Statistical Analysis Plan Cover Page

Official Study Title:	OPSIS: A Phase IIa, Randomized, Double-Masked, Placebo-Controlled, Parallel-Group, Multicenter Study Assessing the Efficacy and Safety of STN1013600 Ophthalmic Solution 0.1% and 0.3% Compared with Placebo in Subjects with Presbyopia
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STATISTICAL ANALYSIS PLAN

OPSIS Study

Protocol Title:	OPSIS: A Phase IIa, Randomized, Double-Masked, Placebo-Controlled, Parallel-Group, Multicenter Study Assessing the Efficacy and Safety of STN1013600 Ophthalmic Solution 0.1% and 0.3% Compared with Placebo in Subjects with Presbyopia
Product:	STN1013600 Ursodiol Ophthalmic Solution
Protocol Number:	101360002IN
Sponsor:	Santen Inc. 6401 Hollis Street, Suite 125 Emeryville, CA 94608 USA.
Date:	August 08, 2023
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CONFIDENTIAL

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ABBREVIATIONS

Table 1: Abbreviations

Abbreviation	Explanation
ADR	Adverse Drug Reaction
AE(s)	Adverse Event(s)
ATC	Anatomical-Therapeutic-Chemical
BCDVA	Best-Corrected Distance Visual Acuity
BID	bis in die (twice a day)
[redacted]	
CI	Confidence Interval
CM	Concomitant Medications
CSR	Clinical Study Report
D	Diopter
[redacted]	
DCNVA	Distance Corrected Near Visual Acuity
eCRF	Electronic Case Report Form
ESI(s)	Event(s) of Special Interest
ET	Early Termination
FAS	Full Analysis Set
IOP	Intraocular Pressure
LogMAR	Logarithm of the Minimum Angle of Resolution
MedDRA	Medical Dictionary for Regulatory Activities
MH	Medical History
mmHg	Millimeter of Mercury
MMRM	Mixed-effect Model for Repeated Measures
MRSE	Manifest Refraction Spherical Equivalent
NAVQ	Near Activity Visual Questionnaire
OD	Oculus Dexter (right eye)
OS	Oculus Sinister (left eye)
OU	Oculus Uterque (both eyes)
PPS	Per-Protocol Set
PT	Preferred Term

Table 1: Abbreviations (Continued)

Abbreviation	Explanation
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SOC	System Organ Class
TEAE	Treatment-emergent adverse event
US	United States
WHO	World Health Organization

1. INTRODUCTION

This statistical analysis plan (SAP) specifies the statistical methods to be implemented for the analysis of data collected from the OPSIS study within the scope of Santen's Protocol 101360002IN, "OPSIS: A Phase IIa, Randomized, Double-Masked, Placebo-Controlled, Parallel-Group, Multicenter Study Assessing the Efficacy and Safety of STN1013600 Ophthalmic Solution 0.1% and 0.3% Compared with Placebo in Subjects with Presbyopia". It applies to the study protocol version Original, dated 23 September 2022, and provides detailed instructions as to how each analysis will be performed.

Results obtained from the analyses specified in the final approved version of the SAP will become the basis of the clinical study report (CSR) for this study. Any deviations from the final approved version of the SAP must be substantiated by sound statistical reasoning and documented in the CSR.

2. OBJECTIVES AND ENDPOINTS

2.1. Objectives

2.1.1. Primary Objectives

To assess the efficacy of two concentrations of STN1013600 ophthalmic solution (0.1% and 0.3%) twice daily dosing when compared to Placebo in subjects aged from 47 to 55 years with presbyopia.

2.1.2. Secondary Objectives

To assess the dose response of STN1013600 ophthalmic solution.

2.1.3. Safety Objective

To assess the safety of two concentrations of STN1013600 ophthalmic solution (0.1% and 0.3%) twice daily dosing when compared to Placebo in subjects aged from 47 to 55 years with presbyopia.

2.1.4. Exploratory Objective

To explore other efficacy outcome measures of two concentrations of STN1013600 ophthalmic solution (0.1% and 0.3%) twice daily when compared to Placebo in subjects aged 47 to 55 years with presbyopia.

2.2. Endpoints

2.2.1. Primary Efficacy Endpoint

The primary efficacy endpoint is the mean change from baseline in Binocular Distance Corrected Near Visual Acuity (DCNVA) at Month 2.

2.2.2. Secondary Efficacy Endpoints

- Mean change from baseline in Study Eye DCNVA at all visits
- Mean change from baseline in Binocular DCNVA at all visits
- Proportion of subjects who improve 1/2/3-lines or more in Study Eye and Binocular DCNVA at each visit without loss of more than 1 line (5 letters) in Best Corrected Distance Visual Acuity (BCDVA)
- Mean change from baseline in quality of life assessed with Near Activity Visual Questionnaire (NAVQ) at Month 2 and Month 3
- Subject treatment satisfaction as assessed by Patient Global Rating of Treatment at Month 2

2.2.3. Safety Endpoints

The safety of STN1013600 ophthalmic solution will be evaluated by adverse events (AEs), Intra-ocular Pressure (IOP), slit-lamp bio-microscopy, ophthalmoscopy, and laboratory tests (serum chemistry, hematology, and urinalysis).

2.2.4. Exploratory Endpoints

3. STUDY DESIGN

3.1. General Study Design

This is a Phase IIa, randomized, double-masked, placebo-controlled, parallel-group study assessing the efficacy and safety of STN1013600 ophthalmic solution (0.1% and 0.3%) in subjects with Presbyopia.

