NOVARTIS

Clinical Development

RFB002/Ranibizumab/Lucentis®

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A randomized, single-blinded, multicenter, phase IV study to compare systemic VEGF protein dynamics following monthly intravitreal injections of 0.5 mg ranibizumab versus 2 mg aflibercept until study week 12 in patients with neovascular (wet) age-related macular degeneration (TIDE AMD)

Statistical Analysis Plan (SAP)

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List of abbreviations

AE	Adverse event
ANCOVA	Analyses of covariance
AMD	Age-related macular degeneration
AUC	Area under the curve
███████	██████████████████████████
BLQ	Below the limit of quantification
BMI	Body mass index
CI	Confident interval
CNV	Choroidal neovascularization
CSF	Case Report Form
CSR	Clinical study report
███	██████████████████
eCRF	electronic Case Report/Record Form
EOS	End of study
EOT	End of treatment
████	███████████████████████
FA	Fluorescein angiography
FAS	Full analysis set
FCP	Foveal center point
FP	Color fundus photography
gCV	Geometric coefficient
gMean	Geometric mean
HbA1c	Glycosylated hemoglobin
IA	Interim analysis
IOP	Intraocular pressure
LS	Least squares
MedDRA	Medical Dictionary for Drug Regulatory Affairs
███	███████████████████
PD	Protocol deviation
███	████████████
PK	Pharmacokinetics
██	██████████████
PRO	Patient-reported outcome
PPS	Per-protocol set
PT	Preferred term
RPE	Retinal pigment epithelium
RS	Randomized set
SAEs	Serious adverse events
SAP	Statistical analysis plan
SD	Standard deviation
████	█████████████
SE	Standard error

SI	Standard international
SOC	System organ class
SRF	Subretinal Hyporeflective Space
TEAE	Treatment emergent AE
VEGF	Vascular endothelial growth factor
wAMD	Neovascular (wet) age-related macular degeneration
WHO	World health organization

1   Introduction

This statistical analysis plan (SAP) describes the statistical analysis according to Section 10 of the study protocol (version no. 2 including Amendment 2) along with any additional analyses, specifications or deviations from this protocol planned before unmasking of the data. ███████

██ Determination of sample size is specified in Section 3.

1.1   Study design

This is a randomized, single-blinded, multicenter, phase IV study to compare systemic vascular endothelial growth factor (VEGF)-A protein levels following monthly intravitreal injections of 0.5 mg ranibizumab versus monthly intravitreal injections of 2 mg aflibercept until study week 12 in patients with active, newly diagnosed, untreated neovascular (wet) age-related macular degeneration (AMD).

From week 12 (visit 10) on all patients will receive monthly intravitreal injections of 0.5 mg ranibizumab until study week 20 (visit 14) in order to compare systemic VEGF-A protein levels from study week 12 to week 24 (visit 16) in patients switching from aflibercept to ranibizumab injections with patients treated with ranibizumab only.

Patients with wAMD will be randomized to one of the following treatment groups:

- Treatment group 1 (n=20): Monthly intravitreal injections of 0.5 mg ranibizumab for six months
- Treatment group 2 (n=20): Monthly intravitreal injections of 2 mg aflibercept for the initial three months followed by monthly intravitreal injections of 0.5 mg ranibizumab for the next three months.

A blinded central laboratory will evaluate systemic VEGF-A plasma levels.

The study requires 16 study visits during 24 weeks and the primary endpoint will assess data from baseline to study week 12 (visit 10).

Figure 1-1   Study outline
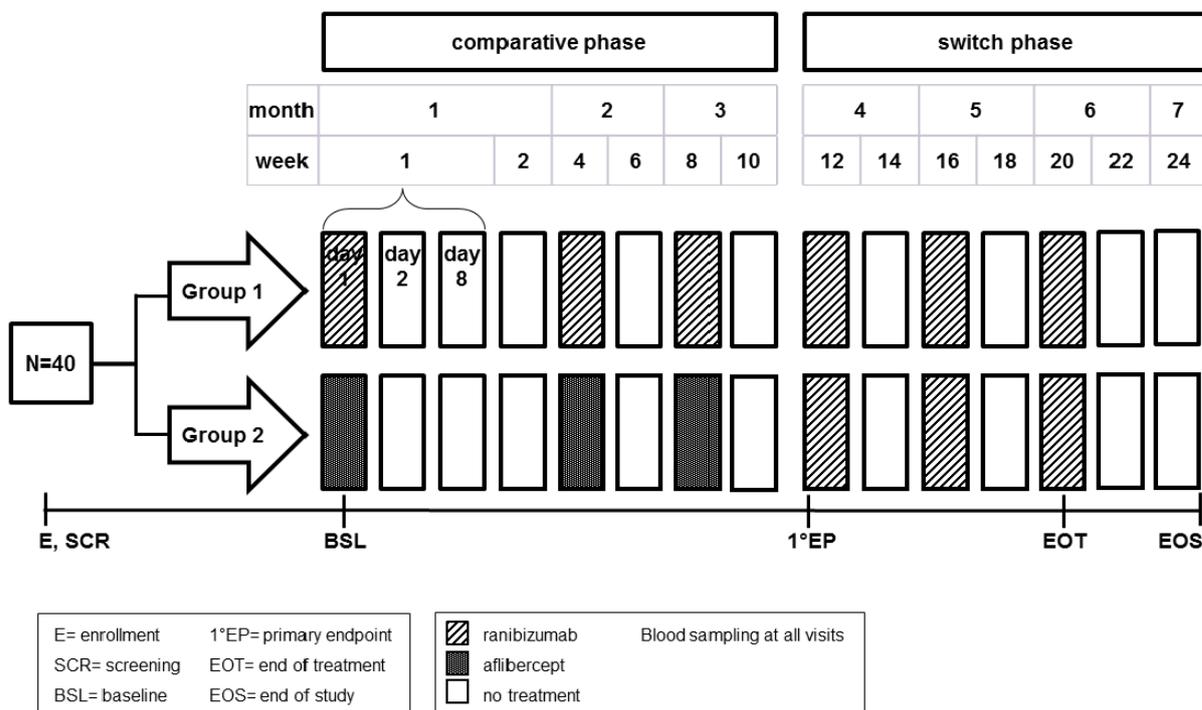
1.2   Study objectives and endpoints

1.2.1   Primary objective

The primary object of this study is as follows:

1. To compare systemic VEGF-A protein levels (based on the SIMOA assay method) following monthly intravitreal injections of 0.5 mg ranibizumab for three months versus monthly 2 mg aflibercept injections for three months as measured by the area under the curve (AUC) from baseline to week 12 (visit 10).

