomization will be stratified by screening ETDRS DRSS (levels 4, 5 and 6).

3.3 Schedule of Evaluations and Analysis Visit Windows

The schedule of visits and procedures are provided in the protocol. The study consists of 48 weeks of study treatment with 4 week of follow-up period. There is a screening visit (Day-28 to -1), baseline visit (Day 1), site visits at every 4 weeks and follow-up/exit visit at week 52. Tables 1-4 show the visit windows to be used for analyses:

Table 1: Visit Windows (Data Collected at All Monthly Visits – BP/HR, BCVA, chemistry)

Visit	Target Day of the Visit	Visit Window (Days) for Analyses
Baseline	1	<= 1
Week 4	29	15-42
Week 8	57	43-70
Week 12	85	71-98
Week 16	113	99-126
Week 20	141	127-154
Week 24	169	155-182

Week 28	197	183-210
Week 32	225	211-238
Week 36	253	239-266
Week 40	281	267-294
Week 44	309	295-322
Week 48	337	323-350

Note: All days are referenced to Day 1

Table 2: Visit Windows (Data Collected at Months 3, 6, 9, 12 – Body System Assessments, Slit Lamp, IOP, Indirect Ophth, SD OCT, FP, OCT Angiography, ERG, Urinalysis, Fecal Occult Blood Test)

Visit	Target Day of the Visit	Visit Window (Days) for Analyses
Baseline	1	< = 1
Week 12	85	82-126
Week 24	169	127-210
Week 36	253	211-294
Week 48	337	295-378

Note: All days are referenced to Day 1
Note: Urinalysis was not collected at Week 12, so it should not have a Week 12 Window; all other visit windows are as shown in the table.

Table 3: Visit Windows (Data Collected at Months 6 and 12 – Height, Weight, Temperature, FA)

Visit	Target Day of the Visit	Visit Window (Days) for Analyses
Baseline	1	< = 1
Week 24	169	85-252
Week 48	337	253-420

Note: All days are referenced to Day 1

Table 4: Visit Windows (Data Collected at Months 2, 4, 6, 8, 10, 12 – Hematology)

Visit	Target Day of the Visit	Visit Window (Days) for Analyses
Baseline	1	<= 1
Week 8	57	29-84
Week 16	113	85-140
Week 24	169	141-196
Week 32	225	197-252
Week 40	281	253-308
Week 48	337	309-364

Note: All days are referenced to Day 1

When multiple observations are captured in a visit window, use the scheduled visit that matches the visit window in the analysis (e.g., if week 24 scheduled visit is in the week 24 window, use this visit for Week 24 analysis visit). If there is no scheduled visit that matches the visit window, the one closest to the target date of the visit will be used to represent the window. If two observations are equidistant from the target date, then the observation that is closer to the upper end of the window visit will be used.

4 ANALYSIS POPULATIONS

4.1 Safety Population

The safety population will include all enrolled subjects who receive at least one dose of study medication. Group assignment will follow actual treatment received. All safety analyses will be conducted using the safety population.

4.2 Modified Intent-to-Treat Population

The modified intent-to-treat (MITT) population will include all enrolled subjects who receive at least one dose of study medication. Group assignment will follow treatment group assigned at randomization. The primary efficacy analysis will be conducted using the MITT population.

4.3 Per Protocol Population

The per protocol (PP) population will consist of all subjects in the MITT population who do not have major protocol deviations considered to affect the primary efficacy variable DRSS, have completed their 48-week visit on treatment, and have been at least 70% compliant. The rules for determining exclusion from the per-protocol population will be made and implemented prior to unmasking. Sensitivity analyses of the primary efficacy variables will be conducted using the PP population.

4.4 Pharmacokinetic Population

The pharmacokinetic population will consist of all subjects with at least one plasma sample collected for AKB-9778 analysis.

4.5 Sub-Study Populations

Sub-study populations include those subjects who participated in the UWF- FA, UWF- Photography, OCT-A, and ERG sub-studies, respectively.

5 STUDY VARIABLES AND COVARIATES

5.1 Primary Efficacy Endpoints

The primary efficacy endpoint is the percentage of subjects with an improvement in study eye severity of diabetic retinopathy (DR) (ETDRS DRSS) of ≥ 2 steps at week 48 compared to baseline.

5.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints include:

- Proportion of subjects with a worsening in the study eye DRSS of ≥ 2 steps at week 48
- Mean change from baseline in DRSS in the study eye at week 48
- Proportion of subjects with an improvement or worsening in the study eye DRSS of ≥ 3 steps at week 48.
- Subjects with criterion step improvement in DRSS at week 48 (≥2 steps improvement in the study eye for patients with non-qualified fellow eye and ≥3 steps improvement on the Person scale for patients with qualified fellow eyes)
- Subjects with criterion step improvement in DRSS at week 48 (≥2 steps improvement in the study eye for patients with non-qualified fellow eye and ≥3 steps improvement on the Binocular scale for patients with qualified fellow eyes)
- Proportion of patients developing center-involved Diabetic Macular Edema (DME) or Proliferative Diabetic Retinopathy (PDR) or PDR-related outcomes during treatment period based on Clinical Data. Due to variation in clinician criteria for reporting DME and PDR adverse events, all events will be reviewed and AEs that were not treated and were not validated in FPRC interpretation of imaging data will be censored from the results.
- Proportion of patients developing center-involved DME or PDR or worsening of ≥2 steps DRSS at week 48 based on Central Image Reading Center Evaluation
- Primary and secondary assessments in qualified fellow eyes and all qualified eyes (study eyes and qualified fellow eyes).

- Primary and secondary endpoints assessed at week 12, 24 and 36.

5.3 Exploratory Efficacy Endpoints

Change from baseline in urine-albumin-to-creatinine ratio (UACR) and estimated glomerular (eGFR) at week 12, 24, 36 and 48 in the overall MITT population and in the subgroup of subjects with renal insufficiency at baseline (UACR >30 µg/mg for UACR analysis; eGFR <90 mL/min/1.73m^2 for the eGFR analysis) is the exploratory efficacy endpoint used to investigate renal function.

For urine albumin values below the lower limit of quantitation, UACRs will be calculated using the 0.5xLLQ value. Due to the positive skewness, UACR data will be log transformed prior to analysis. The primary outcome will be percent change in geometric mean.

5.4 Exploratory Efficacy Sub-Study Endpoints

Exploratory efficacy sub-study endpoints include:

- Mean change from baseline in ERG parameters (LKC Composite Score; Implicit Time (ms) in 16 Td's; Amplitude Value (µV) in 16 TD's) at week 48.
- Mean change from baseline in Retinal Capillary Dropout 3x3, Retinal Capillary Dropout 6x6, FAZ Area (mmsq), Vessel Density (%), Vessel Skeleton Density 6x6 (%), Total Vessel Length 6x6 (mm) and Non-Perfusion Area 6x6 (mmsq) measured by OCT- A at week 48.
- Mean change from baseline in Ischemia Disc (%), Ischemia Macula (%), Leakage Disc (%), Leakage Macula (%) and Microaneurysm Count Macula in the regions ROI 1 and ROI 2 measured by UWF-FA at week 48.
- Subjects (Eyes) with criterion step change in DRSS on UWF-photography (Worsening ≥3, ≥2, Improvement ≥2, ≥3) at week 48

A more detailed presentation of the variables to be analyzed for these sub-studies is provided in the appended sub-study analysis plans.