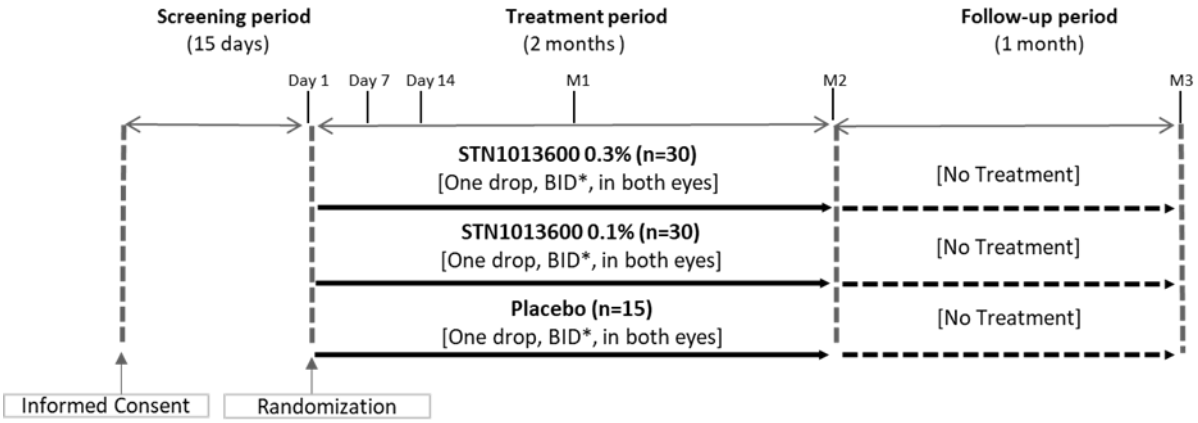
Subjects with Presbyopia who meet all eligibility criteria at Visit 1 (Screening) will be randomized at Visit 2 (Baseline) to receive treatment for 2 months.

Approximately 75 subjects who meet all eligibility criteria at Visit 1 (Screening) will be randomized at Visit 2 (Baseline) in a 2:2:1 ratio to one of the following three treatment arms:

- 0.1% STN1013600 ophthalmic solution BID (08:00 & 20:00 ± 60 min)
- 0.3% STN1013600 ophthalmic solution BID (08:00 & 20:00 ± 60 min)
- Placebo (Vehicle) BID (08:00 & 20:00 ± 60 min)

Subjects will be treated for 2 months, followed by a 1-month safety follow-up without treatment (Figure 1).

Figure 1: Study Design Diagram



* On Day 1, subject should administer 1 dose of assigned medication after study visit at 20:00 ± 60 min. BID dosing starts from Day 2 through Month 2.

This study will consist of a Screening Period of up to 15 days, followed by a 2-month Treatment Period and subsequently a 1-month Treatment-free follow-up Period.

At Visit 1 (Screening), subjects will be screened against the inclusion and exclusion criteria and if subject meets all eligibility criteria, subjects will proceed to Visit 2 (Baseline).

Per study design, subjects who qualify per eligibility criteria will be treated bilaterally (both eyes).

The study eye will be the eye that qualifies with the worse DCNVA per eligibility criteria at Visit 2. If both eyes have the same DCNVA, the right eye will be designated as the study eye.

Treatment Period (2 months):

At Visit 2 (Baseline), approximately 75 eligible subjects will be randomized to receive either 0.1% STN1013600 ophthalmic solution BID or 0.3% STN1013600 ophthalmic solution BID or Placebo BID in a 2:2:1 ratio. Subjects will be treated for 2 months with scheduled assessment visits at Visit 2 (Baseline), Visit 3 (Day 7), Visit 4 (Day 14), Visit 5 (Month 1), and Visit 6 (Month 2).

At Visit 2 (Baseline), subjects will administer their first dose of study medication (study eye drops) as per their assigned/randomized study treatment at 20:00 (± 60 min). The next day

(Day 2), subjects will subsequently dose with their assigned study medication at 08:00 & 20:00 (± 60 min) and continue dosing through Visit 6 (Month 2).

Treatment-free Follow-up Period (1 month):

After Treatment Period, subjects will be followed for 1 month with scheduled study assessment (including safety assessments) visits at Visit 7 (Month 3).

3.2. Randomization and Masking

A permuted-block randomization will be employed to randomize eligible subjects in a 2:2:1 ratio to either 0.1% STN1013600 ophthalmic solution or 0.3% STN1013600 ophthalmic solution or Placebo (vehicle). The randomization will be centralized and stratified by baseline binocular DCNVA (59 ETDRS letters or less; 60 ETDRS letters or more).

The randomization schedule will be generated and implemented using central randomization via Interactive Response Technology (IRT). Each randomized subject will receive numbered study medication kits as assigned by IRT.

This is a double-masked study. The packaging (kit cartons and bottles) will be identical. The subjects, investigators, Examiners, and Santen personnel involved in the conduct of the study will be masked to the study treatment. An authorized unmasked study staff member at the investigative site who is not the investigator or Examiner will dispense and collect study medication(s) and will query about dosing compliance.

In case of a medical emergency, the Principal Investigator may reveal the treatment information by unmasking through IRT to know which treatment the subject has received.

3.3. Sample Size Determination

The sample size of this study is not based on any statistical power calculation due to the exploratory nature of the study and limited knowledge of the treatment effect of study medications. Based on the feasibility of conducting the trial, a total of approximately 75 subjects will be randomized into a 2:2:1 ratio to 0.1% STN1013600, 0.3% STN1013600, and placebo arm. That is, approximately 30 subjects and 15 subjects will be randomized into each of STN1013600 treatment arms and placebo arm, respectively.

Assuming a difference in mean change in DCNVA at Month 2 of 0.08 logMAR, or 4 ETDRS letters between STN1013600 group and placebo group with a standard deviation of 0.12 logMAR or 6 ETDRS letters based on literature data (Korenfeld et al., 2021), the primary analysis with 30 subjects in treatment arm and 15 subjects in control arm will achieve at least 81.5% power to detect such difference. The power calculation for the primary analysis is described in detailed in Section 12.2.

3.4. Visits and Assessments

There are 7 scheduled visits for each enrolled subject. Assessments at each visit and the time/visit window for each post-baseline assessment are specified in the Assessment Schedule (Table 2). For subjects whose study participation is terminated prior to Visit 7 (Month 3), to the extent possible, all assessments scheduled for Visit 7 (Month 3) will be performed at the Exit Visit.

