STATISTICAL ANALYSIS PLAN for Z-AMD

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SIGNATURE PAGE

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ABBREVIATIONS

AE	Adverse Event
AMD	Age-related Macular Degeneration
BCVA	Best-Corrected Visual Acuity
BMI	Body Mass Index
CRT	Central retinal thickness
FAS	Full Analysis Set
nAMD	Neovascular Age-related Macular Degeneration
OCT	Optical Coherence Tomography
PPS	Per Protocol Set
SAE	Serious Adverse Event
T & E	Treat & Extend
TEAE	Treatment Emerging Adverse Event
TESAE	Treatment Emerging Serious Adverse Event
ZA	Zoledronic Acid

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1 Introduction

1.1 Background and rationale

Anti-VEGF therapy stands as the gold standard for managing neovascular age-related macular degeneration (nAMD). With an increasing prevalence of nAMD due to an ageing population, the prolonged and repetitive treatment regimen has led to growing capacity challenges and escalating treatment-related costs. Consequently, there is need for novel strategies aimed at extending treatment duration, thereby reducing the treatment burden.

Zoledronic acid (ZA) is a potent bisphosphonate originally used to treat osteoporosis and other bone diseases. Pre-clinical data have shown that ZA – in addition to its osteoclastic properties- also possesses antiangiogenic properties. Data also suggests that bisphosphonates can supress choroidal neovascularization, which is the hallmark of nAMD. The Z-AMD study aims to explore whether ZA provides sufficient systemic antiangiogenic effect to serve as adjuvant therapy to anti-VEGF therapy for nAMD.

1.2 Trial Objectives

The objective of this pilot study is to study the efficacy and safety of treatment of zoledronic acid as adjuvant therapy for patients with neovascular age-related macular degeneration (AMD).

2 Trial Methods

2.1 Trial Design

The Z-AMD study is designed as a randomized, double blind, placebo-controlled, parallel-group, single-center pilot study. Treatment allocation is a 1:1 ratio. Patients are randomized to either zoledronic acid 5 mg or placebo adjuvant treatment.

One eye per patient will be recruited for the study. If both eyes are eligible for the study, the eye with the worse best-corrected visual acuity (BCVA) will be selected as study eye.
The treatment period is 6 months and the follow-up duration for each patient is 12 months.

2.2 Randomization

Eligible patients are allocated in a 1:1 ratio between zoledronic acid and placebo treatment, using a computer, block-randomization procedure.

Details of block size and allocation sequence generation is provided in a separate document unavailable to those who enroll patients or assign treatment.

The randomization process is described in full within the clinical trial protocol. Details of the randomization including the final random allocation list are held securely and unavailable to unauthorized trial personnel.

2.3 Sample size

A sample size of 20 patients in each group was considered sufficient to obtain preliminary data on efficacy and safety, and for determining an appropriate sample size for a future superiority trial (Julious and Owen, 2006).

2.4 Statistical Framework

This trial is a pilot study, designed to obtain preliminary data on efficacy and safety. It is likely too small to test for superiority, non-inferiority, or clinically relevant differences between the two groups. Accordingly, we will not conduct formal hypothesis testing or define decision rules of superiority or non-inferiority. Summary statistics and simple treatment comparisons with 95% confidence intervals will be presented; however, the results will not be interpreted as confirmatory.

2.5 Statistical Interim Analyses and Stopping Guidance

There will be no interim analysis in this trial.

2.6 Timing of Final Analysis

The main analysis is planned when all included patients either have completed the one-year assessment (at least 51 weeks) or has withdrawn according to protocol procedures, all data have been entered, verified and validated according to the data management plan, and the primary database has been locked.