5.5 Safety Endpoints

Safety endpoints include:

- Incidence and severity of systemic and ocular AEs
- Ocular AEs at Study eyes, all qualified eyes and unqualified fellow eyes during treatment period.
- Shift from baseline in body system assessments for the parameters general appearance, HEENT (Head, Ears, Eyes, Nose and Throat), cardiovascular, respiratory, abdomen/gastrointestinal, musculoskeletal, and dermatologic at week 12, 24, 36, 48.
- Change from baseline in vital sign measurements (SBP, DBP, HR, BMI, Weight and Temperature) at every 4 weeks.
- Change from baseline in ECG parameters (HR, PR, QRS and QTcF) at week 24, 48.
- Change from baseline in clinical laboratory assay results (blood chemistry (at every 4 weeks), hematology (at every 8 weeks), fecal occult blood test (at week 24, 36, 48), and urinalysis (at week 24, 36, 48).
- Mean change from baseline in ETDRS Best Corrected Visual Acuity (BCVA) letter score in the study eyes, all qualified eyes and unqualified fellow eyes at every 4 weeks.
- Proportion of subjects with a loss of ≥15 letters, loss of ≥10 letters and loss of ≥5 letters in BCVA compared to baseline at week 48.
- Mean change from baseline in Spectral Domain Optical Coherence Tomography (SD OCT): Central Subfield Thickness (CST) in the study eyes, all qualified eyes and unqualified fellow eyes at week 12, 24, 36, 48.

- Proportion of subjects with increase of ≥100 µm and increase of ≥50 µm SD OCT: CST in the study eyes, all qualified eyes and unqualified fellow eyes at week 48.
- Change from baseline in Intraocular Pressure (IOP), with additional sub-group analysis with baseline IOP <16 mmHg and baseline IOP ≥ 16 mmHg in the study eyes and fellow eyes at week 12, 24, 36, 48.
- Shift from baseline in Slit Lamp Biomicroscopy examination for the parameters Eyelids, Cornea, Conjunctiva, Anterior Chamber, Iris/Pupil and Lens in all qualified eyes and unqualified fellow eyes at week 12, 24, 36, 48.
- Shift from baseline in Dilated Indirect Ophthalmoscopy examination for the parameters Vitreous, Macula, Optic Nerve, and Peripheral Retina in all qualified eyes and unqualified fellow eyes at week 12, 24, 36 and 48.
- Change from baseline in Fluorescein Angiogram (FA) for the parameters total area of capillary non-perfusion, and leakage within ETDRS grid, change in fluorescein leakage from screening, NVD and NVE within 7 standard field in all qualified eyes and unqualified fellow eyes at week 24, 48.
- Shift from baseline in Fundus Photograph (FP) for the parameters clinically significant ME (ETDRS), hard exudate presence within grid, and hemorrhages and/or microaneurysms within grid in all qualified eyes and unqualified fellow eyes at 12, 24, 36, 48.

6 DATA PREPARATION

Except for the laboratory data (clinical laboratory assays, PK and biomarker data) and imaging data, all reported study data will be recorded on the eCRFs supplied by TrialRunners (TR) using iMedNet Electronic Data Capture (RDC),. Only the Principal Investigator and authorized study staff according to the Delegation of Responsibilities log are entitled to make entries on the eCRF. Data from source documents will be entered into RDC by site personnel. The laboratory and imaging data will be incorporated into the study database electronically. The details of the laboratory data transfer can be found in the Data Transfer Agreements (DTAs) and TR's Standard Operating Procedure (SOP) on electronic data importation.

All ocular image data (fundus photographs, FA and SD-OCT) will be transmitted to the Eyekor Excelsior data platform. The Wisconsin Fundus Photograph Reading Center (FPRC) will assess all screening images to confirm subject eligibility. The FPRC will also grade all 7-field or 4-field wide color fundus photographs for DRSS, FA data, and SD-OCT data. Details are contained in the FPRC image grading charter. Ultra-wide field fluorescein angiography data will be graded by the Campane Center for Advanced Imaging at the Cole Eye Institute (CCAI) using their custom software. Details are contained in the CCAI UWF-FA charter. Electroretinographic data will be evaluated by LKC Technologies using their custom software. The FPRC, CCAI and LKC Technologies will store graded data in Excelsior and this data will be transferred to TR per the DTA and incorporated into the study database electronically. OCT-A and UWF-color photographs will be graded by the Boston Image Reading Center (BIRC) using their custom software. Data will be stored at BIRC and transferred TR per the DTA and incorporated into the study database electronically. Source raw images will be stored at study sites. Image grading source data will be stored at the reading centers.

All analyses outlined in this document will be carried out after the following has occurred:

- All data management requirements are met according to TR standard operating procedures, including data entry, performance of edit and validation checks, documentation and resolution of data queries, and database lock with written authorization provided by appropriate TR and Sponsor personnel;
- Protocol deviations have been identified and status defined (major/minor deviations);
- Analysis populations have been determined, and

- Randomized treatment codes have been unmasked.

7 DATA CONVENTION

All data analysis will be performed by Trial Runners, LLC after the study is completed and database has been locked and released for unmasking. Statistical programming and analyses will be performed using SAS® Version 9.4 or higher. Output will be provided in RTF format for tables and PDF format for tables, listings, and figures using landscape orientation. All study data will be listed by subject, treatment, and visit (as applicable), based on all enrolled subjects.

7.1 Missing Data

Efficacy data related to eye anatomy or visual function that is collected after rescue treatment (i.e., treatment for DME or PDR other than study medication) will be treated as missing data. Unless, otherwise stated, missing data will be handled using the following approach:

- For start date, partial dates will have first day of the month imputed if day is missing, and January of that year will be imputed if month is missing. If both day and month are missing, January 01 will be imputed.
- For end date, partial dates will have last day of the month imputed if day is missing, and December of that year will be imputed if month is missing. If both day and month are missing, December 31 will be imputed.
- Missing data are imputed using Last Observation Carried Forward (LOCF) for all visits except screening/baseline; screening/baseline values are not carried forward.
- For vision related efficacy measurements (Tables 14.2.1.x, 14.2.2.x, 14.2.3.x, 14.2.4.x, 14.2.5.x, 14.2.6.x, 14.2.7.x,14.2.8.x, 14.2.10.x, 24.2.11.x, 14.2.12.x, 14.2.13, 14.3.12.3, 14.3.13.3, 14.3.14.2x, 14.3.14.5) subjects receiving rescue treatment will be handled differently to reflect their status as a treatment failure and to avoid confounding of the effects of rescue treatment with the effects of study drug. Specifically, last pre-rescue data are carried forward for LOCF analysis. If rescue and DRSS occur on the same day, DRSS from that day is carried forward. For Observed Case (OC) analyses, results post-rescue are omitted, and last pre-rescue data are carried forward. Note that for DRSS scales involving both eyes (Person and Binocular scale) the carry forward rules apply only to the rescued eye in calculation of the combined score.
- Where severity or relationship is missing for adverse events, most conservative imputation will be made.