Table 2: Assessment Schedule

		Treatment Period					Follow Up Without Treatment
Study Procedures	**Visit 1 / Screening Up to 15 days before Day 1**	**Visit 2 Baseline / Day 1**	**Visit 3 Day 7 ± 2 days**	**Visit 4 Day 14 ± 2 days**	**Visit 5 Month 1 ± 3 days**	**Visit 6 Month 2 ± 3 days**	**Visit 7 Month 3 ± 3 days / Early Discontinuation**
Informed consent[a]	X						
Inclusion and Exclusion criteria	X	X					
Symptom Assessment iN Dry Eye questionnaire (SANDE)[b]	X						
Near Activity Visual Questionnaire (NAVQ)[b]		X				X	X
Demographics information	X						
Ocular and systemic medical history	X						
Vital signs (blood pressure, heart rate, temperature)	X	X	X	X	X	X	X
Height and weight	X						
Previous and current concomitant ocular and systemic medications	X	X	X	X	X	X	X
Objective refraction test	X[c]	X	X	X	X	X	X
Subjective refraction test	X	X	X	X	X	X	X
Distance Corrected Near Visual Acuity (DCNVA) test[d]	X	X	X	X	X	X	X
[redacted]		[redacted]					[redacted]

Table 2: Assessment Schedule (Continued)

		Treatment Period					Follow Up Without Treatment
Study Procedures	**Visit 1 / Screening Up to 15 days before Day 1**	**Visit 2 Baseline / Day 1**	**Visit 3 Day 7 ± 2 days**	**Visit 4 Day 14 ± 2 days**	**Visit 5 Month 1 ± 3 days**	**Visit 6 Month 2 ± 3 days**	**Visit 7 Month 3 ± 3 days / Early Discontinuation**
Best Corrected Distance Visual Acuity (BCDVA) test[d]	X	X	X	X	X	X	X
[redacted]		[redacted]					
[redacted]	[redacted]						
[redacted]		[redacted]					
Slit lamp biomicroscopy	X	X	X	X	X	X	X
Pupil size	X	X	X	X	X	X	X
Tear film break-up time (TFBUT)	X						
Corneal Fluorescein Staining (CFS) assessment (with Oxford Scale)	X						
Intraocular pressure (IOP)	X	X	X	X	X	X	X
Ophthalmoscopy	X[g]	X	X	X	X	X	X[g]
Patient global rating of treatment						X	
Post-study clinical trial experience survey							X
Urine pregnancy test (women of childbearing potential only)[h]	X					X	X
Blood chemistry and Hematology (CBC, CMP)	X				X	X	
Hemoglobin A1c	X						
Urinalysis	X				X	X	

Table 2: Assessment Schedule (Continued)

		Treatment Period					Follow Up Without Treatment
Study Procedures	**Visit 1 / Screening Up to 15 days before Day 1**	**Visit 2 Baseline / Day 1**	**Visit 3 Day 7 ± 2 days**	**Visit 4 Day 14 ± 2 days**	**Visit 5 Month 1 ± 3 days**	**Visit 6 Month 2 ± 3 days**	**Visit 7 Month 3 ± 3 days / Early Discontinuation**
Adverse events (AEs)	X	X	X	X	X	X	X
Dispense study medication		X[i]			X		
Medication compliance diary[j]			X	X	X	X	

[a] Informed Consent Form must be signed and dated before study procedures are performed.
[b] NAVQ and SANDE questionnaires are to be performed at the beginning of the study visit, in the order reflected on Schedule of Assessments Table.
[c] During screening, cycloplegic refraction (objective refraction with dilation) should be performed; At visit 7 objective refraction can be performed with or without cycloplegia.
[d] DCNVA, [redacted] and BCDVA assessments should be performed on OD, OS, and OU.
[e] [redacted]
[g] Dilated ophthalmoscopy required at Screening and Exit visits.
[h] Urine pregnancy test (women of childbearing potential only) may be repeated at each visit as per request of IRB/IECs or at the Investigator's discretion.
[i] Only patients fulfilling eligibility criteria will be enrolled and receive the study medication.
[j] Dispense medication compliance diaries with when study medication is dispensed and instruct subjects on the proper completion of the diary. Collect paper diaries at specified visits and review for accuracy and completeness. Dosing for randomized subjects on Visit 2 (Day 1/Baseline) is after the study visit in the evening (20:00 ± 60 min), and Day 2 through Month 2 is daily BID (08:00 ± 60 min and 20:00 ± 60 min). Re-educate the subject on diary completion and/or medication instillation procedures as needed.

4. TIME-RELATED TERMS

4.1. Baseline Visit

The *Baseline Visit* is Visit 2 (Day 1) when the subject is randomized.

4.2. Treatment Period, Treatment Start Date, and Treatment End Date

This study has two study periods: Treatment Period and Treatment-free Follow-up Period. The start date and end date for each period are defined as follows in Table 3.

Table 3: Definitions for Period Start and End Dates by Study Period

Study Period	Period Start Date	Period End Date
Treatment Period	The date at which a randomized subject takes the first dose of study drug	The date at which a subject takes the last dose of study drug. If the date of the last dose is missing, The day of the Visit 6 (Month 2) date will be considered as the end date The day before the Study Exit date will be used for subjects who prematurely discontinued the study before Visit 6 (Month 2). If the Study Exit date of a non-completer is not available, then the day of the last available visit date will be considered the treatment end date
Treatment-free Follow-up Period	The day after the Visit 6 (Month 2) date.	The date of Visit 7 (Month 3)

Treatment start date and *Treatment end date* are the start date and the end date of the Treatment Period, defined in Table 3.

4.3. Study Day and Analysis Visit

The *study day* describes the relative day of an observation starting with the reference date designated as Study Day 1. In this study, the treatment start date is the reference date. Thus, the study day will be calculated as:

- For a pre-baseline date, Study Day = Date – Treatment Start Date
- For a post-baseline date, Study Day = Date – Treatment Start Date + 1

4.4. Analysis Visit and Analysis Window

Analysis visit is a timing variable to be used for analyses involving visits. For each analysis visit, an *analysis window* is set up to determine the analysis visit to which a measurement should be mapped (Table 4). The analysis visit of a measurement will be determined based on the study day of the measurement and specified analysis windows and is not necessarily the same as the

study visit at which the measurement was collected. For example, an out-of-window measurement collected at the Day 7 study visit will be mapped to the Day 14 analysis visit, if the study day of the measurement falls into the analysis window of Day 14.

The following *analysis windows* will be applied to minimize the amount of missing data for analysis purposes:

Table 4: Analysis Visit and Analysis Window

Analysis Visit (Target Study Day)	Protocol Visit Window	Analysis Window		Patient Global Rating of Treatment	Blood Chemistry, Hematology, Urinalysis	Other Parameters
Baseline (Day 1)	[1, 1]	[redacted]	[redacted]	NA*	[- , 1]	[- , 1]
Day 7 (Day 7)	[5, 9]			NA*	NA*	[2, 11]
Day 14 (Day 14)	[12, 16]			NA*	NA*	[12, 23]
Month 1 (Day 30)	[27, 33]			NA*	[2, 49]	[24, 49]
Month 2 (Day 60)	[57, 63]			[2, -]	[50, -]	[50, day of Month 2 Visit]
Month 3 (Day 90)	[87, -]			NA*	NA*	[day of Month 2 Visit + 1, -]

*Not collected at this visit.