2.7 Timing of Outcome Assessments

For all clinically planned measures, visits should occur within a window of the scheduled visit. Visits outside visit window is regarded a protocol deviation. The target day and visits window are defined in the protocol as:

Visit Label	**Target Day**	**Definition (Day window)**
Visit 1: Screening/Baseline	Day 0	Day 0
Visit 2: Randomization and infusion of study medication	7 (Randomization)	Target day ± 3 days
Visit 3	28	Target day ± 7 days
Follow-up visits according to the Treat & Extend (T&E) protocol described in section 5 of the protocol	T & E protocol	Target day ± 7 days
Infusion of study medication	182	Target day ± 7 days
Year 1 visit/Study exit	364	Target day ± 7 days
Early discontinuation visit		

3 Statistical Principles

3.1 Confidence Intervals and p-values

All efficacy estimates will be presented with two-sided 95% confidence intervals. No hypothesis testing will be performed. As there is only one primary outcome in this trial, there will be no adjustments for multiplicity.

3.2 Adherence and Protocol Deviations

3.2.1 Adherence to Allocated Treatment

Compliance is defined as having received both intravenous infusions of the randomized treatment, i.e. either zoledronic acid or 0.9% NaCl. Infusions occur at baseline (one week (+/- 3 days) after starting anti-VEGF treatment) and again after 26 weeks (+/- 1 week).

3.2.2 Protocol Deviations

The following are pre-defined major protocol deviations regarded to affect the efficacy of the intervention:

- Entering the trial when the eligibility criteria should have prevented trial entry
- Being non-compliant to the allocated treatment (see Section 3.2.1)
- Received or used other intervention than allocated
- Deviation from planned anti-VEGF treatment
- Blinding not maintained
- Failure to follow up a Serious Adverse Event (SAE)

Protocol deviations are classified prior to unblinding of treatment. The number (and percentage) of patients with major and minor protocol deviations will be summarized by treatment group with details of type of deviation provided. The patients that are included in the Full Analysis Set (see Section 3.3) will be used as the denominator to calculate the percentages.

3.3 Analysis Populations

The Enrolled set will include all patients who have provided informed consent and have been included into the study data base.

The Full Analysis Set (FAS) will be defined as all patients randomly assigned to a treatment group having received at least one study treatment infusion after randomization.

The Safety Analysis Set will include all patients having received at least one study treatment infusion after randomization.

The Per Protocol Analysis Set (PPS) will include all randomized patients meeting the study eligibility criteria and with no major protocol deviations affecting the treatment efficacy.

4 Trial Population

4.1 Screening Data, Eligibility and Recruitment

The total number of screened patients and reasons for not entering the trial will be summarized and tabulated.

A CONSORT flow diagram will be used to summarize the number of patients who were:

- eligible at screening
- ineligible at screening*
- eligible and randomized

- eligible but not randomized*
- received the randomized allocation
- did not receive the randomized allocation*
- lost to follow-up*
- discontinued the intervention*
- randomized and included in the primary analysis
- randomized and excluded from the primary analysis*

*reasons will be provided.

4.2 Withdrawal/Follow-up

The status of eligible and randomized patients at trial end will be tabulated by treatment group according to

- completed intervention and assessments
- completed assessments but not intervention
- withdrew consent
- lost to follow-up

4.3 Baseline Patient Characteristics

The patient demographics and baseline characteristics to be summarized include age in years, gender, medical history (myocardial infarction/stroke/transient ischemic attack), hypertension (normal/suspect/definite), best corrected visual acuity (logMAR and decimal equivalent), and central retinal thickness.

Patient demographics and baseline characteristics will be summarized by randomized treatment arm and overall using descriptive statistics. There will be no statistical analysis of treatment difference. Any clinical important imbalance between the treatment groups will be noted.

5 Analysis

5.1 Outcome Definitions

5.1.1 Best-Corrected Visual Acuity

Best corrected visual acuity (BCVA) is measured on a digital acuity chart with logarithmic line progression, with logMAR values used for statistical analyses. This is regarded as a continuous outcome.