7.2 Descriptive Statistics

For continuous variables, descriptive statistics will include the number of subjects (n), mean, standard deviation (SD), median, minimum and maximum. Categorical variables will be summarized using absolute and relative frequencies (i.e., counts and percentages). For categorical analyses, eyes that receive rescue therapy will be considered as treatment failures. Safety and PK data will be analyzed as collected.

All efficacy data will be summarized by treatment groups and overall using appropriate descriptive statistics. Summaries will be presented for both the MITT and PP populations, where applicable. Due to the small number of subjects t enrolled at each center, all summaries and analyses will be performed using data pooled across centers. Unscheduled data, such as information from unscheduled visits or investigator comments, will be included in the data listings. In general, these data will be excluded from the summary tables unless otherwise specified.

7.3 Study Days Relative to First Day of Treatment

Baseline results will be those recorded just prior to dosing. In cases where more than one pre-dose observation has been recorded, the last recording will be identified as the baseline result. Study days will be numbered relative to the first day of dosing. The start of study (Day 1) will be defined as the date on which a subject takes the first dose of any study medication, as recorded on the CRF. Relative to study start, days will be numbered ...,-2, -1, 1, 2, ... with Day 1 data collected on the first day of dosing prior to the start of study medication (Pre-Dose). Recorded data will be assigned to evaluation/assessment windows.

8 DISPOSITION AND EXIT STATUS

8.1 Subject Disposition

Subjects who are enrolled, complete the study, discontinue study medication, or withdraw from the study including reasons for study medication discontinuation or study withdrawal will be summarized along with the number and percentage of subjects in each of the analysis populations (safety, PP, PK and sub-study) in tabular format by treatment group and for all subjects. The number and percentage of subjects who completed 1-month follow-up visit will be summarized in the Safety population. The reasons for active treatment period discontinuation that will be summarized include: adverse events, treatment for PDR or DME, subject withdrew consent, lost to follow-up, protocol deviation, termination of study and other. Individual subject disposition data will be listed.

8.2 Extent of Study Drug Exposure

Exposure to treatment and treatment compliance will be summarized by treatment group and for all subjects. A listing will present all product accountability details. Duration of exposure is determined from the dispensed and return date information. Treatment compliance is determined as the number of actual doses received over the number of expected doses multiplied by 100. Expected subcutaneous study medication doses is twice the date of the last date of dosing minus the date of the Day 1 visit plus 1 (minus allowed days of non-dosing, i.e., days on which subject was intentionally not dosing per Investigator instruction/allowance, due to safety/tolerability or concomitant illness/procedure/hospitalization). The number and percentage of subjects who are at least 70% compliant will be summarized.

8.3 Protocol Deviations

The number and percentage of subjects with major protocol deviations will be summarized by treatment groups and for all subjects for the safety population. The protocol deviations that will be summarized include: informed consent, inclusion/exclusion and randomization, study drug administration and assignment at site, improper protocol procedures at site (missed, repeated, not per protocol), site's failure to report SAE/AE, visit out of window (missed, early, late), subject's non-compliance with subcutaneous study drug (<70% of expected doses administered), subject's use of prohibited concomitant medication, subject's failure to follow instructions and other. A subject listing will be provided that includes the date of the deviation, the deviation description and the classification of whether the deviation was judged to be major or minor.

8.4 Exclusion from Analysis Populations

The number and percentage of subjects that are excluded from Per Protocol Population due to protocol deviations, did not complete study and non-compliant will be summarized for the MITT population by treatment groups and for all subjects. The number of subjects with percentage excluded from pharmacokinetic population because of non-evaluable data will also be presented for MITT population by

treatment groups and for all subjects. A listing for the subjects excluded from MITT population will be generated.

9 BASELINE CHARACTERISTICS

9.1 Demographics and Baseline/Screening Characteristics

All demographic and baseline data will be summarized per treatment group and overall. These will be presented for the safety population and MITT population separately. The demography presentation will include Age (years), Sex, Ethnicity, Race, and BMI (kg/m^2). Summary statistics of duration of diabetics (years), and the types of diabetics will be presented. The duration of NPDR (years), DRSS and BCVA will be summarized for the study eye. Number and percentage of subjects with right eye (OD) and left eye (OS), and prior treatment of PDR or DME in study eye will also be summarized. Additionally, the number and percentage of subjects who were taking insulin and ACEi/ARB will be presented by the treatment group and for all subjects.

9.2 Prior and Concomitant Medications

Prior and concomitant medications will be presented for each subject coded per the World Health Organization (WHO) Anatomical Therapeutics Chemical (ATC) Drug dictionary. Prior medication are those medications that were started anytime and were stopped within 30 days prior to visit 1. Concomitant medications are those medications that were started anytime after visit 1 and before the last study visit or started anytime before visit 1 and were ongoing on or after visit 1. The data will be summarized by ATC dictionary (WHO DDE B2 September 1, 2017) drug class and generic name and classified as non-ocular and ocular prior and concomitant medications. Further, ocular prior and concomitant medications are presented for study eyes, qualified fellow eyes and unqualified fellow eyes. Descending sorting order by the frequency of ATC drug classification, and within that descending order by the frequency of generic name will be followed. A separate listing for both non-ocular and ocular prior and concomitant medication will present be presented.

Medications with partial dates that do not allow determination of whether prior or concomitant will be considered concomitant. Prior and concomitant medications are presented for safety population.

9.3 Medical History

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 20.1 Non-ocular medical history will be summarized using discrete summary statistics and presented by treatment groups and for all subjects by System Organ Class (SOC) and Preferred Term (PT) using safety population. Ocular medical history will be similarly summarized by treatment groups and for all subjects for the study eyes, qualified fellow eyes, and unqualified fellow eyes separately. Subjects with multiple medical histories in the same SOC or PT will be counted only once for the respective SOC or PT. Subject listings of medical history will be generated separately for ocular and non-ocular data.

10 EFFICACY ANALYSIS

Efficacy analysis will be performed after the last patient has completed the study. Data to be included in this analysis include Subject Disposition, Extent of Drug Exposure, Demographic and Baseline Characteristics, Primary Outcome (MITT & PP population, LOCF & OC), all secondary efficacy endpoints (MITT, LOCF), OCT-A (MITT, LOCF & OC), UACR (MITT & PP population, LOCF & OC), eGFR (MITT & PP population, LOCF & OC), ERG (MITT, LOCF & OC), and UWF-FA (MITT, LOCF & OC). The primary efficacy analysis will only contain data on events that occurred at timepoints up to and including the EOT visit (week 48).