1.2.2   Secondary objective(s)

The secondary objectives of this study are as follows:

1. To compare systemic VEGF-A protein levels (based on the SIMOA assay method) in patients switching from monthly 2 mg aflibercept injections for first three months to monthly 0.5 mg ranibizumab injections for the next three months compared to patients treated with monthly 0.5 mg ranibizumab for six months at specific time points as measured by the AUC from week 12 (visit 10) to week 24 (visit 16).

2. To explore whether systemic VEGF-A levels (based on the SIMOA assay method) of patients switching from monthly 2 mg aflibercept injections for first three months to monthly 0.5 mg ranibizumab injections for the next three months will adjust to levels comparable to baseline or to levels comparable as in patients treated with monthly 0.5 mg ranibizumab for six months at specific time points from week 12 (visit 10), over time up to week 24 (visit 16).
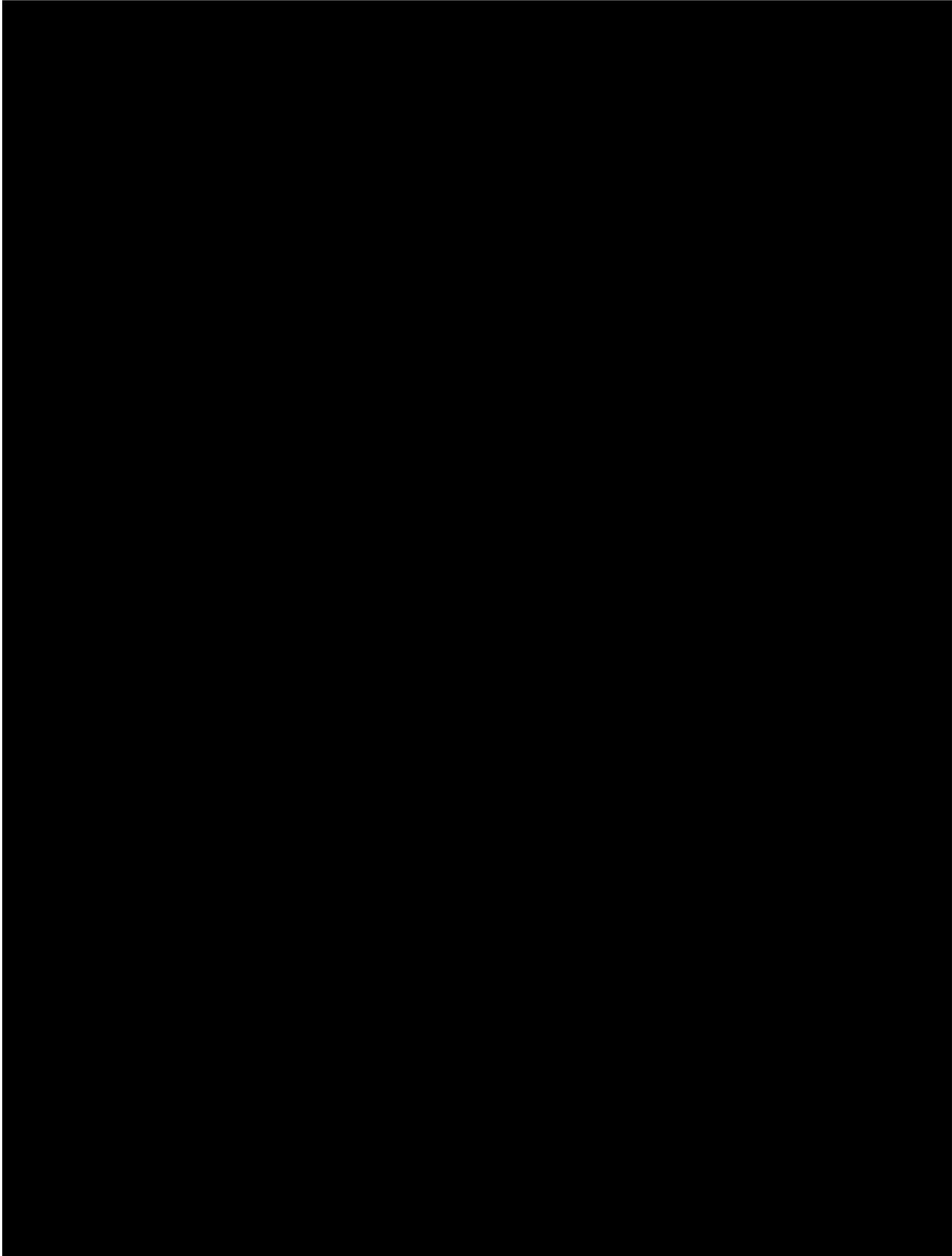
2 Statistical methods

2.1 Data analysis general information

The analysis will be performed by ████████████████████████████. SAS®
statistical software (Version 9.4 or a more recent version) will be used for analysis.

Data will be summarized with respect to demographic and baseline characteristics, efficacy observations and measurements, safety observations and measurements, and pharmacodynamic measurements.

Descriptive statistics (the number of non-missing observations, mean, median, standard deviation [SD], minimum and maximum values) will be presented for continuous variables. The following number of decimal places will be used: mean and median values to 1 more decimal place than the raw data; minimum and maximum to the same number of decimal places as the raw data and SD to 2 more decimal places than the raw data. If required (e.g. for AUC), the geometric mean (gMean) will be derived by calculating the mean of the natural log transformed individual values. This value will then be back transformed to give the geometric mean.

The study eye is the eye selected by the investigator at baseline (according to the protocol) to receive the study treatment. The fellow eye is the non-study eye.

Assessments documented in the database as occurring in "both eyes" will be summarized for each eye separately.

Medications, Significant Non-drug Therapies and Adverse Events (AEs) will be defined as ocular or non-ocular according to the investigator's response to 'ophthalmic relevance' on the relevant electronic Case Report/Record Form (eCRF) page.

The safety observation period for the exposure to treatment will be presented separately for:

1. Comparative phase: From baseline (Day 1) to the last visit the patient attended up to and including the week 12 visit (note: although the date of the week 12 visit is used for derivation of period when injections are summarized the week 12 injection is not part of comparative phase)

2. Switch phase: From week 12 to the last visit the patient attended up to and including the week 24 visit (week 24).

3. Overall treatment period: From baseline to the last visit the patient attended up to and including the week 24 visit.

Safety observation period (days) is defined as (date of study completion – date of first administration of treatment) + 1.

Change from baseline will only be summarized for patients with both baseline and post-baseline values at the relevant visit and will be calculated as:

Change from baseline = post-baseline value – baseline value.