If there are two or more visits that fall into the same analysis window, then the visit closest to the target assessment day will be selected for that visit window. In the case that two visits are equidistant to the target assessment day, i.e., one is before and one is after the target assessment day, the latter will be selected for that visit.

4.5. Extent of Exposure

The *extent of exposure* to study medication will be assessed by duration of treatment exposure, derived as:

Duration of treatment exposure = Treatment end date – Treatment start date + 1

5. GENERAL CONSIDERATIONS

All measures will be summarized descriptively. Continuous variables will be summarized using descriptive statistics such as number of observations (n), mean, standard deviation, standard error, median, minimum, and maximum. Categorical variables will be tabulated using frequency (n) and percent (%).

Unless otherwise specified, the following conventions will be followed in reporting the decimal places.

Reporting Statistics	**Decimal places**
Range (Low Value, High Value)	Recorded Decimal Places
Mean, Median	Recorded value + 1 Decimal Places
Confidence Interval, Standard Deviation, Standard Error	Recorded value + 2 Decimal Places
p-Value	4 Decimal Places (ex. 0.0021)

The statistical testing will be conducted at a significance level of 0.10 (two-sided) and the 90% confidence interval will be shown, unless specified otherwise. No statistical testing will be conducted for safety measures.

All data manipulations, descriptive summaries, and statistical hypothesis testing will be performed using Statistical Analysis System (SAS) Version 9.4 or later. Individual data, including relevant derived variables, will be listed.

Additional analyses not specified in this SAP may be conducted if deemed necessary and will be documented in the CSR.

5.1. Adjustments for Covariates

Details in covariates to be included in the statistical model for each individual statistical analysis are provided Section 8.

5.2. Handling of Missing Data

5.2.1. Efficacy Measures

The primary analysis of DCNVA will be based on observed cases; hence, missing data will not be imputed. As a sensitive analysis of the primary efficacy endpoint, an analysis using mixed-effect model for repeated measures (MMRM) will be performed. The MMRM model will be applied using observed cases and no imputation will be needed.

For continuous secondary endpoints where MMRM-based analysis will be performed, no imputation will be necessary. For binary secondary endpoints with response status, the analysis will be based on observed cases; hence, no imputation will be performed.

5.2.2. Safety Measures

Descriptive summaries of safety measures will be based on observed data only. No imputation of missing scores will be implemented.

5.2.3. Dates for Medical Events and Medications

Completely or partially missing onset and resolution dates for AEs, Concomitant Medications (CM), and Medical History (MH) will be imputed in a conservative fashion as follows:

Incomplete Adverse Event Onset Date

1. *Year imputation*
   - If *year* is missing (or AE onset date is completely missing), then the onset date will not be imputed.
2. *Month imputation*
   - If *year* is not missing but *month* is missing, then:
     - If *year* = year of first study dose date, then set the *month* and *day* to the day and month of first study dose
     - Else if *year* ≠ year of first study dose: set *month* to January
3. *Day imputation*
   - If *day* is missing (*month and year* not missing), then:
     - If *year* = year and *month* = *month* of first study dose, then set *day* to day of first study dose
     - Else if *year* ≠ *year* and *month* ≠ *month* of first study dose, then set *day* to first day of the *month* in the year

Incomplete Adverse Event Resolution Date

- Do not impute if any resolution date is missing
- If the duration of AE is needed, the following approach may be considered:
  - If *year* is missing (or AE resolution date is completely missing): do not impute
  - If *year* is not missing but *month* and *day* are missing: impute December 31st for missing *month* and *day*
  - If *year* and *day* are not missing but *month* is missing: impute December for missing *month*
  - If *year* and *month* are not missing but *day* is missing: impute last day of the *month* for missing *day.*

Incomplete CM or MH Onset Date

1. If *year* is missing (or CM/MH onset date is completely missing): do not impute
2. If *year* is not missing but *month* and *day* are missing: impute January 1st for missing *month* and *day*
3. If *year* and *day* are not missing but *month* is missing: impute January for missing *month*
4. If year and month are not missing but day is missing: impute 01 for missing day

Incomplete CM or MH Resolution Date

- Do not impute if any resolution date is missing.

5.3. Multi-Center Studies

This is a multi-center study enrolling subjects from 12 US sites. The number of subjects per site might be small. Therefore, there are no analyses adjusting for sites.

5.4. Multiple Comparisons / Multiplicity

This trial plans to have only one hypothesis testing, which is for the primary efficacy endpoint. Therefore, multiple comparisons do not apply and there is no need to adjust for multiplicity.

5.5. Interim Analysis

Following the Santen Steering Committee' recommendation, no interim analysis is planned for this study.

6. STUDY POPULATION

6.1. Safety Population

The Safety Population will include all subjects who signed informed consent, met the eligibility criteria at baseline (Visit 2, Day 1), and received at least one dose of the study medication. The safety analyses will be performed on the Safety Population.

6.2. Full Analysis Set

The Full Analysis Set (FAS) will include all Safety population who had at least one post-baseline DCNVA measurement. The efficacy analyses will be performed using the FAS or a subset of the FAS.

6.3. Per-Protocol Set

The Per-Protocol Set (PPS) is a subset of the FAS, restricted to the subjects who fulfill the protocol in the terms of eligibility, interventions, and other assessment. It will be the analysis population for some sensitivity analyses.

Before database lock, Santen's study team will review all protocol deviations, identify subjects with any protocol deviation that could impact the efficacy outcome, and determine whether to exclude the subject from the PPS.

7. SUMMARY OF STUDY POPULATION DATA

7.1. Subject Disposition

The disposition of all randomized subjects will be summarized by treatment and overall. The summary will include the number and percentage of subjects in the Safety population, FAS, and PPS. The disposition summary will also include the number and percentage of subjects who completed the treatment period, who completed all study visits, subjects who discontinued the study prematurely before the end of treatment period (Visit 6, Month 2), as well as the number and percentage of subjects who discontinued from the study prior to Month 3 Visit by the primary discontinuation reason separately.