10.1 Primary Efficacy Endpoint Analysis

The primary hypotheses to be tested is that AKB-9778 15 mg twice daily and AKB-9778 15 mg once daily will be superior to placebo in the improvement of DR as measured by the ETDRS severity scale change from baseline at 48 weeks. Specifically, the primary endpoint is the percentage of subjects with an improvement in study eye severity of DR (ETDRS DRSS) of ≥ 2 steps at 48 weeks without need for treatment of PDR or DME (by IVT, laser, or vitrectomy) in the study eye. DRSS scores used for all analyses will be obtained from final adjudicated grading by the central reading center (Fundus Photograph Reading Center for the standard field images).

The primary hypotheses will be tested in the MITT population using a Fisher's Exact Test with a 2-sided 5% significance level. AKB-9778 15 mg twice daily will be tested first. If this is found to be statistically significant, then AKB-9778 15 mg once daily will be tested for statistical significance, at the same significance level. Cochran-Mantel-Haenszel (CMH) test stratified for baseline ETDRS DRSS (levels 4, 5 and 6) will also be used for testing primary hypothesis in the MITT population. Missing data will be imputed using LOCF; baseline values will not be carried forward.

As a sensitivity analysis, primary hypothesis will be tested using Fisher's Exact test, and CMH test stratified for baseline ETDRS DRSS (levels 4, 5 and 6) on OC in the PP population.

10.2 Secondary, Exploratory and Exploratory Sub-Study Efficacy Endpoint Analysis

Secondary efficacy endpoints are summarized in Section 5.2, and involve alternative analyses of DRSS results, showing the proportion of patients who develop sight-threatening complications of DR (DME & PDR). Measures of kidney function (UACR and eGFR) are exploratory efficacy endpoints. Exploratory sub-study endpoints are derived from OCT-Angiography, Ultra-wide field fluorescein angiography, Ultra-wide field color photographs, and electroretinography. Details of these variables and their analysis are contained in separate SAPs.

Normality assumption will be tested for any endpoint of continuous nature. If normality assumption is violated, appropriate transformation and/or non-parametric analysis will be employed. Otherwise, all secondary, exploratory and exploratory sub-study endpoints of continuous nature will be analyzed using ANCOVA model, and all inferential analyses will use a 2-sided 5% significance level. For the exploratory efficacy endpoints UACR and eGFR, for internal decision-making purposes, a 1-sided 10% significance level will be used. Whenever possible, a mixed model repeated measures (MMRM) model will be utilized to analyze this data. The model will include change from baseline as the dependent variable. The p-value and 95% CI from the model is baseline parameter adjusted.

For MMRM, visit will be treated as the repeated variable within a subject. Within-subject error, an unstructured variance-covariance will be applied; in case of non-convergence, compound symmetry will be used. MMRM model for UACR and eGFR is adjusted by the baseline HbA1c, Baseline Systolic BP, log-transformed Baseline UACR, and ACEi/ARB use.

Secondary, exploratory and exploratory sub-study endpoints of a dichotomous nature will be analyzed using the same approach as outlined for the primary efficacy endpoint.

11 SAFETY ANALYSIS

The safety and tolerability of AKB-9778 will be determined by incidence and severity of treatment emergent AEs and changes from baseline in ECG, vital sign measurements, clinical laboratory assay results (blood chemistry, hematology, fecal occult blood, and urinalysis), BCVA, IOP, slit lamp examinations, funduscopic examinations and FA. Continuous and qualitative variables will be summarized indicating change from baseline and shift tables, where appropriate. Some data will be summarized through treatment period as

well as throughout the study (treatment period plus follow-up period). Also, individual subject safety data will be listed.

11.1 Safety Endpoint Analysis

11.1.1 Adverse Events

All AEs reported during the study and follow-up period will be recorded and coded using MedDRA version 20.1 terminology. An AE will be considered as treatment-emergent if it has an onset during the treatment period or is pre-existing and worsens after the first dose of study medication is administered. When the date of AE is same as date of randomization, the site will be queried to ensure timing of AE start relative to first dose is determined.

The incidence of all reported treatment-emergent adverse events (TEAEs) will be summarized using system organ class and preferred term by treatment group and overall, using counts and percentages. All adverse events will also be presented by relationship and severity. Further, serious adverse events and adverse events leading to discontinuation will also be presented.

Separate analyses will be performed for ocular and non-ocular TEAEs. For ocular TEAEs, the subject will be considered to have had the TEAE if at least one eye had the TEAE. Ocular TEAEs will be summarized separately for all eyes, study eyes, all qualified eyes (study eyes and qualified fellow eyes) and unqualified fellow eyes.

Subjects may have more than one AE per system organ class and preferred term. At each level of subject summarization, a subject will be counted once if they reported 1 or more events and will be reported with the highest severity. In cases where severity or relationship is missing, the most conservative approach will be taken (i.e. highest severity and assumed to be related).

A listing of TEAEs will be generated showing adverse event location (OD, OS or non-ocular), duration of TEAEs with onset date, severity, relationship with study medication and change in status of ongoing event. Also, listing for serious adverse events, and adverse events leading to discontinuation will be generated.

11.1.2 Electrocardiogram (ECG)

Change from baseline for ECG parameters will be presented along with their associated 95% confidence intervals. Potentially clinically significant findings will also be presented based on the following categories:

- The proportion of patients obtaining treatment-emergent absolute QTcF values > 450 ms and ≤ 480 ms; > 480 ms and ≤ 500 ms; and > 500 ms.
- The proportion of patients obtaining a QTcF increase from baseline values >30 and ≤60 ms; and > 60 ms.
- The proportion of patients obtaining a QRS change from baseline > 25% resulting in QRS > 120 ms
- The proportion of patients obtaining a PR interval change from baseline >25% reaching a value >220 ms
- The proportion of patients obtaining a heart rate change from baseline > 25% decrease resulting in a heart rate < 50 beats per minute (bpm) and heart rate change from baseline > 25% increase resulting in a heart rate > 100 bpm

12 PHAMACOKINETIC ANALYSIS

AKB-9778 plasma concentrations will be summarized by treatment group and nominal sampling time using descriptive statistics. Individual subject pharmacokinetic data will be listed. Additional analyses including

the potential relationship of plasma concentration and selected safety and efficacy parameters and/or subject demographic variables may be performed.

13 PHARMACODYNAMICS ANALYSIS

Blood samples collected at baseline and Month 12 End of Treatment (EOT) will be retained for exploratory analysis of biomarkers in plasma. Once results of the study are known, the Sponsor will determine the value of conducting these analyses.

14 EXPLORATORY GENETIC ANALYSIS

For subjects who have provided consent for genetic sampling, a blood sample will be retained for exploratory genetic analysis that may provide insight into the role of Tie2 in diabetic retinopathy and an individual subject's response to AKB-9778 treatment. Once results of the study are known, the Sponsor will determine the value of conducting these analyses.