All data will be listed by patient, unless stated otherwise.

2.1.1 General definitions

This study will consist of the following epochs (refer to Figure 1-1):

- a screening period,
- a treatment period (comparative and switch phases).

The treatment comparative phase is from Day 1 (visit 2) up to week 12 (visit 10). Treatment switch phase is from week 12 (visit 10) up to week 24 (visit 16).

Study treatment refers to:

- Ranibizumab (Lucentis®)
  - The investigational treatment in treatment group 1 from week one (visit 2) to week 24 (visit 16) and for treatment group 2 from week 12 (visit 10) to week 24 (visit 16) is 0.5 mg ranibizumab (Lucentis®).
- Aflibercept (Eylea®)
  - Investigational treatment in treatment group 2 from week one (visit 2) to week 8 (visit 8) is 2 mg aflibercept (Eylea®).

Baseline is the date of first administration of study treatment in the study eye. Baseline value will be considered as the value of the last non-missing assessment collected prior to start of treatment (i.e. data from screening or baseline). If a patient is randomized but not treated then the baseline value for a variable is the last available non-missing value collected prior to randomization.

Some baseline assessments may be recorded on the day of the baseline visit (visit 2). However the time of each of these assessments may not be recorded in the eCRF. In this case, only the assessments which, according to the protocol, should have been conducted pre-dose will be assumed to have been done before the first study treatment in the study eye when deriving baseline values recorded on the day of the baseline visit (visit 2).

All data collected after Day 1 are defined as post-baseline.

2.1.2 End of study visit

Analyses presented by visit will use the visits and visit numbers as recorded in the database. No visit windows will be defined except for the End of Study (EOS) visit (see below). EOS visits will be denoted by visit number 777 in the database.

The study day for the EOS visit will be allocated to the nearest planned visit.

Study day will be calculated relative to Day 1:

Study day = (Date of visit) – (Date of baseline visit) + 1.

Table 2-1   Allocation of end of study visit

Visit number	Analysis Visit	Scheduled visit day	Visit window (study days)
1	Screening	<1	-14 - (-1)
2	Baseline/Day 1	1	1
3	Day 2	2	2 - 4

Visit number	Analysis Visit	Scheduled visit day	Visit window (study days)
4	Week 1	8	5 - 11
5	Week 2	15	12 - 21
6	Week 4	29	22 - 35
7	Week 6	43	36 - 49
8	Week 8	57	50 - 63
9	Week 10	71	64 - 77
10	Week 12	85	78 - 91
11	Week 14	99	92 - 105
12	Week 16	113	106 - 119
13	Week 18	127	120 - 133
14	Week 20	141	134 - 147
15	Week 22	155	148 - 161
16	Week 24	169	≥162

If data for the nearest planned visit already exist then the EOS visit will be assigned to the next visit.

For example, consider a patient with planned visits at study days -7, 1, 2, 8, 15 and 29. If this patient's EOS visit were at day 57 then the allocated scheduled visit would be 8. If however the EOS visit were at day 40 then the allocated scheduled visit would be 7.

2.1.3 Unscheduled visits

All data collected at unscheduled visits will be listed.

Exposure and AEs data collected at unscheduled visits will be summarized. No other data from unscheduled visits will be summarized.

2.2 Analysis sets

The **Randomized Set (RS)** will consist of all patients who were randomized to one of the treatment arms.

The **Full Analysis Set (FAS)** will consist of all patients as randomized who receive at least one application of study treatment and have at least one post-baseline assessment of the primary efficacy variable (plasma VEGF-A concentration). Following the intent-to-treat principle, patients will be analyzed according to the treatment they were assigned to at randomization. No data will be excluded from the FAS analyses because of protocol deviations.

The **Safety Set** will consist of all patients that received at least one application of study treatment and had at least one post-baseline safety assessment. Patients will be analyzed according to treatment received. Of note, the statement that a patient had no adverse events also constitutes a safety assessment.

The **Per-Protocol Set (PPS)** will consist of all patients in FAS who did not show major deviations from the protocol procedures that might have an impact on the study outcome.

Criteria that are assumed to have such an impact will be defined in the data validation document (VAP) and in the Blind Review Protocol before unblinding.

The primary analysis will be performed for both, the FAS and the PPS. The PPS is regarded as primary. FAS will be interpreted in terms of supportive analysis.

Rule of exclusion criteria of analysis sets see Appendix 5.4.

Table 2-2   **Analysis data sets for specific outputs**

	All screened patients	RS	FAS	PPS	Safety Set
Screening failures	x				
Demographics and baseline characteristics			x	x	
Analysis sets		x			
Patient disposition	x	x			
Protocol deviations		x			
Medical history		x			
Concomitant medication					x
Primary endpoint			x	x	
Secondary endpoints			x	x	
████████████					
Safety analyses					x
Exposure					x
Subgroup analyses				x	
Sensitivity analyses			x		

The number of patients included in each analysis set will be presented.

A listing will be produced to show the patient inclusion/exclusion into each of the analysis sets with the corresponding reason(s) for exclusion.

The number of patients with clinical study report (CSR) reportable protocol deviations (PDs) as defined in the data review plan will be summarized by category and PD. Patients with multiple occurrence of a PD will be presented once for that PD and patients with multiple occurrences of PDs within a category will be only counted once per category.

The PD categories are as follows:
- Patient did not satisfy inclusion criteria
- Patient met exclusion criteria
- Patient received the wrong treatment or incorrect dose
- Patient met compliance violation (unblinding)
- Patient participated in treatment relevant visits out of schedule
- Patient had missing values in the primary variable
- Patient developed withdrawal criteria during the study, but not withdrawn
- Patient took an excluded concomitant medication

● Others.

Patients removed from the RS will not appear in the summary of PDs. These patients will be documented in the output summarizing the patients excluded from analysis sets.

Criteria defining PDs is outlined in the Appendix 5.4.

2.2.1 Subgroup of interest

Descriptive subgroup analyses will be prepared to explore gender differences for the primary endpoint.

Patients with missing baseline values used to define the subgroups will be presented in a category "unknown" in the respective subgroup analyses.

2.3 Patient disposition, demographics and other baseline characteristics

Patient disposition will be presented for RS and all the demographics and baseline characteristics will be presented for the FAS and PPS by treatment group and overall.

2.3.1 Patient disposition

The number and percentage of patients who are screen failures and reasons for screen failure will be presented for all patients screened.

The number and percentage of patients who completed and discontinued the study (including primary reason for study discontinuation) will be summarized for the RS by treatment and overall.

All patient disposition data will be listed by treatment, center and patient number for the RS.