7.2. Demographics and Baseline Characteristics

Subject demographics and baseline characteristics will be descriptively summarized for FAS. Specifically, for subject demographics, the following variables will be summarized:

- Age at enrollment (continuous and categorical: 47-50 years or 51-55 years)
- Sex (categorical: Male or Female)
- Ethnicity (categorical: Hispanic/Latino or Not)
- Race (categorical: American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, White, or Other)

For baseline characteristics, the following variables will be summarized for both eyes (if applicable), study eye and fellow eye, separately:

- Best corrected distance visual acuity
- [redacted]
- Distance corrected near visual acuity
- Objective refraction (spherical/cylinder/axis)
- Subjective refraction (spherical equivalent, spherical, cylinder, and axis)
- Refractive status (categorical: Myopes/Emmetropes/Hyperopes)

7.3. Medical and Surgical History

For this study, medical and surgical history and adverse events will be coded using MedDRA 25.1, September 2022. Each medical event will be classified into a system organ class (SOC) and mapped to a Preferred Term (PT).

The medical and surgical history will be summarized for the FAS population. Subjects reporting any medical and surgical history at baseline will be tabulated by SOC and PT for each planned treatment and overall.

7.4. Protocol Deviations

In this study, protocol deviations are categorized as follows:

- Informed Consent
- Inclusion/Exclusion Criteria
- Concomitant Treatment
- Investigational Product
- Procedures/Tests/Assessments
- Laboratory
- Time Window
- Other

A protocol deviation is considered significant if it may affect the subject's rights, safety, or well-being, and/or the completeness, accuracy, or reliability of the study data. Santen's study team will review all protocol deviations and determine the list of significant protocol deviations prior to database lock. All FAS subjects with any significant protocol deviation(s) will be tabulated by deviation category for each planned treatment and overall. In addition, two listings will be provided: (1) all significant protocol deviations and (2) subjects excluded from the per protocol population.

7.5. Impact of the COVID-19 Pandemic

The impact of the COVID-19 pandemic is defined as any disruption to the study and subject participation, such as changes in study visits, missed visits, subject discontinuations, etc. All Safety Population subjects who experienced any of such impact will be summarized and listed along with the description of how their participation was altered.

7.6. Prior and Concomitant Medications

Non-study medications will be categorized into prior medications and concomitant medications. Specifically, *prior medication* is defined as any non-study medication taken and ended prior to the study medication start date. *Concomitant medication* is defined as any non-study medication taken concurrently while receiving study medication, i.e., the period from first dose to last dose of a concomitant medication taken by a subject must overlap with the period from first dose to last dose of the study medication.

For this study, non-study medications, including prior and concomitant medications, will be coded using World Health Organization (WHO) Drug Global, Version September 2018, format B3. Each non-study medication will be classified using the Anatomical-Therapeutic-Chemical (ATC) classification system and mapped to a WHO Drug preferred drug name.

Non-study medications will be summarized for the Safety population. Subjects taking any prior medications will be tabulated by ATC level 3, level 4, and preferred drug name. A subject will be counted at most once for each prior medication, even if the subject took the same prior medication on multiple occasions. Subjects taking any concomitant medications will be tabulated similarly. In addition, prior medications and concomitant medications will also be listed, separately.

7.7. Treatment Compliance

For compliance calculation, there will be three study intervals in the Treatment Period:

- Baseline Visit to Day 7
- Day 7 to Day 14
- Day 14 to Month 1
- Month 1 to Month 2

The compliance rate for a subject will be calculated as follows for each study interval:

$$\text{Compliance Rate (\%)} = (2 \times \text{Duration} - \sum\text{Miss}) / (2 \times \text{Duration}) \times 100$$

Where

Duration: The number of days subject should have administered study medication calculated as:

Study Interval	**Equation for Duration Calculation**
Baseline to Day 7	Day 7 Visit date – Date of first study drug dispensation
Day 7 to Day 14	Day 14 Visit date – Day 7 Visit date
Day 14 to Month 1	Month 1 Visit date – Day 14 Visit date
Month 1 to Month 2	Month 2 Visit date – Month 1 Visit date

Miss: The number of missed doses since the last visit.

A subject is fully compliant with the study treatment if his/her overall compliant rate is 100% over the Treatment Period (between Baseline and Month 2). The compliance rate of subjects in the FAS will be summarized by study intervals and over the Treatment Period for each planned treatment and overall.

7.8. Exposure to Study Medication

The duration of exposure to a study medication is measured by days on treatment as derived in Section 4.5. For subjects in the Safety Population, the duration of exposure will be summarized using descriptive statistics, and frequency and percentage of subjects will be tabulated by duration category (1-30 days, 31-60 days, or ≥ 61 days) for each actual treatment received.

8. EFFICACY ANALYSES

8.1. Efficacy-Related Definitions

8.1.1. Study Eye and Fellow Eye

The *study eye* of a treated subject will be the eye that received the study medication and has a lower score of DCNVA as study eye at Visit 2 (Baseline, Day 1). If both eyes have the same score of DCNVA, the right eye will be designated as the study eye. The other eye will be the non-study eye, or *fellow eye*.

8.1.2. Baseline Score

The *baseline score* is the observed measurement at Visit 2 (Baseline, Day 1). If a baseline score is missing, the last observed measurement or derived score prior to the first dose of study medication will be used to impute the baseline score.

8.1.3. Change and Percent Change from Baseline

The change and the percent change from baseline in a measure at a post-baseline visit will be derived as:

- Change = (Score at the Post-Baseline Visit) – (Baseline Score)
- Percent Change from Baseline = 100 × Change / (Baseline Score)

8.1.4. Efficacy Measures

Table 5 lists all the efficacy measures that will be evaluated in this study.

Table 5: Efficacy Measures

Efficacy Measures	Note
Distance-corrected Near Visual Acuity (DCNVA)	DCNVA will be measured in the right eye, left eye and both eyes at all visits using the distance manifest refraction. The total number of letters at 40 cm will be recorded.
[redacted]	[redacted]
Near Activity Visual Questionnaire (NAVQ): Rasch score and overall satisfactory score	Any N/A responses are scored according to the median overall score. The summated score from the main body of 10 questions is adjusted to a Rasch score from 0 to 100 using a conversion table (Section 12.1) such that 0 indicates no difficulty at all with any near tasks, and 100 indicates extreme difficulty with all near activities.

Table 5: Efficacy Measures (Continued)

Efficacy Measures	Note
Patient Global Rating of Treatment	At Visit 6 (month 2), patients will be asked to rate their overall satisfactory with the treatment on a 4-point Likert scale with responses including 'Unsatisfactory', 'Not very satisfactory', 'Satisfactory', and 'Very satisfactory'.
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
Objective and Subjective Refraction	Objective refraction will be performed as per standard of care using autorefractor available at clinical site at all study visits. Subjective refraction with "push plus" approach will be performed at all visits to determine the best correction for a subject to perform the visual acuity tests.