15 VALIDATION

Derived datasets will be independently reprogrammed by a second programmer. The separate datasets produced by the two programmers must match 100%. Tables will be independently reprogrammed by a second programmer for numeric results. Statisticians will be involved in the process of programming and validating tables that include inferential statistical results. Figures will be checked for consistency against corresponding tables and listings, or independently reprogrammed if there are no corresponding tables or listings.

16 APPENDIX 1

Person level	Level worse eye	Level better eye
1	1	1
2	2	1
3	2	2
4	3	<3
5	3	3
6	4	<4
7	4	4
8	5	<5
9	5	5
10	6	<6
11	6	6
12	7	<7
13	7	7
14	8	<8
15	8	8
16	>8	<8
17	>8	>8

17 APPENDIX 2 GLOSSARY OF ABBREVIATIONS

AE	Adverse event
ANCOVA	Analysis of Covariance
ATC	Anatomical Therapeutics Chemical
BCVA	Best Corrected Visual Acuity
BID	Twice Daily
BIRC	Boston Image Reading Center
BMI	Body Mass Index
BP	Blood Pressure
bpm	Beats Per Minute
CCAI	Campane Center for Advanced Imaging
CMH	Cochran-Mantel-Haenszel
CRF	Case Report Form
CST	Central Sub-Field Thickness
DTA	Data Transfer Agreement
DBP	Diastolic Blood Pressure
DME	Diabetic Macular Edema
DR	Diabetic Retinopathy
DRSS	Diabetic Retinopathy Severity Score
ECG	Electrocardiogram
eGFR	Estimated Glomerular Filtration Rate
EOT	End of Treatment
ERG	Electroretinogram
ETDRS	Early Treatment Diabetic Retinopathy Study
FA	Fluorescein Angiography
FP	Fundus Photograph
FPRC	Fundus Photograph Reading Center
HR	Heart Rate
IOP	Intra-ocular Pressure
IVT	Intra-Vitreal Therapy
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
MITT	Modified Intent-to-Treat
MMRM	Mixed Model Repeated Measures
NPDR	Non-proliferative Diabetic Retinopathy
OC	Observed Cases
OCT-A	Optical Coherence Tomography Angiography
OD	Right Eye
OS	Left Eye
PBS	Phosphate Buffered Saline
PDR	Proliferative Diabetic Retinopathy
PK	Pharmacokinetic
PP	Per Protocol
PT	Preferred Term
QD	Once Daily
RDC	Remote Data Capture
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event

SBP	Systolic Blood Pressure
SC	Subcutaneous
SD	Standard Deviation
SD OCT	Spectral Domain Optical Coherence Tomography
SOC	System Organ Class
SOP	Standard Operating Procedure
TEAE	Treatment-Emergent Adverse Event
TR	Trial Runners
Td's	Troland Seconds
UACR	Urine-albumin-to-creatinine Ratio
UWF	Ultra-wide Field
WHO	World Health Organization

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TrialRunners

Note to File

Clinical Study Protocol AKB-9778-CI-5001: PHASE 2 DOUBLE-MASKED, PLACEBO-CONTROLLED STUDY TO ASSESS THE SAFETY AND EFFICACY OF SUBCUTANEOUSLY ADMINISTERED AKB 9778 15MG ONCE DAILY OR 15MG TWICE DAILY FOR 12 MONTHS IN PATIENTS WITH MODERATE TO SEVERE NON-PROLIFERATIVE DIABETIC RETINOPATHY

To: Statistical Analysis Plan, Final Version 27Feb2019

Date: 03-Mar-2019

Re: Criteria used to define Progression to Vision-Threatening Complications (DME and/or PDR)

Criteria used to define Progression to Vision-Threatening Complications (DME and/or PDR):

Qualified eyes were included as progressors if one or more of the following conditions occurred during the trial:

1. An AE of DME was entered that was either treated or was confirmed by the reading center as having either center involved retinal cyst, center involved retinal edema, center involved subretinal fluid, or central subfield thickness >300 microns.

2. An AE of PDR, NVE, NVD, or vitreous hemorrhage

3. IVT with anti-VEGF therapy or corticosteroid with indication for treatment documented as either DME or PDR

4. PRP, Grid laser, or focal laser with indication for treatment documented as either DME or PDR

5. An increase in central subfield thickness of ≥20% at last observation

6. DRSS score of >53, indicating PDR, at last observation

AERPIO SIGNATURE

03-MAR-2019
DATE

Statistical Analysis Plan

OCT-Angiography Efficacy Sub-study

Study AKB-9778-CI-5001

PHASE 2 DOUBLE-MASKED, PLACEBO-CONTROLLED STUDY TO ASSESS THE SAFETY AND EFFICACY OF SUBCUTANEOUSLY ADMINISTERED AKB-9778 15MG ONCE DAILY OR 15MG TWICE DAILY FOR 12 MONTHS IN PATIENTS WITH MODERATE TO SEVERE NON-PROLIFERATIVE DIABETIC RETINOPATHY

Prepared for:

Aerpio Therapeutics, Inc.

Version and Author details:

Version Date:	28 February 2019
Version Number	1.0
Author:	Jatinder Singh

SIGNATURES

The undersigned have approved this Statistical Analysis Plan for use in this study.

Jatinder Singh, Biostatistician, Trial Runners, LLC	Date
Steve Pakola, Chief Medical Officer, Aerpio Therapeutics, Inc.	Date
Mitchell Brigell, Vice-President of Clinical Development, Aerpio Therapeutics, Inc.	Date

OCT-Angiography (OCT-A) images were obtained in study AKB-9778-CI-5001 as an exploratory sub-study. Images (3mm x 3mm and 6mm x 6mm images, centered on the fovea) from study and fellow eyes were obtained at pretreatment Baseline, Week 12, Week 24, Week 36 and Week 48/EOT visits. Data acquisition and transmission protocols were specified in study manuals for Optovue, Zeiss, and Topcon systems. All images were uploaded to the Eyekor Excelsior platform. Images were graded by the Boston Image Reading Center (BIRC) using their custom software. Parameters graded are shown in Table 1. Outcome variables of primary interest are FAZ area, % vessel density, % vessel skeleton density, total vessel length, and non-perfusion area.

Continuous sub-study endpoints of interest will be analyzed using an ANCOVA model, and all inferential analyses will use a 2-sided 5% significance level. Whenever possible, a mixed model repeated measures (MMRM) model will be utilized to analyze this data. The model will include change from baseline as the dependent variable. The p-value and 95% CI from the model is baseline parameter adjusted. For MMRM, visit will be treated as the repeated variable within a subject. Within-subject error, an unstructured variance-covariance will be applied; in case of non-convergence, compound symmetry will be used.

Categorical variables will be tested using a Fisher's Exact Test with a 2-sided 5% significance level. AKB-9778 15mg twice daily will be tested first. If this is found to be statistically significant, then AKB- 9778 15mg once daily will be tested for statistical significance, at the same significance level. Cochran-Mantel-Haenszel (CMH) test stratified for baseline values will also be used for testing. Missing data will be imputed using Last Observation Carried Forward (LOCF); baseline values will not be carried forward.