2.3.2 Background and demographic characteristics

Demographics and baseline data collected and documented at screening (visit 1) and/or baseline (visit 2) will be summarized for the FAS and PPS by treatment group and overall.

The time from a pre-screening event (e.g. diagnosis of a condition or taking a medication) in days will be calculated in relation to date of informed consent.

Descriptive statistics will be provided for the below demographics and baseline characteristics:

Demographic variables and vital signs

Categorical variables:
- Sex (Male, Female)
- Predominant race (Caucasian, Black, Asian, Other)
- Age group (<65, ≥65 years)
- Smoking status (Never smoked, Current smoker, Ex-smoker)
- Pregnancy testing for women of child-bearing potential (Negative, Positive, Not applicable)

Continuous variables:

- Age (years)
- Vital signs: Height (cm), Weight (kg), Pulse (bpm), Systolic sitting blood pressure (mmHg), Diastolic sitting blood pressure (mmHg)
- Body mass index (BMI) [kg/m²]
- Estimated number of cigarette pack years

Baseline ocular characteristics for study eye

Categorical variables:

- Assessed by the site:
  - Study eye (left, right)
  - AMD baseline characteristics:
    - Presence of active CNV lesion due to neovascular AMD (Yes, No)
    - Type of CNV lesion (Predominantly classic, Minimally classic, Occult, Not evaluable)
    - Location of CNV lesion (Subfoveal, Juxtafoveal, Extrafoveal, Not evaluable)
- Assessed by central reading center:
  - OCT variable:
    - Presence of Macular Edema (Yes, No, Questionable, Cannot grade) (study eye)
      - If Yes or Questionable: presence of Cystoid spaces (Yes, No, Questionable, Cannot grade)
    - Presence of Subretinal Hyporeflective Space (SRF) (Yes, No, Questionable, Cannot grade)
    - Presence of Epiretinal Membrane (Yes, No, Questionable, Cannot grade)
    - Presence of Vitreomacular Traction (Yes, No, Questionable, Cannot grade)
  - Color fundus photography (FP) variable:
    - Presence of Intra-/subretinal or sub-Retinal pigment epithelium (RPE) Hemorrhage (Yes, No, Questionable, Cannot grade)
      - If Yes or Questionable: is it subfoveal (Yes, No, Questionable, Cannot grade)
  - Fluorescein Angiography (FA) variable:
    - Presence of CNV (Yes, No, Questionable, Cannot grade)
      - If Yes or Questionable: presence of active CNV leakage (Yes, No, Questionable, Cannot grade)
    - Type of CNV lesion (Predominantly classic, Minimally classic, Occult, Cannot grade)
    - Location of CNV lesion (Subfoveal, Juxtafoveal, Extrafoveal, Cannot grade)
  - General reading center:
    - Presence of other pathology (Yes, No, Questionable, Cannot grade)
      - If Yes or Questionable: other pathologies
    - Patients eligibility (Yes, No, Questionable, Cannot grade)
      - If No or Questionable: the reasons

Continuous variables:

- Assessed by site:
  - ████████████████████
  - Intraocular pressure (IOP) (mmHg)
  - AMD baseline characteristics:
    - Time since diagnosis of wAMD (weeks)
- ██
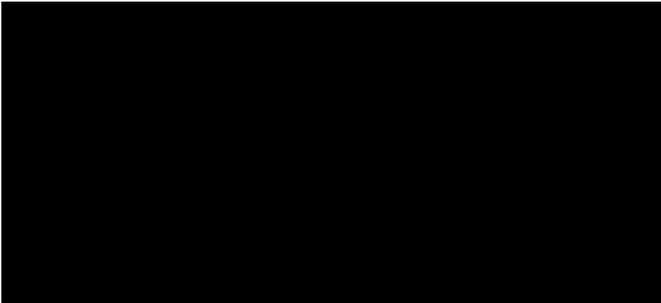
All demographic and baseline data will be listed by treatment, center and patient number for the FAS.

2.3.3 Medical history

The number and percentage of patients from the RS with relevant medical history and current medical conditions will be tabulated separately by system organ class (SOC) and preferred term (PT) from the current Medical Dictionary for Regulatory Activities (MedDRA). The SOCs will be presented in alphabetical order. PTs will be ordered for each treatment in decreasing proportion in the ranibizumab treatment group of interest (treatment group 1).

Separate tables will be provided for ocular and non-ocular histories and conditions. Ocular histories and conditions will also be presented separately for study and fellow eye.

Furthermore, medical history data will be listed by treatment, center, and patient number for the RS.

2.4 Treatments (study treatment, concomitant therapies, compliance)

Unless otherwise stated, all analyses will be based on patients in the Safety Set.

2.4.1 Study treatment / compliance

All treatment exposure will be presented for the study eye. It is not planned for the fellow eye to receive study treatment.

Exposure is defined as the number of ranibizumab and/or aflibercept injections received for the safety observation period of interest.

Descriptive statistics will be provided for exposure to study treatment for each safety observation epoch (see Section 2.1) using the Safety Set. The number of ranibizumab and aflibercept injections will be presented by treatment arm in frequency tables by visit and cumulatively.

2.4.2 Prior and concomitant therapies

The number and percentage of patients taking concomitant therapies will be summarized by PT according to the World Health Organization (WHO) Drug Reference List dictionary. Summaries of the following will be presented:

- Prior medications will be defined as drugs taken and stopped prior to baseline
- Any medication given at least once between baseline and the last day of study visit will be a concomitant medication, including those which were started pre-baseline and continued into the treatment period.

For both, prior and concomitant therapies, summary tables will be presented for ocular therapies (separated for study eye and fellow eye) and non-ocular therapies.

For handling of missing or incomplete start and end dates, see Section 5.1.2 of this document.

By-patient listings for prior and concomitant therapies will be provided.

2.5 Analysis of the primary objective

Only the study eye will be evaluated for efficacy.

2.5.1 Primary endpoint

The primary variable is the AUC of systemic VEGF-A protein concentration in plasma (based on the SIMOA assay method) following monthly injections of ranibizumab or aflibercept monotherapy measured between Day 1 and study week 12.

AUC will be calculated using the trapezoidal rule, where all available measurements between Day 1 and week 12 will be used.

$$\mathbf{AUC_{0-12weeks}} = \sum_{k=2}^{9} \left(\frac{1}{2} \times (V_{k+1} + V_k) \times (d_{k+1} - d_k) \right)$$

Where d_k represents scheduled day of visit k and V_k represents systemic VEGF-A levels at visit k. The AUC will be standardized by dividing the calculated value of the individual follow-up time (the number of days from first to last measurement).

