VISUS Therapeutics
Protocol #: VT-001

A 3-Dose, Multicenter, Randomized, Double-Masked, Crossover Phase 2 Safety and Efficacy Study of BRIMOCHOL™ (Carbachol/Brimonidine Tartrate Fixed-Dose Combination) Topical Ophthalmic Solution vs. BRIMOCHOL™ F (Carbachol /Brimonidine Tartrate Fixed-Dose Combination) Topical Ophthalmic Solution vs. Monotherapy with Carbachol Topical Ophthalmic Solution in Subjects with Emmetropic Phakic and Pseudophakic Presbyopia

Statistical Analysis Plan

Version 1.0
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Approved by:

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[redacted]

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I. Introduction

A. Background

Presbyopia is an inevitable, age-related, gradual loss in the ability to focus at intermediate and near targets without spectacle or surgical correction due to a progressive loss of elasticity in the crystalline lens. The known pharmacology and the results of previous investigator-initiated clinical studies of BRIMOCHOL suggest the addition of an alpha-2 agonist to a cholinergic agent, could have additive direct and indirect effects on both the iris constrictor and dilator muscles leading to robust and durable miosis and improvement in near visual acuity over monotherapy alone. Prior clinical studies and more recent nonclinical studies conducted by Visus demonstrate that the fixed-dose combination of BRIMOCHOL and BRIMOCHOL F both demonstrate a contribution of elements vs. the individual monotherapies formulated similarly not only on pupil size, but that iris/ciliary body carbachol area under the curve (AUC) concentrations are ~50% higher with BRIMOCHOL than carbachol alone. Ex vivo studies in bovine ciliary muscle suggest alpha-2 receptors inhibit the contraction of cholinergically innervated ciliary muscle that does not occur when the ciliary muscle is at rest. These findings support the rationale for combining an alpha-2 agonist with a cholinergic agent to minimize the adverse events (AEs) of browache/headache, myopic shift, and intraocular pressure (IOP) changes associated with cholinergics alone, and indeed, clinical studies with BRIMOCHOL suggest this is the case. There are currently no Food and Drug Administration (FDA)-approved drug products to treat presbyopia. The protocol describes the general approach to analysis of data from the study. This analysis plan describes additional detail needed to complete such an analysis.

B. Protocol and Amendment History

This Statistical Analysis Plan (SAP) is based on Protocol VT-001 Amendment 8 Version 9 dated on 28 July 2021.

VISUS/ Protocol: VT-001
[redacted] #: UZA81693

Version	Approval Date	Salient Changes, if any*
[redacted]		

[redacted]

*Only changes related to statistical analysis are included here.

This SAP will govern the analysis of data from this study. The plan may be modified until the time of treatment unblinding. Any deviations from the analysis plan, including any after the time of treatment unblinding, will be documented as such in the study report.

II. Protocol Objectives

The main objectives of this study are:

1. To compare the efficacy, pharmacodynamics (PD), safety, [redacted] t of topically administered, BRIMOCHOL 2.75% (carbachol 2.75%/brimonidine tartrate fixed-dose combination 0.1% with benzalkonium chloride [BAK]) vs. BRIMOCHOL F 2.75% (carbachol 2.75%/brimonidine tartrate fixed-dose combination 0.1% BAK free) vs. carbachol 2.75% monotherapy (BAK free) topical ophthalmic solutions administered [redacted] in inducing miosis and improving near visual acuity among subjects with emmetropic phakic and pseudophakic presbyopia
2. To characterize the systemic pharmacokinetic (PK) profile of fixed-dose combinations BRIMOCHOL and BRIMOCHOL F topical ophthalmic solutions in subjects with emmetropic phakic and pseudophakic presbyopia

III. Study Endpoints

A. Efficacy Endpoints

Primary Efficacy Endpoints:

a. Proportion of subjects with a ≥15 Early Treatment Diabetic Retinopathy Study (ETDRS) letter gain from Baseline in binocular uncorrected near visual acuity (BUCNVA) without a ≥5 ETDRS letter loss in binocular uncorrected distance visual acuity (BUCDVA) at Hour 1 under mesopic conditions
b. Proportion of subjects with a ≥15 ETDRS letter gain from Baseline in BUCNVA without a ≥5 ETDRS letter loss in BUCDVA at [redacted] under mesopic conditions
c. Proportion of subjects with a ≥15 ETDRS letter gain from Baseline in BUCNVA without a ≥5 ETDRS letter loss in BUCDVA at [redacted] under mesopic conditions
d. Proportion of subjects with a ≥15 ETDRS letter gain from Baseline in BUCNVA without a ≥5 ETDRS letter loss in BUCDVA at [redacted] under mesopic conditions
e. Proportion of subjects with a ≥15 ETDRS letter gain from Baseline in BUCNVA without a ≥5 ETDRS letter loss in BUCDVA at [redacted] under mesopic conditions

[redacted]

B. Safety Endpoints

[redacted]

c. Ocular and non-ocular AEs

C. Pharmacodynamic Endpoints

Change from Baseline in pupil size in each eye at all timepoints

D. Pharmacokinetic Endpoints

Concentration and the following PK parameters of carbachol and brimonidine tartrate in plasma:

a. Area under the plasma concentration-time curve from time 0 to time of last quantifiable concentration [AUC(0-last)]
b. Observed maximum plasma concentration (Cmax)
c. Time of maximum plasma concentration (tmax)

E. [redacted]

[redacted]