8.1.5. Visual Acuity Response Endpoints

Binocular DCNVA response endpoints are defined as follows:

- Binocular DCNVA 1-line gain response: having an increase in DCNVA of 5 letters (1 line) or more from baseline and having no decrease in BCDVA of more than 5 letters (1 line). That is, change from baseline in DCNVA $\geq$ 5 letters and change from baseline in BCDVA $\geq$ -5 letters.
- Binocular DCNVA 2-line gain response: having an increase in DCNVA of 10 letters (2 lines) or more from baseline and having no decrease in BCDVA of more than 5 letters (1 line). That is, change from baseline in DCNVA $\geq$ 10 letters and change from baseline in BCDVA $\geq$ -5 letters.
- Binocular DCNVA 3-line gain response: having an increase in DCNVA of 15 letters (3 lines) or more from baseline and having no decrease in BCDVA of more than 5 letters (1 line). That is, change from baseline in DCNVA $\geq$ 15 letters and change from baseline in BCDVA $\geq$ -5 letters.

The three Study Eye DCNVA response endpoints are defined similarly. [redacted]

For a response endpoint, the response rate at a given post-baseline visit is calculated as the proportion of subjects who met the response criterion at that visit.

8.1.6. Treatment Satisfactory Response Endpoints

The treatment satisfactory response endpoint is defined as having the response of either 'Satisfactory' or 'Very Satisfactory' when asked to rate the overall satisfactory with the treatment under Patient Global Rating of Treatment questionnaire.

8.1.7. Refractive Status and Manifest Refraction Spherical Equivalent Endpoint

Manifest refraction spherical equivalent (MRSE) is calculated by adding the spherical power with half of the cylindrical power, where spherical and cylindrical power are measured from the subjective refraction test.

Based on the MRSE, a refractive status of an eye will be defined as follows:

- Myopia: MRSE < -0.25D
- Emmetropia: -0.25D ≤ MRSE ≤ +0.25D
- Hyperopia: MRSE > +0.25D

8.1.8. Lens and Corneal Elasticity

Five parameters for lens elasticity are (1) width of the bottom plateau, (2) width of the top plateau, (3) height of the plateau, (4) slope of the anterior cortex, and (5) slope of the posterior cortex. Lens elasticity will be measured in triplicate on each eye at each visit. Measurement values across multiple scans of each parameter will be averaged and used for the analysis. Any unsuccess scans whose all 5 parameters are zeros will be excluded before the averaging calculation.

Corneal elasticity will be measured in duplicate on each eye at Baseline and Month 2 visit. The average value of corneal elasticity across multiple scans will be used for analysis.

[redacted]

8.2. Analyses of Primary Endpoint

8.2.1. Historical Control Data

Searching the clinicaltrials.gov database for presbyopia clinical studies with pharmacological treatment with the exposure duration of 2 months or longer, the study team identified three studies with similar study populations and inclusion/exclusion criteria. Out of three studies, only one (NCT02516306) has reported the study results (Korenfeld, 2011) with the same endpoint as this study's primary endpoint. Based on the historical data of 23 subjects in the control group, the estimated mean ± standard deviation of the change from baseline in binocular DCNVA at Month 2 is reported as -0.085 ± 0.087 logMAR, or 4.25 ± 4.35 EDTRS letters read.

A power prior with a power parameter w, presenting the prior knowledge learned from a study with n_0 subjects, will be derived based on the estimated mean $\widehat{\mu_0}$, and standard deviation s_0 as follows:

$$\mu \sim \mathrm{N}\left(\widehat{\mu_0}, \frac{s_0^2}{w \cdot n_0}\right),$$

where μ is the mean change from baseline in binocular DCNVA, $\mathrm{N}(\cdot, \cdot)$ denotes the normal distribution with the first and second parameter indicating mean and variance, respectively. Hence, the effective sample size borrowing from the historical control data will be $w \cdot n_0$.

8.2.2. Primary Analyses

The primary efficacy endpoint is the change from Baseline (Day 1) in binocular DCNVA at Month 2 (Visit 6).

For comparison of 0.1% STN1013600 vs Placebo and of 0.3% STN1013600 vs Placebo, the primary endpoint will be evaluated under the Bayesian framework in accordance with the following null (versus alternative) hypothesis:

$$\mathrm{H_0}: \mu_T \leq \mu_C$$

versus

$$\mathrm{H_A}: \mu_T > \mu_C$$

where μ_T and μ_C is the mean value of change from baseline binocular DCNVA at Month 2 of the treatment group and the control group, respectively.

For the primary analysis, a power prior with power parameter w [redacted] will be used for the control group mean and a non-informative prior, $p(\mu_T) \propto 1$, for the treatment group mean. Based on the posterior means and variances for treatment and control groups, the probability of (Treatment mean – Control mean > 0) given the data i.e., $P(\mu_T - \mu_C > 0 \mid \text{Data})$ will be calculated. The null hypothesis $\mathrm{H_0}$ will be rejected and the efficacy of the treatment will be declared when $P(\mu_T - \mu_C > 0 \mid \text{Data}) > 0.95$. The details for historical data borrowing analysis are in Section 12.2 .

8.2.3. Sensitivity Analyses

To assess the robustness of results from the primary analysis in the presence of prior-data conflict (i.e., the mean of historical data is different from the observed mean of the placebo arm), a mixed-effect model for repeated measures (MMRM) will be performed in observed cases up to Month 3 for the primary endpoint. The model will include treatment, analysis visit, and treatment-by-visit interaction as fixed effects, and baseline binocular DCNVA at baseline as a covariate, and subject as a random effect. The denominator degrees of freedom for fixed effects will be estimated using the Kenward-Roger approximation. An unstructured covariance matrix will be used to model the within-subject errors.

If there are convergence issues, covariance matrix structures other than unstructured will be tried in the following order: (1) heterogeneous Toeplitz (TOEPH), (2) heterogeneous autoregressive of order 1 (ARH(1)), (3) heterogeneous compound symmetry (CSH), and (4) compound symmetry (CS). The first covariance structure that converges will be used for this sensitivity analysis. Least squares mean change from baseline at Month 2 and differences between least squares treatment arm means and associated 90% confidence intervals will be reported.