Range calculated as:

$$\mathbf{d_{last} - d_{first}}$$

Where d_{last} and d_{first} represents scheduled day of last and first systemic VEGF-A measurements in the visit range 2 to 10.

The analysis of the primary variable will be performed on the PPS. A further supportive analysis will be performed in the FAS (see Section 2.5.4).

2.5.2 Statistical hypothesis, model, and method of analysis

The null and alternative hypothesis for the primary analysis is as follows:

H_0: $AUC_{Ranibizumab} - AUC_{Aflibercept} = 0$

H_1: $AUC_{Ranibizumab} - AUC_{Aflibercept} \neq 0$

The primary analysis will be performed by an analysis of covariance (ANCOVA) model including study treatment and center as factors and baseline systemic VEGF-A level as a covariate. The analysis will be performed at the two-sided 5% significance level. Least squares (LS) ("raw" and "adjusted") means with corresponding two-sided asymptotic 95% confidence intervals (CIs) and p-values will be calculated as point estimates for the treatment contrasts.

The assumptions of the above ANCOVA model (i.e. errors were independently normally distributed with constant variance) will be evaluated. Homogeneity of variance between/among treatment groups will be evaluated visually. If the assumptions for ANCOVA are not met, a non-parametric ANCOVA analysis will be performed.

Descriptive statistics including the geometric mean and geometric coefficient of determination (gCV) for AUC based on systemic VEGF-A levels from Day 1 up to week 12 (visit 10) will also be presented.

Furthermore, summary statistics will be presented for systemic VEGF-A levels by visit. Absolute values, change from baseline, and percent change from baseline values for systemic VEGF-A levels will be displayed.

Individual VEGF-A level profiles will be listed and also presented using line plots. Line plots will also be produced for the mean change from baseline values.

2.5.3   Handling of missing values/censoring/discontinuations

For the primary endpoint, there will be no imputation of missing data as the AUC of systemic VEGF-A levels will be standardized by dividing by the calculated value of the individual follow-up time (the number of days from first to last measurement). For the primary analysis, missing baseline VEGF-A level covariate values will be imputed by the mean value of non-missing baseline VEGF-A level from all the patients with values.

Patients with no visit 2 to 10 VEGF-A level data will be omitted from the primary endpoint analysis as per the PPS definitions given above (see Section 2.2).

2.5.4   Supportive analyses

To assess for robustness of the results, the same ANCOVA model as specified for the primary analysis will be repeated for the FAS (on observed data).

In addition, the same ANCOVA model will be repeated without VEGF-A level covariate imputation for the PPS.

Furthermore, gender subgroup analysis will be done for the primary analysis (based on PPS).

2.6   Analysis of the key secondary objective

There is no key secondary objective in this study.

2.7 Analysis of secondary objective(s)

2.7.1 Secondary endpoints

The secondary endpoints are:

The AUC of systemic VEGF-A protein levels following monthly injections of ranibizumab monotherapy in both treatment groups measured between week 12 (visit 10) to week 24 (visit 16).

The values of systemic VEGF-A protein levels following monthly injections of ranibizumab monotherapy in both treatment groups at each visit between week 12 (visit 10) and week 24 (visit 16).

The analysis set for these analyses will be the PPS and FAS.

2.7.2 Statistical hypothesis, model, and method of analysis

There will be no hypothesis testing for the secondary endpoints and all analysis is to be interpreted in a purely descriptive manner.

The descriptive analysis will be performed following the same methods as for the primary analysis taking into account the levels measured between week 12 (visit 10) and 24 (visit 24) (see Section 2.5.2).

In addition, summary statistics will be produced for the systemic VEGF-A protein baseline values, absolute values and the (percentage) change from baseline values from week 12 up to week 24 (visit 16) by visit.

The course over time for the absolute values and for changes from baseline will be presented using line plots for the treatment groups for variable, showing the mean and standard error (SE) per visit.

Individual VEGF-A level profiles will be listed and as well presented using line plots.

2.7.3 Handling of missing values/censoring/discontinuations

Handling of missing values in the secondary analysis will follow the similar approach as in the primary (see Section 2.5.3). There will be no imputation of missing data for the AUC of systemic VEGF-A protein levels. For the secondary analysis, missing baseline VEGF-A level covariate values will be imputed by the mean value of non-missing baseline VEGF-A level from all the patients with values. Patients with no visit 10 to 24 VEGF-A level data will be omitted from the analysis.

2.8 Safety analyses

Safety parameters will include AEs, vital signs, laboratory evaluations, ophthalmic examinations and IOP.

All safety analyses will be summarized by treatment using the Safety Set. No missing data will be imputed for safety analyses.

2.8.1 **Adverse events (AEs)**

AEs will be deemed treatment emergent if the onset date is on or after the date of first treatment with investigational drug in the study eye or events present prior to start of study treatment but increased in severity on or after the first administration of study treatment in the study eye.

Only treatment emergent AEs (TEAEs) will be summarized. If any event has an incomplete onset date, this will be handled as described in the Appendix 5.1.1.

AEs will be coded by primary SOC and PT according to the latest version of MedDRA available at time of analysis. Patients who experienced multiple AEs for a PT will be counted once, similarly for patients with multiple AEs per SOC, unless specified otherwise.

The number and percentage of patients with ocular (study eye, fellow eye) and non-ocular AEs will be summarized separately by treatment group for the following categories:

- All AEs
- Serious adverse events (SAEs)
- AEs suspected to be related to the study treatment
- AEs suspected to be related to the ocular injection
- AEs leading to study discontinuation
- AEs by maximum severity (mild, moderate, severe)

For the legal requirements of ClinicalTrials.gov and EudraCT, two required tables on TEAEs which are not serious adverse events with an incidence greater than 5% and on treatment emergent SAEs and SAEs suspected to be related to study treatment will be provided by SOC and PT on the Safety Set.

Furthermore, all AEs (AEs during the screening period, TEAEs, AEs during the post-treatment period) will be listed by treatment, center and patient number for the RS. This will also include details of the AE (e.g., verbatim given by the investigator as well as the primary SOC and PT), onset date, end date, severity, relationship to study treatment, relationship to ocular injection, whether it constitutes a SAE.

The following AE listings will be provided:

- All AEs
- Deaths
- SAEs
- AEs leading to study treatment discontinuation
- AEs suspected to be related to investigational study treatment and/ or ocular injection.

2.8.2 **Deaths**

Deaths (recorded on study completion CRF) will be listed as part of study disposition.

2.8.3   **Laboratory data**

Serum pregnancy testing, pharmacodynamics will be presented using standard international (SI) units. Laboratory data - observed values or changes from baseline values at each visit - will be summarized using descriptive statistics.