The relation between change in DRSS from baseline to Week 48/EOT visit and baseline FAZ Area, baseline Vessel Skeleton Density (6x6), baseline Total Vessel Length (6x6), and baseline Non-perfusion Area (6x6) will also be calculated.

TABLE: OCT-A Parameters. Parameters of primary interest are noted in red.

Parameter	Unit	Picklist or Numerical Value	Picklist or Numerical Value (addtl)
OCTA_FAZ Area	mmsq	Numerical Value	XXX.XXX
OCTA_FAZ Perimeter	mm	Numerical Value	XXX.XXX
OCTA_Acircularity Index	score	Numerical Score	XXX.XXX
OCTA_FAZ Remodeling 3x3	-	Yes/No/Can't Grade	If YES, Select Sector: superotemporal, superonasal, inferotemporal, inferonasal
OCTA_Microaneurysm 3x3	-	Yes/No/Can't Grade	If YES, Select Sector: superotemporal, superonasal, inferotemporal, inferonasal
OCTA_Microaneurysm 6x6	-	Yes/No/Can't Grade	If YES, Select Sector: superotemporal, superonasal, inferotemporal, inferonasal
OCTA_Vascular Tortuosity 3x3	-	Yes/No/Can't Grade	If YES, Select Sector: superotemporal, superonasal, inferotemporal, inferonasal
OCTA_Vascular Tortuosity 6x6	-	Yes/No/Can't Grade	If YES, Select Sector: superotemporal, superonasal, inferotemporal, inferonasal
OCTA_IRMA 3x3	-	Yes/No/Can't Grade	If YES, Select Sector: superotemporal, superonasal, inferotemporal, inferonasal
OCTA_IRMA 6x6	-	Yes/No/Can't Grade	If YES, Select Sector: superotemporal, superonasal, inferotemporal, inferonasal
OCTA_Retinal Capillary Dropout 3x3	-	Yes/No/Can't Grade	If YES, Select Sector: superotemporal, superonasal, inferotemporal, inferonasal
OCTA_Retinal Capillary Dropout 6x6	-	Yes/No/Can't Grade	If YES, Select Sector: superotemporal, superonasal, inferotemporal, inferonasal
OCTA_Retinal Neovascularization 3x3	-	Yes/No/Can't Grade	If YES, Select Sector: superotemporal, superonasal, inferotemporal, inferonasal
OCTA_Retinal Neovascularization 6x6	-	Yes/No/Can't Grade	If YES, Select Sector: superotemporal, superonasal, inferotemporal, inferonasal
OCTA_Vessel Density 3x3	percent	Numerical Value	XXX.XXX
OCTA_Vessel Density 6x6	percent	Numerical Value	XXX.XXX
OCTA_Vessel Skeleton Density 3x3	percent	Numerical Value	XXX.XXX

OCTA_Vessel Skeleton Density 6x6	percent	Numerical Value	XXX.XXX
OCTA_Total Vessel Length 3x3	mm	Numerical Value	XXX.XXX
OCTA_Total Vessel Length 6x6	mm	Numerical Value	XXX.XXX
OCTA_Non-perfusion Area 3x3	mmsq	Numerical Value	XXX.XXX
OCTA_Non-perfusion Area 6x6	mmsq	Numerical Value	XXX.XXX
Evolutive Assessment			
OCTA_Retinal Capillary Dropout 3x3	-	Better/Worse/Unchanged/Can't Grade	
OCTA_Retinal Capillary Dropout 6x6	-	Better/Worse/Unchanged/Can't Grade	

Statistical Analysis Plan

Electroretinogram Efficacy Sub-study

Study AKB-9778-CI-5001

PHASE 2 DOUBLE-MASKED, PLACEBO-CONTROLLED STUDY TO ASSESS THE SAFETY AND EFFICACY OF SUBCUTANEOUSLY ADMINISTERED AKB-9778 15MG ONCE DAILY OR 15MG TWICE DAILY FOR 12 MONTHS IN PATIENTS WITH MODERATE TO SEVERE NON-PROLIFERATIVE DIABETIC RETINOPATHY

Prepared for:

Aerpio Therapeutics, Inc.

Version and Author details:

Version Date:	28 February 2019
Version Number	1.0
Author:	Jatinder Singh

SIGNATURES

The undersigned have approved this Statistical Analysis Plan for use in this study.

Jatinder Singh, Biostatistician, Trial Runners, LLC	Date
Steve Pakola, Chief Medical Officer, Aerpio Therapeutics, Inc.	Date
Mitchell Brigell, Vice-President of Clinical Development, Aerpio Therapeutics, Inc.	Date

Electroretinograms were obtained in study AKB-9778-CI-5001 as an exploratory efficacy sub-study. Light-adapted flicker responses and pupillary responses were obtained to 3 stimulus strengths, from the study and fellow eyes, at pretreatment Baseline, Week 12, Week 24, Week 36, and Week 48/EOT visits, using the LKC RETeval system. Data acquisition and transmission protocols were specified in a study manual. All responses were uploaded to the Eyekor Excelsior platform. Responses were graded by LKC Technologies (Gaithersburg, MD). Parameters graded are shown in Table 1. Outcome measures of primary interest are LKC Composite score, age adjusted pupil area ratio, age adjusted fundamental implicit time for stimulus strengths 4, 16 and 32 Troland-seconds (Td's), age adjusted Fundamental amplitude for stimulus strengths 4, 16 and 32 Td's. Given the multitude of variables, inferential statistics will not be performed on non-age adjusted parameters. Non-Age adjusted parameters will be summarized by descriptive statistics only.

Sub-study ERG endpoints of interest will be analyzed using an ANCOVA model without adjustment for multiplicity, and all inferential analyses will use a 2-sided 5% significance level. Whenever possible, a mixed model repeated measures (MMRM) model will be utilized to analyze this data. The model will include change from baseline as the dependent variable. The p-value and 95% CI from the model is baseline parameter adjusted. For MMRM, visit will be treated as the repeated variable within a subject. Within-subject error, an unstructured variance-covariance will be applied; in case of non-convergence, compound symmetry will be used.

Categorical variables will be tested using a Fisher's Exact Test with a 2-sided 5% significance level. AKB-9778 15mg twice daily will be tested first. If this is found to be statistically significant, then AKB- 9778 15mg once daily will be tested for statistical significance, at the same significance level. Cochran-Mantel-Haenszel (CMH) test stratified for baseline values will also be used for testing. Missing data will be imputed using Last Observation Carried Forward (LOCF); baseline values will not be carried forward.

The relation between change in DRSS from baseline to Week 48/EOT visit and baseline Pupil Area Ratio, LKC Composite Score, and baseline Fundamental Implicit Time and Fundamental Amplitude for each stimulus strength will also be calculated.

Table: ERG Parameters. Those of primary interest are noted in red.