In addition, the following sensitivity analyses will be performed to assess the robustness of the results from the primary analysis:

- The primary analysis will be repeated on the PP Population.
- [redacted]

8.3. Analyses of Secondary Efficacy Endpoints

8.3.1. Analyses of Continuous Secondary Endpoints

The following secondary endpoints will be analyzed using MMRM on observed cases up to Month 3:

- Mean change from baseline in Study Eye DCNVA at all visits
- Mean change from baseline in Binocular DCNVA at all visits
- Mean change from baseline in Rasch score at Month 2 and Month 3 as measured by Near Activity Visual Questionnaire (NAVQ)
- Mean change from baseline in the NAVQ overall satisfactory rating of near vision at Month 2 and at Month 3

For the analysis of the above secondary endpoints, the MMRM model will include treatment, visit, and treatment-by-visit as fixed effects, baseline as covariate and subject as random effect. The denominator degrees of freedom for fixed effects will be estimated using the Kenward-Roger approximation. An unstructured covariance matrix will be used to model the within-subject errors. When non-convergence issue arises, covariance structure in the following order will be tried: (1) heterogeneous Toeplitz (TOEPH), (2) heterogeneous autoregressive of order

1 (ARH(1)), (3) heterogeneous compound symmetry (CSH), and (4) compound symmetry (CS). The first covariance structure that converges will be used for the analysis.

8.3.2. Analyses of Binary Secondary Endpoints

The following binary secondary endpoints will be summarized descriptively:

- Having Binocular DCNVA 1/2/3-line gain from baseline at each visit
- Having Study Eye DCNVA 1/2/3-line gain from baseline at each visit
- Proportion of subjects with treatment satisfactory assessed by Patient Global Rating of Treatment at Month 2

The responder rates will be tabulated using frequencies and percentages based on observed cases.

For exploratory purposes, comparison between 0.1% STN1013600 vs Placebo and between 0.3% STN1013600 vs Placebo in response rate will be performed using the Fisher's Exact test for a 2x2 contingency table.

8.4. Subgroup Analyses

The homogeneity of treatment effects among prospectively defined subgroups will be assessed via descriptive statistics of the change from baseline in Binocular DCNVA by analysis visit for the following subgroups:

- Age (47-50 or 51-55 years)
- Sex (males or females)
- Race (White or non-White)
- Baseline Binocular DCNVA (< 60 or ≥ 60 ETDRS letters)
- Baseline refractive status of the Study Eye (Myopia or Emmetropia or Hyperopia)

Other subgroup analyses may be performed as deemed necessary.

8.5. Exploratory Analyses

[redacted]

- [redacted]

9. SAFETY ANALYSES

9.1. Safety-Related Definitions

9.1.1. Adverse Event

Under Protocol 101360002IN, an AE is defined as any *on*-study untoward medical occurrence (e.g., sign, symptom, disease, syndrome, intercurrent illness) that occurs in a study subject, regardless of the suspected cause and regardless of the timing of the study medication administration. An on-study AE can occur any time after the date of informed consent through the last study visit. An AE will be considered as *treatment-emergent* if the AE occurred on or after the treatment start date up to 8 days after treatment end date (or the last study visit). Treatment-emergent AEs (TEAEs) are a subset of on-study AEs. Both on-study and TEAEs will be collected, but only TEAEs will be tabulated.

The severity of each AE will be graded by the Clinical Investigator as Mild, Moderate, or Severe. AEs will also be rated by the Investigator as to their causality/relationship to the study drug.

Each AE will be classified into a system organ class (SOC) and coded to a preferred term (PT) using Medical Dictionary for Regulatory Activities (MedDRA), version 25.1 published in September 2022.

9.1.1.1. Ocular Adverse Event

An AE will be counted as an *ocular AE* if the Clinical Investigator selected "Ocular" under 'Event Location' on the AE eCRF.

9.1.1.2. Adverse Drug Reaction

An AE will be counted as an *adverse drug reaction* (ADR) if the Clinical Investigator answered 'Related' to the AE eCRF question "Relationship to Study Drug."

9.1.1.3. Serious Adverse Event

An AE will be counted as a *serious adverse event* (SAE) if the Clinical Investigator selected "Yes" to the question 'Is the adverse event serious?' on the AE eCRF. Any AE is considered a SAE if it fulfills one or more of the following criteria:

- Death (i.e., the AE caused or led to death)

- Life threatening (i.e., immediately life-threatening)
- It required prolonged inpatient hospitalization.
- It resulted in a persistent or significant disability/incapacity (i.e., the AE resulted in a substantial disruption of the subject's ability to carry out normal life functions).
- It resulted in a congenital anomaly/birth defect in the offspring of a study subject who was exposed to study therapy prior to conception or during pregnancy.
- It is a medically significant event(s), which may include "sight-threatening events," that may not meet any of the above serious criteria but may jeopardize the subject or require medical or surgical intervention to prevent one of the outcomes listed above.

9.1.1.4. Events of Special Interest

For this study, *events of special interest* (ESI) are pregnancy and clinically significant study medication administration error.

9.1.2. Safety Measures

Table 6 lists the safety measures to be evaluated for this study.

Table 6: Safety Assessments

Safety Measures	***Note***
Intraocular pressure (IOP)	The examiner will measure IOP using a Goldmann Applanation Tonometer in each eye at least two, and sometimes three, consecutive times. IOP will be the mean of 2 readings or median of 3 readings of the first 2 readings are 3 mmHg or more apart.
Slit-lamp Biomicroscopy	Slit-lamp biomicroscopy examinations (severity scores for 12 parameters: anterior chamber cells, anterior chamber flare, lid hyperemia, lid edema, conjunctival hyperemia, conjunctival chemosis, corneal edema, corneal staining, keratic precipitates, lens, anterior synechiae of iris, posterior synechiae of iris) will be performed and graded at all visits. Cataract severity will be assessed for subjects with phakic lens.
Ophthalmoscopy	The ophthalmoscopy (fundus) examination will be performed with dilation at Visit 1 (Screening) and Visit 7 (Month 3) and without dilation at other study visits. Variables from ophthalmoscopy are cup-to-disc ratio, and assessments of vitreous, retina, macula, choroid, and optic nerve.

Table 6: Safety Assessments (Continued)

Safety Measures	*Note*
Pupil measurement	Pupil measurement will be performed with ambient lighting conditions (10-12 lux) with an automated device. The same device should be used for all visits on a subject.
Best Corrected Distance Visual Acuity (BCDVA)	BCDVA will be recorded for right eye, left eye and both eyes at all visits using ETDRS charts. The total number of letters at 4 meters and 1 meter will be recorded. If a subject's visual acuity is so poor that he/she cannot read any chart letters when tested at 1 meter, then the subject's ability to count fingers, detect hand motion, or have light perception should be evaluated.