A shift table will be produced to summarize the shift from baseline to any laboratory values that are abnormally low or high. The clinical critical values are defined in Appendix 5.3. Baseline critical values are defined by the absolute values only. All data, including data from unscheduled visits, will be considered when identifying abnormal values.

Patient listings will be provided for all laboratory data. Values outside the clinical normal ranges will be flagged.

2.8.4   **Other safety data**

2.8.4.1   **Vital signs**

Vital signs (sitting systolic and diastolic blood pressure, pulse rate, height and weight) will be summarized by descriptive statistics, by visit, presenting absolute and change from baseline values. Change from baseline will only be summarized for patients with both baseline and post-baseline values.

Clinically notable values for certain vital sign measurements are defined in Appendix 5.3. Using these critical values, a shift table will be produced to summarize the shift from baseline to any vital signs values that are abnormally low or high. All data, including data from unscheduled visits, will be considered when identifying abnormal values.

Patient listings will be provided for all vital signs data and values outside the clinical normal ranges will be flagged.

2.8.4.2   **Intraocular Pressure**

IOP measurements (pre-injection and post-injection) will be summarized descriptively, by visit for absolute values and change from baseline (each visit for the study eye; final visit for fellow eye).

Change from baseline will only be summarized for patients with both baseline and post-baseline values.

In addition, the number and percentage of patients with IOP greater than or equal to ($\geq$) 25 mmHg in the study eye will be presented by visit (pre-injection and post-injection).

All IOP data will be listed for all patients and IOP values $\geq$ 25 mmHg will be flagged.

2.8.4.3   **Other safety assessments**

Child bearing potential and serum and urine pregnancy test results (if applicable) will be listed for female patients.

Results from ophthalmic examination and post-injection safety assessments (not including IOP) results will be listed.

2.9   Pharmacokinetic endpoints

There are no pharmacokinetic endpoints in this study.
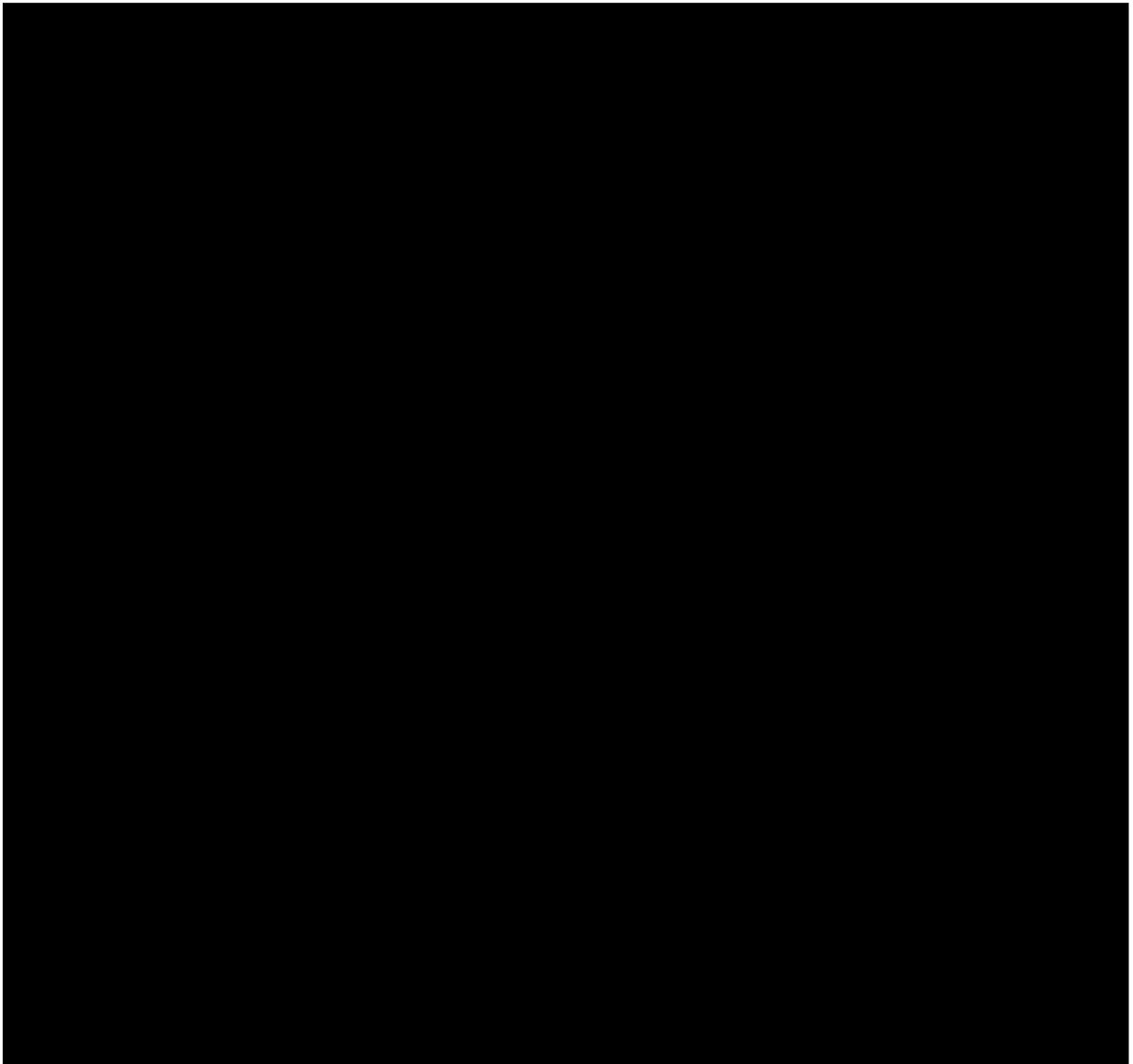
2.10   PD and PK/PD analyses

Refer to Section 2.5 and Section 2.7 for PD analyses (AUC of systemic VEGF-A levels).

There are no PK/PD analyses planned in this study.

2.11   Patient-reported outcomes

There are no patient-reported outcomes (PRO) analyses planned in this study.

2.14   Interim analysis

Interim analysis (IA) was performed in order to verify the effect size assumed in the sample size calculation. Statistical analysis for IA is outlined in the IA SAP.

3   Sample size calculation

In the past, measuring systemic VEGF protein levels was performed differently in the literature available. While some authors used blood plasma (Avery 2013; Carneiro 2012; Zehetner 2013), others used blood serum (Chakravarthy 2012). In addition, individual systemic VEGF levels seem to be very variable, depending e.g. on the state of health, hormone levels or menstrual cycle.