Parameter	Picklist or Numeric Value	Field Description
Derived measures		
Pupil Area Ratio Value Not Age Adjusted	Number (populated by .json import)	Ratio of the pupil area between the 4 Td-s stimulus and the 32 Td-s stimulus. Unit: none
Pupil Area Ratio Value Age Adjusted	Number (populated by .json import)	Ratio of the pupil area between the 4 Td-s stimulus and the 32 Td-s stimulus. Unit: none
LKC Composite score	Number (populated by .json import)	Combined measure of vision-threatening diabetic retinopathy. Scores above 20 have increased risk of VTDR. Unit: none
Stimulus Strength 32		
32 Fundamental Implicit Time Value Not Age Adjusted	Number (populated by .json import)	Time from flash to peak of best-fitting sine wave (fundamental). Unit: ms. Stimulus: 32 Td-s flicker with no background
32 Fundamental Implicit Time Value Age Adjusted	Number (populated by .json import)	Time from flash to peak of best-fitting sine wave (fundamental). Unit: ms. Stimulus: 32 Td-s flicker with no background
32 Fundamental Amplitude Value Not Age Adjusted	Number (populated by .json import)	Peak to peak amplitude of the best-fitting sine wave (fundamental). Unit: µV. Stimulus: 32 Td-s flicker with no background
32 Fundamental Amplitude Value Age Adjusted	Number (populated by .json import)	Peak to peak amplitude of the best-fitting sine wave (fundamental). Unit: µV. Stimulus: 32 Td-s flicker with no background
32 Harmonic Implicit Time Value Not Age Adjusted	Number (populated by .json import)	Time from flash to peak of waveform. Unit: ms. Stimulus: 32 Td-s flicker with no background
32 Harmonic Implicit Time Value Age Adjusted	Number (populated by .json import)	Time from flash to peak of waveform. Unit: ms. Stimulus: 32 Td-s flicker with no background
32 Harmonic Amplitude Value Not Age Adjusted	Number (populated by .json import)	Peak to peak amplitude of the waveform. Unit: µV. Stimulus: 32 Td-s flicker with no background

Parameter	Picklist or Numeric Value	Field Description
32 Harmonic Amplitude Value Age Adjusted	Number (populated by .json import)	Peak to peak amplitude of the waveform. Unit: µV. Stimulus: 32 Td-s flicker with no background
32 Pupil Diameter	Number (populated by .json import)	Steady-state pupil diameter for the 32 Td-s stimulus. Unit: mm
Stimulus Strength: 16		
16 Fundamental Implicit Time Value Not Age Adjusted	Number (populated by .json import)	Time from flash to peak of best-fitting sine wave (fundamental). Unit: ms. Stimulus: 16 Td-s flicker with no background
16 Fundamental Implicit Time Value Age Adjusted	Number (populated by .json import)	Time from flash to peak of best-fitting sine wave (fundamental). Unit: ms. Stimulus: 16 Td-s flicker with no background
16 Fundamental Amplitude Value Not Age Adjusted	Number (populated by .json import)	Peak to peak amplitude of the best-fitting sine wave (fundamental). Unit: µV. Stimulus: 16 Td-s flicker with no background
16 Fundamental Amplitude Value Age Adjusted	Number (populated by .json import)	Peak to peak amplitude of the best-fitting sine wave (fundamental). Unit: µV. Stimulus: 16 Td-s flicker with no background
16 Harmonic Implicit Time Value Not Age Adjusted	Number (populated by .json import)	Time from flash to peak of waveform. Unit: ms. Stimulus: 16 Td-s flicker with no background
16 Harmonic Implicit Time Value Age Adjusted	Number (populated by .json import)	Time from flash to peak of waveform. Unit: ms. Stimulus: 16 Td-s flicker with no background
16 Harmonic Amplitude Value Not Age Adjusted	Number (populated by .json import)	Peak to peak amplitude of the waveform. Unit: µV. Stimulus: 16 Td-s flicker with no background

Parameter	Picklist or Numeric Value	Field Description
16 Harmonic Amplitude Value Age Adjusted	Number (populated by .json import)	Peak to peak amplitude of the waveform. Unit: µV. Stimulus: 16 Td-s flicker with no background
16 Pupil Diameter	Number (populated by .json import)	Steady-state pupil diameter for the 16 Td-s stimulus. Unit: mm
Stimulus strength: 4		
4 Fundamental Implicit Time Value Not Age Adjusted	Number (populated by .json import)	Time from flash to peak of best-fitting sine wave (fundamental). Unit: ms. Stimulus: 4 Td-s flicker with no background
4 Fundamental Implicit Time Value Age Adjusted	Number (populated by .json import)	Time from flash to peak of best-fitting sine wave (fundamental). Unit: ms. Stimulus: 4 Td-s flicker with no background
4 Fundamental Amplitude Value Not Age Adjusted	Number (populated by .json import)	Peak to peak amplitude of the best-fitting sine wave (fundamental). Unit: µV. Stimulus: 4 Td-s flicker with no background
4 Fundamental Amplitude Value Age Adjusted	Number (populated by .json import)	Peak to peak amplitude of the best-fitting sine wave (fundamental). Unit: µV. Stimulus: 4 Td-s flicker with no background
4 Harmonic Implicit Time Value Not Age Adjusted	Number (populated by .json import)	Time from flash to peak of waveform. Unit: ms. Stimulus: 4 Td-s flicker with no background
4 Harmonic Implicit Time Value Age Adjusted	Number (populated by .json import)	Time from flash to peak of waveform. Unit: ms. Stimulus: 4 Td-s flicker with no background
4 Harmonic Amplitude Value Not Age Adjusted	Number (populated by .json import)	Peak to peak amplitude of the waveform. Unit: µV. Stimulus: 4 Td-s flicker with no background

Parameter	Picklist or Numeric Value	Field Description
4 Harmonic Amplitude Value Age Adjusted	Number (populated by .json import)	Peak to peak amplitude of the waveform. Unit: µV. Stimulus: 4 Td-s flicker with no background
4 Pupil Diameter	Number (populated by .json import)	Steady-state pupil diameter for the 4 Td-s stimulus. Unit: mm

Statistical Analysis Plan

Ultra-Wide Field Color Photograph Efficacy Sub-study

Study AKB-9778-CI-5001

PHASE 2 DOUBLE-MASKED, PLACEBO-CONTROLLED STUDY TO ASSESS THE SAFETY AND EFFICACY OF SUBCUTANEOUSLY ADMINISTERED AKB-9778 15MG ONCE DAILY OR 15MG TWICE DAILY FOR 12 MONTHS IN PATIENTS WITH MODERATE TO SEVERE NON-PROLIFERATIVE DIABETIC RETINOPATHY

Prepared for:

Aerpio Therapeutics, Inc.

Version and Author details:

Version Date:	28 February 2019
Version Number	1.0
Author:	Jatinder Singh

SIGNATURES

The undersigned have approved this Statistical Analysis Plan for use in this study.