5.1.2 The number of anti-VEGF intravitreal injections

Eyes will be examined and injected every 4 weeks until no signs of active AMD are present on optical coherence tomography (OCT). If no signs of active AMD are present, an injection is given and the treatment interval is extended by 2 weeks at a time, up to a maximum interval of 12 weeks. If active AMD recurs at an extended interval, treatment is shortened to every 4 weeks. If again no signs of active AMD are present, the treatment interval is extended again by 2 weeks at a time to a maximum of 2 weeks less than the previous recurrence interval. Thus, in a 52-week period, the minimum number of injections is 8 (baseline + after intervals of 4, 6, 8, 10, 12, and 12 weeks) and the maximum number of injections is 14 (baseline + after intervals of 4 weeks x 13). This is regarded as a discrete numerical outcome.

5.1.3 Refractory nAMD

Refractory AMD is defined as residual macular fluid on OCT despite bevacizumab injections every 4 weeks for 6 consecutive months (Yang et al., 2016). This is regarded as a dichotomous outcome.

5.1.4 EQ-5D

Patient-reported outcome measuring health-related quality of life. EQ-5D-5L contains five questions on physical function, pain and anxiety/depression with five graded response options. Only the global sum score (called the index value) will be used as an outcome. The index value ranges from 0-1, where 0 designates “dead” and 1 designates “perfect health”. This is regarded as a continuous outcome.

5.1.5 NEI-VFQ-25

National Eye Institute Visual Functioning Questionnaire 25 (NEI-VFQ 25) is a patient-reported outcome instrument to assess vision-related quality of life. It contains 25 questions on visual function in daily life and is sensitive to changes in visual acuity. NEI-VFQ 25 has 12 subcategories: 1) General health; 2) General vision; 3) Ocular pain; 4) Near vision activities; 5) Distance activities; 6) Social functioning; 7) Mental health; 8) Role difficulties; 9) Dependency; 10) Driving; 11) Color vision; and 12) Peripheral vision. Only the global sum score (called the composite score) will be used as an outcome. The composite score ranges from 0 to 100, where 100 reflects best vision-specific health. This is regarded as a continuous outcome.

5.1.6 Central Retinal Thickness

Central retinal thickness (CRT, measured in µm) is obtained from OCT scans, serving as a surrogate endpoint for macular fluid. This is regarded as a continuous outcome.

5.1.7 Adverse Events of Special Interest

Adverse events of special interest are osteonecrosis of the jaw or atypical femoral fracture, endophthalmitis or orbital, scleral, or serious intraocular inflammation (grade 4 aqueous cells/FLARE). This is regarded as a dichotomous outcome.

5.2 Overview of Outcomes

Level	Outcome	Timepoint	Type
Primary	Change from baseline in BCVA (measured by logMAR)	52 weeks*	Continuous
Secondary	The number of anti-VEGF intravitreal injections	0-52 weeks*	Discrete numerical
	Change from baseline in BCVA of 0.3 logMAR or more	52 weeks*	Dichotomous
	Occurrence of refractory nAMD/Switching from Avastin to Eylea	After at least 6 injections until 52 weeks*	Dichotomous
	Change from baseline in EQ-5D index value	52 weeks*	Continuous

Change from baseline in NEI-VFQ-25 composite score	52 weeks*	Continuous
Change from baseline in central retinal thickness (CRT)	52 weeks*	Continuous
Occurrence of an adverse event of special interest	0-52 weeks*	Dichotomous

*The timing of the last measurement will differ for each patient, based on the results of the Treat & Extend protocol for the anti-VEGF intravitreal injection (see Section 2.7 and Section 5.1.2). In the case of multiple measurements during the follow-up, the last measurement will be used, which occurs at approximately 52 weeks.

5.3 Analysis Methods

All outcomes will be analyzed on the FAS. An additional analysis of the primary outcome will be done on the PPS.

5.3.1 Continuous outcomes

The primary outcome (BCVA) and all other continuous outcomes (EQ 5D, NEI-VFQ-25, and CRT) will be analyzed with linear regression, where the last measurement (at approximately 52 weeks follow-up) will be the dependent variable and treatment (Zoledronic acid vs placebo) and baseline measurement will be the independent variables. The treatment effect will be the between-group difference in change from baseline to the last measurement, estimated by the coefficient for treatment in the linear regression model.

The mean and standard deviation of the outcome at baseline and 52 weeks and the change from baseline to 52 weeks will be presented per treatment group, together with the estimated treatment effect with a 95% confidence interval.