To date, only one study investigated the pharmacodynamic effect of aflibercept on systemic VEGF protein levels (Avery 2013). Given that aflibercept features an Fc part, a similar transport mechanism for aflibercept over the blood-retina-border as for bevacizumab can be hypothesized.

Given that individual systemic VEGF-A levels seem to be variable, a change of ±20% from baseline to study week 12 is considered clinically irrelevant. It is expected that the change of systemic VEGF-A protein levels from baseline to study week 12 following aflibercept treatment is >20%. A difference in change of ≥25% of systemic VEGF-A protein reduction between the two treatment arms from baseline to study week 12 is considered reasonable to show different effects of the two drugs on systemic VEGF-A plasma levels.

While the calculation of the sample size was performed using percentages due to the huge variability of baseline VEGF-A levels in the present data, it is assumed that the equivalent effect size will result from the AUC as endpoint variable.

A large standardized effect size of 1,25 was assumed (which would lead to an estimated common standard deviation of the change in VEGF-A levels of 20%). 15 patients per group are required to achieve 90% power on a 2-sided 5% significance level. To compensate for some dropouts and other protocol violations such as treatment of the fellow eye with anti-VEGF treatments, 20 patients per treatment arm (40 total) should be recruited into this trial.

As only baseline values and standard deviation for systemic VEGF-A levels are variable in all published studies to date, a blinded pooled analysis of the variance for the primary endpoint parameter will be performed when 20 patients (10 in each treatment arm) completed study week 12. This analysis is intended to adjust the sample size if necessary while limiting the interventions (blood sampling) to as little patients as necessary.

4   Change to protocol specified analyses

Protocol section 3.1 determined the primary variable AUC of VEGF-A protein concentration in plasma to be measured between baseline and study week 12 whereas protocol section 10.4.1 claimed the measure between Day 2 to study week 12. The assumption was made that section 10.4.1 mixed day 2 with visit 2 so decision was made to use AUC values from baseline to study week 12.

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Analyses on patient demographics will be based on the FAS and PPS rather than RS as defined in the protocol.

No other changes from the protocol specified analysis have been made.

5   Appendix

5.1   Imputation rules

5.1.1   AE date imputation

5.1.1.1   AE start date imputation

The following table explains the notation used in the logic matrix. Please note that completely missing start dates will not be imputed.

	Day	Month	Year
Partial Adverse Event Start Date	Not used	MON	YYYY
Treatment Start Date	Not used	TRTM	TRTY

The following matrix explains the logic behind the imputation.

	MON MISSING	MON < TRTM	MON = TRTM	MON > TRTM
YYYY MISSING	(1) No convention	(1) No convention	(1) No convention	(1) No convention
YYYY < TRTY	(2.a) Before Treatment Start	(2.b) Before Treatment Start	(2.b) Before Treatment Start	(2.b) Before Treatment Start
YYYY = TRTY	(4.a) Uncertain	(4.b) Before Treatment Start	(4.c) Uncertain	(4.c) After Treatment Start
YYYY > TRTY	(3.a) After Treatment Start	(3.b) After Treatment Start	(3.b) After Treatment Start	(3.b) After Treatment Start

Before imputing AE start date, find the AE start reference date.

1. If the (imputed) AE end date is complete and the (imputed) AE end date < investigational study treatment start date then AE start reference date = min (informed consent date, earliest visit date).

2. Else AE start reference date = investigational study treatment start date

Impute AE start date -

1. If the AE start date year value is missing, the date uncertainty is too high to impute a rational date. Therefore, if the AE year value is missing, the imputed AE start date is set to NULL.

2. If the AE start date year value is less than the treatment start date year value, the AE started before treatment. Therefore:

a. If AE month is missing, the imputed AE start date is set to the mid-year point (01JULYYYY).

b. Else if AE month is not missing, the imputed AE start date is set to the mid-month point (15MONYYYY).

3. If the AE start date year value is greater than the investigational study treatment start date year value, the AE started after treatment. Therefore:

a. If the AE month is missing, the imputed AE start date is set to the year start point (01JANYYYY).

b. Else if the AE month is not missing, the imputed AE start date is set to the later of (month start point (01MONYYYY), AE start reference date + 1 day).

4. If the AE start date year value is equal to the investigational study treatment start date year value:

a. And the AE month is missing the imputed AE start date is set to the AE reference start date + 1 day.

b. Else if the AE month is less than the investigational study treatment start month, the imputed AE start date is set to the mid-month point (15MONYYYY).

c. Else if the AE month is equal to the investigational study treatment start date month or greater than the treatment start date month, the imputed AE start date is set to the later of (month start point (01MONYYYY), AE start reference date + 1 day).

If complete (imputed) AE end date is available and the imputed AE start date is greater than the (imputed) AE end date, then imputed AE start date should be set to the (imputed) AE end date.

5.1.1.2   AE end date imputation

For the purpose of date imputation, the study treatment follow-up period date is defined as the last available visit date, i.e. including unscheduled visits after the EOS visit.

1. If the AE end date month is missing, the imputed end date should be set to the earliest of the (study treatment follow-up period date, 31DECYYYY, date of death).

2. If the AE end date day is missing, the imputed end date should be set to the earliest of the (study treatment follow-up period date, last day of the month, date of death).

3. If AE year is missing or AE is ongoing, the end date will not be imputed.

If the imputed AE end date is less than the existing AE start date then use AE start date as AE end date.

5.1.2   Concomitant medication date imputation

5.1.2.1   CM start date imputation

The following table explains the notation used in the logic matrix. Please note that completely missing start dates will not be imputed.

	Day	Month	Year
Partial CM Start Date	Not used	MON	YYYY
Treatment Start Date	Not used	TRTM	TRTY

The following matrix explains the logic behind the imputation.

	MON MISSING	MON < TRTM	MON = TRTM	MON > TRTM
YYYY MISSING	(1) Uncertain	(1) Uncertain	(1) Uncertain	(1) Uncertain
YYYY < TRTY	(2.a) Before Treatment Start	(2.b) Before Treatment Start	(2.b) Before Treatment Start	(2.b) Before Treatment Start
YYYY = TRTY	(4.a) Uncertain	(4.b) Before Treatment Start	(4.a) Uncertain	(4.c) After Treatment Start
YYYY > TRTY	(3.a) After Treatment Start	(3.b) After Treatment Start	(3.b) After Treatment Start	(3.b) After Treatment Start

1. If the CM start date year value is missing, the imputed CM start date is set to one day prior to treatment start date.