Jatinder Singh, Biostatistician, Trial Runners, LLC	Date
Steve Pakola, Chief Medical Officer, Aerpio Therapeutics, Inc.	Date
Mitchell Brigell, Vice-President of Clinical Development, Aerpio Therapeutics, Inc.	Date

Ultra-wide field Color Photographs were obtained in study AKB-9778-CI-5001 as an exploratory sub-study. Images were obtained from both eyes, at pretreatment Baseline, Week 24, and Week 48/EOT visits, using either an Optos 200Tx or Optos California system. Data acquisition and transmission protocols were specified in a study manual. All images were uploaded to the Eyekor Excelsior platform. Images were graded by the Boston Image Reading Center using their custom software. Grading will follow ETDRS DRSS criteria, but the analysis area will include the entire ultra-wide field rather than be limited to the central 7-fields. DRSS grades ranging from 10-85 will be converted to an ordinal score ranging from 1-11. Monocular scores will be converted to Person and Binocular scores in subjects with qualified fellow eyes as is specified for the primary DRSS outcome.

The primary endpoint of this substudy is the percentage of subjects with an improvement in study eye severity of DR (ETDRS DR Severity Score or DRSS) of ≥ 2 steps at 48 weeks without need for treatment of PDR or DME (by IVT, laser, or vitrectomy) in the study eye.

The primary hypotheses will be tested in the MITT population using a Fisher's Exact Test with a 2-sided 5% significance level. AKB-9778 15mg twice daily will be tested first. If this is found to be statistically significant, then AKB- 9778 15mg once daily will be tested for statistical significance, at the same significance level. Cochran-Mantel-Haenszel (CMH) test stratified for baseline ETDRS DRSS (levels 4, 5 and 6) will also be used for testing primary hypothesis in the MITT population. Missing data will be imputed using Last Observation Carried Forward (LOCF); baseline values will not be carried forward. For rescued eyes the last observation pre-rescue will be carried forward.

As a sensitivity analysis, Fisher's Exact test and CMH test stratified for baseline ETDRS DRSS (levels 4, 5 and 6) on Observed Cases (OC carrying forward pre-rescue values) in the Per Protocol (PP) population will also be performed. Secondary analyses will be performed on study eyes and all qualified eyes on derived DRSS Person and Binocular scales.

Statistical Analysis Plan

Ultra-wide Field Fluorescein Angiography Efficacy Sub-study

Study AKB-9778-CI-5001

PHASE 2 DOUBLE-MASKED, PLACEBO-CONTROLLED STUDY TO ASSESS THE SAFETY AND EFFICACY OF SUBCUTANEOUSLY ADMINISTERED AKB-9778 15MG ONCE DAILY OR 15MG TWICE DAILY FOR 12 MONTHS IN PATIENTS WITH MODERATE TO SEVERE NON-PROLIFERATIVE DIABETIC RETINOPATHY

Prepared for:

Aerpio Therapeutics, Inc.

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SIGNATURES

The undersigned have approved this Statistical Analysis Plan for use in this study.

Jatinder Singh, Biostatistician, Trial Runners, LLC	Date
Steve Pakola, Chief Medical Officer, Aerpio Therapeutics, Inc.	Date
Mitchell Brigell, Vice-President of Clinical Development, Aerpio Therapeutics, Inc.	Date

Ultra-wide field fluorescein angiography was obtain6ed in study AKB-9778-CI-5001 as an exploratory sub-study. Time-series Images were obtained from the study eye, at pretreatment Baseline, Week 24, and Week 48/EOT visits, using either an Optos 200Tx or Optos California system. Data acquisition and transmission protocols were specified in a study manual. All images were uploaded to the Eyekor Excelsior platform. Images were graded by the Campane Center for Advanced Imaging at the Cole Eye Institute (CCAI) using their custom software. Parameters graded are shown in Table 1. Outcome variables of primary interest are Ischemic index in macula centered ROI 1 (Region of Interest 1) and ROI 2, Leakage index in macula centered ROI 1 and ROI 2, and Microaneurysm counts in macula centered ROI 1 and ROI 2.

Continuous sub-study endpoints of interest will be analyzed using an ANCOVA model, and all inferential analyses will use a 2-sided 5% significance level. Whenever possible, a mixed model repeated measures (MMRM) model will be utilized to analyze this data. The model will include change from baseline as the dependent variable. The p-value and 95% CI from the model is baseline parameter adjusted. For MMRM, visit will be treated as the repeated variable within a subject. Within-subject error, an unstructured variance-covariance will be applied; in case of non-convergence, compound symmetry will be used.

Categorical variables will be tested using a Fisher's Exact Test with a 2-sided 5% significance level. Cochran-Mantel-Haenszel (CMH) test stratified for baseline values will also be used for testing. Missing data will be imputed using Last Observation Carried Forward (LOCF); baseline values will not be carried forward.

The relation between change in DRSS from baseline to Week 48/EOT visit and baseline Ischemia %, baseline Total Leakage Index, and total Aneurysms will also be calculated.

Table: UWF-FA Parameters. Those of primary interest are noted in red.

Parameter	Unit
Ischemia %	%, 2 decimals
Ischemia Disc Centered ROI 1 Ischemia Index	%, 2 decimals
Ischemia Disc Centered ROI 2 Ischemia Index	%, 2 decimals
Ischemia Disc Centered ROI 3 Ischemia Index	%, 2 decimals
Ischemia Disc Centered ROI 1+2 Ischemia Index (Derived)	%, 2 decimals
Ischemia Macula Centered ROI 1 Ischemia Index	%, 2 decimals
Ischemia Macula Centered ROI 2 Ischemia Index	%, 2 decimals
Ischemia Macula Centered ROI 3 Ischemia Index	%, 2 decimals
Ischemia Macula Centered ROI 1+2 Ischemia Index (Derived)	%, 2 decimals
Leakage Total Leakage Index	%, 2 decimals
Leakage Disc Centered ROI 1 Leakage Index	%, 2 decimals
Leakage Disc Centered ROI 2 Leakage Index	%, 2 decimals
Leakage Disc Centered ROI 3 Leakage Index	%, 2 decimals
Leakage Disc Centered ROI 1+2 Leakage Index (Derived)	%, 2 decimals
Leakage Macula Centered ROI 1 Leakage Index	%, 2 decimals
Leakage Macula Centered ROI 2 Leakage Index	%, 2 decimals
Leakage Macula Centered ROI 3 Leakage Index	%, 2 decimals
Leakage Macula Centered ROI 1+2 Leakage Index (Derived)	%, 2 decimals
Total Aneurysms	Number
MA Disc Centered ROI I	Number
MA Disc Centered ROI 2	Number
MA Disc Centered ROI 3	Number
MA Disc Centered Aneurysms ROI 1 + ROI 2 (Derived)	Number
MA Macula Centered ROI 1	Number
MA Macula Centered ROI 2	Number
MA Macula Centered ROI 3	Number
MA Macula Centered Aneurysms ROI 1 + ROI 2 (Derived)	Number