The raw data of the primary outcome (BCVA) and the secondary outcome CRT, both of which is measured at each of the anti-VEGF intravitreal injection visits (see Section 2.7 and Section 5.1.2), will be presented in spaghetti plots, with one line per patient showing the measurements across the follow-up period.

5.3.2 Discrete numerical outcomes

The number of anti-VEGF intravitreal injections will be analyzed with a two-sample t-test with adjustment for unequal variances (Fagerland et al., 2011). A contingency table with two rows (Zoledronic acid and placebo) and seven columns (one for each of the possible outcomes: 8, 9, 10, 11, 12, 13, and 14 injections) will be presented. We will also present the mean and standard deviation of the number of injections for each group, and the difference between the treatment groups in mean number of injections with a 95% confidence interval.

5.3.3 Dichotomous outcomes

Dichotomous outcomes will be presented with the number and percentage of patients with an occurrence of the outcome by treatment group. The between-group difference in the probabilities of the outcome will be given with a 95% Newcombe hybrid score confidence interval (Chapter 4 of Fagerland et al., 2017).

5.3.4 Assumption Checks and Alternative Analyses

For the continuous outcomes analyzed by linear regression, we will assess the distribution of the model residuals with histograms and descriptive statistics. If the residuals deviate considerably from the normal distribution, median regression with bootstrap confidence intervals will be used instead of linear regression. Median and differences in medians will then be reported instead of means and difference in means.

The discrete numerical outcome (number of anti-VEGF injections) is limited to seven possible values, and as such, there is no concern about the distribution of the outcomes, except for the situation where almost all patients have the same number and there is not enough variation in the data to get a meaningful result from the statistical methods. In that case, the outcome will be presented with the contingency table only.

The confidence interval method for the dichotomous outcomes was chosen for its ability to handle small-sample and sparse data, for which it is robust, including to situations with zero cell counts.

5.3.5 Missing Data

Because of the small sample of patients and the close and meticulous follow-up of each patient, we expect no missing data for the outcome variables, and we thereby do not plan for statistical analyses to take missing data into account. In the case that missing data still occurs, the number and percentages of patients with missing data will be presented; however, the statistical analyses will be performed as presented above on the complete case data.

5.3.6 Sensitivity Analyses

The primary outcome will be analyzed on the PPS.

6 Safety Analyses

The safety assessments and outcomes include monitoring, recording, follow-up, and reporting of adverse events. Adverse events will be tabulated and presented for all patients in the safety set.

The investigator records the maximum intensity of each adverse event using the levels mild, moderate and severe. Adverse events with missing intensity will be considered to be severe. For tabulations, only treatment emerging adverse events (TEAEs) will be presented. TEAEs are defined as AEs with a start date on or after date of first randomized treatment. Any AEs prior to treatment will be listed but not tabulated.

The number (%) of subjects with any TEAEs, with 1, 2 or > 3 TEAEs, with treatment related TEAEs, with treatment emerging serious AEs (TESAE) and TEAEs leading to study drug withdrawal will be summarized by treatment group. The number of events and number (%) of subjects with adverse events by system organ class and preferred term will be summarized by treatment group, overall, for severe AEs and for AEs leading to study discontinuation. A detailed patient narrative will be given for any serious adverse event in the clinical study report in addition to listing.

7 Statistical Software

All statistical analyses will be done in Stata version 18 (StataCorp LLC, College Station, TX, USA).

8 References

Fagerland MW, Lydersen S, Laake P. *Statistical Analysis of Contingency Tables*. Chapman & Hall/CRC, 2017, Boca Raton, FL.

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Julious SA, Owen R J. Sample size calculations for clinical studies allowing for uncertainty about the variance. *Pharmaceutical Statistics* 2006; 5(1): 29-37.

Yang S, Zhao J, Sun X. Resistance to anti-VEGF therapy in neovascular age-related macular degeneration: a comprehensive review. *Drug Design, Development and Therapy* 2016; 10: 1857-1867.