2. If the CM start date year value is less than the investigational study treatment start date year value, the CM started before treatment. Therefore:
   a. If the CM month is missing, the imputed CM start date is set to the mid −year point (01JULYYYY).
   b. Else if the CM month is not missing, the imputed CM start date is set to the mid-month point (15MONYYYY).

3. If the CM start date year value is greater than the investigational study treatment start date year value, the CM started after treatment. Therefore:
   a. If the CM month is missing, the imputed CM start date is set to the year start point (01JANYYYY).
   b. Else if the CM month is not missing, the imputed CM start date is set to the month start point (01MONYYYY).

4. If the CM start date year value is equal to the investigational study treatment start date year value:
   a. And the CM month is missing or the CM month is equal to the treatment start date month, then the imputed CM start date is set to one day prior to treatment start date.
   b. Else if the CM month is less than the treatment start date month, the imputed CM start date is set to the mid-month point (15MONYYYY).
   c. Else if the CM month is greater than the treatment start date month, the imputed CM start date is set to the month start point (01MONYYYY).

If complete (imputed) CM end date is available and the imputed CM start date is greater than the (imputed) CM end date, then imputed CM start date should be set to the (imputed) CM end date.

5.1.2.2 CM end date imputation

1. If the CM end date year value is missing, the date uncertainty is too high to impute a rational date. Therefore, if the CM end year value is missing or ongoing, the imputed CM end date is set to NULL.

2. Else, if the CM end date month is missing, the imputed end date should be set to the earliest of the (treatment follow up period date, 31DECYYYY, date of death).

3. If the CM end date day is missing, the imputed end date should be set to the earliest of the (treatment follow up period date, last day of the month, date of death).

4. If the imputed CM end date is less than the existing CM start date, use the CM start date as the imputed CM end date.

5.1.3 **Other imputations**

Other missing baseline data will not be imputed.

5.2 **Efficacy variables and populations for analysis**

Table 5-1 describes the primary and secondary efficacy endpoints with the respective methods and sets for analysis.

Table 5-1   Primary and secondary efficacy endpoints

Table	Variable	Analysis			
		Type	Method	Population (method of imputation)	Missing VEGF-A level baseline values imputation
Primary					
AUC of systemic VEGF-A levels	Mean AUC systemic VEGF-A levels from Day 1 to week 12 (visit 10)	Main	ANCOVA[1]	PPS (observed data)	Mean value of non-missing baseline VEGF-A level from all the patients with values
		Sensitivity	ANCOVA[1]	FAS (observed data)	Mean value of non-missing baseline VEGF-A level from all the patients with values
		Sensitivity	ANCOVA[2]	PPS (observed data)	Mean value of non-missing baseline VEGF-A level from all the patients with values
		Sensitivity	ANCOVA[1]	PPS (observed data)	No imputation
Secondary					
AUC of systemic VEGF-A levels	Mean AUC systemic VEGF-A levels from week 12 (visit 10) to week 24 (visit 16)	Main	ANCOVA[1]	PPS (observed data)	Mean value of non-missing baseline VEGF-A level from all the patients with values
		Main	ANCOVA[1]	FAS (observed data)	Mean value of non-missing baseline VEGF-A level from all the patients with values

[1] The model includes the systemic VEGF-A baseline level as a linear covariate and the following categorical variables: treatment, center

[2] The model includes the systemic VEGF-A baseline level as a linear covariate and the following

Table	Variable	Analysis			
		Type	Method	Population (method of imputation)	Missing VEGF-A level baseline values imputation
Primary					
categorical variables: treatment, center, gender					

5.2.1 **SAS code for analysis**

SAS code for ANCOVA analysis

The ANCOVA model will contain 'treatment' and 'center' as categorical variables, and 'baseline systemic VEGF-A' as continuous.

For the appropriate treatment difference, the treatments should be sorted or coded appropriately before using this model.

The ANCOVA analysis will be performed using SAS procedure PROC MIXED as below. Note that for this illustrative example:

- the data are stored in the file data_set
- treatmentvar = treatment variable
- responsevar = dependent variable
- stratavar = stratification variable
- bsl = baseline value of the responsevar
- alpha = significance level (i.e. 0.05 produces two-sided 95% CI)
- control = control group

```
PROC MIXED DATA = <data_set>;
CLASS <stratavar> <treatmentvar>;
MODEL <responsevar> = <stratavar> <treatmentvar> <bsl>;
LSMEANS <treatmentvar> / PDIFF = all CL alpha = <alpha> OM;
RUN;
```

5.3 **Clinically notable laboratory values and vital signs**

Table 5-2   Vital signs critical values

Variable	Type of abnormality	Criterion
Systolic blood pressure (mmHg)	High	Either >180 with an increase from baseline > 30 or > 200 absolute
	Low	Either < 90 with a decrease from baseline > 30 or < 75 absolute
Diastolic blood pressure (mmHg)	High	Either >105 with an increase from baseline > 20 or > 115 absolute
	Low	Either < 50 with a decrease from baseline > 20 or < 40 absolute
Pulse rate (bpm)	High	Either > 120 with an increase from baseline > 25 or > 130

Variable	Type of abnormality	Criterion
		absolute
	Low	Either < 50 with a decrease from baseline > 30 or < 40 absolute

5.4   **Rule of exclusion criteria of analysis sets**

Table 5-3   Protocol Deviation categories used

PD Category	PD Code
Patient did not satisfy inclusion criteria	I
Patient met exclusion criteria	E
Patient received the wrong treatment or incorrect dose	S
Patient met compliance violation (unblinding)	C
Patient participated in treatment relevant visits out of schedule	T
Patient had missing values in the primary variable	P
Patient developed withdrawal criteria during the study, but not withdrawn	D
Patient took an excluded concomitant medication	M
Others	O

Table 5-4   Protocol deviations that cause subject to be excluded

Deviation code	Text description	Action for analysis
S1	In case of treatment group 1 & 2: Application of study drug from the wrong treatment arm group in study eye at visit 2, 6, or 8	Exclude from PPS
S6	The interval between two intravitral injections should not be shorter than 28 days	Exclude from PPS if difference is <= 23 days

Reference

Avery R (2013) Comparison of Systemic Pharmacokinetics Post-Anti-VEGF Intravitreal Injections of Ranibizumab, Bevacizumab and Aflibercept. Data presented at ASRS Annual Meeting, Toronto CA, 25th August 2013.

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Zehetner C, Kirchmair R, Huber S et al. (2013) Plasma levels of vascular endothelial growth factor before and after intravitreal injection of bevacizumab, ranibizumab and pegaptanib in patients with age-related macular degeneration, and in patients with diabetic macular oedema. Br J Ophthalmol, vol. 97 (4), p. 454-9.

References are available upon request.