The safety-related measures collected in this study include AEs, slit-lamp biomicroscopy (severity scores for 12 parameters), ophthalmoscopy (cup-to-disc ratio, and assessments of vitreous, retina, macula, choroid, and optic nerve), intraocular pressure, pupil diameter, and BCDVA. The Safety population will be used for all summaries.

All the safety-related measures will be summarized descriptively by actual treatment received. Except AEs and BCDVA, the descriptive summary of each ocular safety-related measure and the change from baseline in that measure will be performed for study eyes and fellow eyes separately.

9.2. Adverse Events

Subjects with any AE(s) will be tabulated by type of AE(s) for each actual treatment received and overall. Unless otherwise specified, any AE experienced by either eye or both eyes will be counted once for that AE. In addition to the overall AE summary, subjects with any AE(s) will be tabulated by SOC and preferred term. A subject who experienced multiple AEs within a SOC or preferred term will be counted only once for that SOC or preferred term. Ocular AEs, SAEs, ADRs, Serious ADRs, non-serious AEs (including number of events) will be tabulated similarly.

AEs, AEs leading to death, AEs leading to discontinuation, SAEs, ADRs, serious ADRs, ocular AEs, and non-TEAEs, if any, will be listed separately.

9.3. Intraocular Pressure (IOP)

IOP (mmHg) and changes from baseline will be summarized by analysis visit for study eyes and fellow eyes, separately. In addition, any worsening of ≥ 10 mmHg from baseline will be listed.

9.4. Pupil Measurement

Pupil diameter (mm) and changes from baseline will be summarized by analysis visit for study eyes and fellow eyes, separately. Any clinically significant change from baseline in pupil diameter (more than 2.5 mm) will also be listed.

9.5. Best Corrected Distance Visual Acuity (BCDVA)

BCVDA (ETDRS letters) and changes from baseline will be summarized by analysis visit for both eyes, study eyes, and fellow eyes, separately. In addition, any worsening of 10 letters (2 lines) or more from baseline will be listed.

9.6. Slit-lamp Biomicroscopy

Twelve parameters for slip-lamp biomicroscopy are (1) anterior chamber cells, (2) anterior chamber flare, (3) lid hyperemia, (4) lid edema, (5) conjunctival hyperemia, (6) conjunctival chemosis, (7) corneal edema, (8) corneal staining, (9) keratic precipitates, (10) lens (cataract), (11) anterior synechiae of iris, (12) posterior synechiae of iris. Any additional findings from biomicroscopy examination will be combined into a "Other" category for summary purpose. For each biomicroscopy parameter, frequency and percentage of rating scores will be summarized by analysis visit for study eyes and fellow eyes, separately. The rating score of "Other" category of an eye at a visit will be the most severe rating of all findings within the category. In addition, any worsening (increase) of ≥ 2 units from baseline in severity for all parameters except anterior chamber cells and anterior chamber flare, and any worsening from baseline in severity for anterior chamber cells and anterior chamber flare will be listed.

9.7. Ophthalmoscopy

Cup-to-disc ratio will be summarized with n, mean, standard deviation, median, minimum, and maximum by analysis visit for study eyes and fellow eyes separately. In addition, subjects with at least 0.2 increase in cup/disc ratio from baseline will be listed.

Retina, macula, choroid, vitreous, and optic nerve will be assessed as normal or abnormal. Frequency and percentage of rating will be summarized by analysis visit for study eyes and fellow eyes, separately. In addition, subjects with change from baseline from normal to abnormal in these parameters will be listed.

10. SUMMARY OF CHANGES TO THE PROTOCOL

10.1. Secondary and Exploratory Endpoints

10.1.1. Secondary efficacy endpoints

Based on the communication with regulatory agency, a clarification was added to the secondary efficacy endpoint "Proportion of subjects who improve 1/2/3-lines or more in Study Eye and Binocular DCNVA at each visit". The new definition of this endpoint will be "Proportion of subjects who improve 1/2/3-lines or more in Study Eye and Binocular DCNVA at each visit without loss of more than 1 line (5 letters) in Best Corrected Distance Visual Acuity (BCDVA)."

10.1.2. Safety endpoints

Additional safety endpoints were added to further assess the safety of the study drug: pupil size and best corrected distance visual acuity.

10.1.3. Exploratory endpoints

[redacted]

10.2. Primary Analysis

In the protocol, the superior of the treatment arm over placebo arm would be establish of the probability of (Treatment mean – Placebo mean > 0) given the data is greater than 90%. In this SAP, the criterion was revised to be more stringent with 95% will be used to establish the superior of the treatment over the placebo. The new threshold of 95% will guarantee the (Bayesian) type I error to be lower than or equal to 0.10.

Analysis will be based on observed cases instead of multiple imputation approached imputed values due to the following rationales:

- In general, discrepancy in analysis results due to different imputation approaches are minimal when the amount of missing data is small. Based on masked review of subject disposition, few of than 5% of subjects with missing data due to missed visits and premature study discontinuation.

11. REFERENCE

1. Korenfeld, M. S., Robertson, S. M., Stein, J. M., Evans, D. G., Rauchman, S. H., Sall, K. N., Venkataraman, S., Chen, B. L., Wuttke, M., Burns, W. (2021) Topical lipoic acid choline ester eye drop for improvement of near visual acuity in subjects with presbyopia: a safety and preliminary efficacy trial. Eye (Lond).

12. APPENDIX

12.1. Conversion Table for Near Activity Visual Questionnaire (NAVQ)

The summated NAVQ score for the initial 10 questions can be converted to Rasch score using the following table:

Non-adjusted score	Rasch score	Non-adjusted score	Rasch score
0	0	16	56.89
1	10.72	17	58.89
2	18.13	18	60.84
3	23.07	19	62.77
4	26.96	20	64.68
5	30.30	21	66.81
6	33.3	22	68.58
7	36.08	23	70.62
8	38.70	24	72.77
9	41.22	25	75.09
10	43.65	26	77.68
11	46.02	27	80.72
12	48.32	28	84.59
13	50.56	29	90.55
14	52.73	30	100
15	54.84		

Any N/A responses are scored according to the median overall score for the subject.