F. [redacted]

IV. Study Design

A. Design Overview

This is a 3-dose, multicenter, randomized, crossover, Phase 2 safety and efficacy study in emmetropic phakic or pseudophakic adults with presbyopia at study sites in the United States. There will be 5 study visits with total participation for each subject of up to approximately 30 days. Subjects will be randomly allocated in a 1:1:1 ratio to one of 6 crossover treatment sequences. Each subject will receive the following topical ophthalmic solutions:

- BRIMOCHOL 2.75% (carbachol 2.75%/brimonidine tartrate fixed-dose combination 0.1% with BAK)
- BRIMOCHOL F 2.75% (carbachol 2.75%/brimonidine tartrate fixed-dose combination 0.1% BAK free)
- Carbachol 2.75% monotherapy (BAK free)

[redacted]

[redacted]

B. Study Population

Approximately [redacted] subjects with visually significant emmetropic phakic or pseudophakic presbyopia will be enrolled. This will include enrolling approximately [redacted] in the PK sampling group.

A subject in the PK sampling stratum who discontinues at any time during [redacted]

C. Sample Size Predictions

The sample size was not calculated based on statistical considerations for this study.

D. Treatment Randomization

Subjects will be randomly allocated to 1 of the following crossover treatment sequences:

[redacted]

E. Assessment Schedule

[redacted] and procedures Table 1.2 for details.

V. Interventions

Study Procedures

The study procedures for the key study outcomes are as follows:

1. *Visual Function Assessments:* Visual acuity tests will be performed using the early treatment diabetic retinopathy study (ETDRS) test. The table below shows the characteristics of each visual function assessment performed

Table 2: Summary of Visual Function Assessments

Visual Function Assessments	Laterality	Distance	Light Level	Timepoints of measurements
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

2. *Pupillometry*: An objective pupillometer will be used to measure the diameter of the pupil . The following results will be reported for each eye:
   - Mean diameter of the pupil in
   - Standard deviation of the pupil diameter

3.

4. Ocular Health Assessments: Ocular Health will be assessed with slit-lamp biomicroscopy and dilated ophthalmoscopy
   - Slit-lamp Assessments: Assessments will be performed at the screening visit and visit 2, 3, 4 and 5 after final evaluation. Slit-

lamp biomicroscopy findings of the Conjunctiva, Cornea, Anterior Chamber, Iris/Pupil, Lens are categorized as: 1) Normal, 2) Abnormal, Not Clinically Significant and 3) Abnormal, Clinically Significant. Cell grade findings are categorized as 0,0.5: 1-5,1: 6-15, 2: 16-25,3: 26-50, and 4: > 50. Shaffer grade are categorized as Grade 0: 0^0, Grade 1: 10^0, Grade 2: 20^0, Grade 3: 20^0-35^0, Grade 4: 35^0-40^0

- Ophthalmoscopy Assessments: to be performed at the screening visit and at visit 5 after final evaluation. Ophthalmoscopy findings of the Macula, Retina, Choroid, Vitreous and Optic nerve are categorized as: 1) Normal, 2) Abnormal, Not Clinically Significant and 3) Abnormal, Clinically Significant.

VI. General Analytical Considerations

A. Data Sources

Data are recorded on electronic case report form (eCRF) for each subject screened/enrolled in this study. These forms will be used to transmit information collected during the study to the Sponsor and regulatory authorities, as applicable. Pupillometry data, visual function data, laboratory data, ECG and blood samples data are collected separately by the selected vendors. Section 11 of the protocol provides additional details regarding data handling and record keeping.

Statistical analysis will be performed following [redacted] standard operating procedures and on the [redacted] computer network. All statistical analysis will be performed using SAS Version 9.4 with program code prepared specifically for the project by qualified [redacted] statisticians and SAS programmers.

All observed and derived variables that are analyzed or summarized will be listed by subject. Descriptive statistics will provide an overview of the efficacy and safety results. For categorical parameters, the number and percentage of subjects in each category will be presented. For continuous parameters, descriptive statistics will include number of subjects, mean, SD, median, minimum, and maximum.

B. Definition of Baseline

[redacted]

[redacted]

C. Analysis Visit Window

All study medications will be administered at the study site, therefore, no analysis visit window mapping based on actual study day will be derived. All efficacy and safety endpoints will be summarized and analyzed according to their nominal visit. Unless otherwise specified, data of unscheduled visits will not be considered for the by-visit summary statistics but will be included in the by-subject listings.

D. Missing Data

Missing efficacy data will be imputed as following:

Observed Cases (OC): [redacted]

[redacted]

Multiple Imputation (MI): [redacted]

[redacted]

The example SAS code for PROC MI are shown in Appendix A.

The imputation model may be modified based on the actual data if there is an issue in model convergence.

Last Observation Carried Forward: [redacted]

[redacted] will be imputed as per Section XI.

E. Multiple Comparisons

Aside from the control of Type I error in the primary analysis of the primary endpoint, no control for the effect of multiple comparisons is planned for secondary and exploratory analysis.

F. Analysis Populations

Several analysis populations are defined for use with various analyses

a. Safety Population

All enrolled subjects who received any amount of study drug will be included in the Safety population. Subjects will be analyzed as treated.

b. Modified Intent-to-treat Population

The modified intent-to-treat (mITT) population will include all subjects who receive at least 1 dose of study drug, have the primary efficacy assessments available at Baseline (pre-dose at Hour 0 on a dosing day) and at Hour 1 (on the same dosing day) for at least 1 dosing day. Subjects will be analyzed as treated.

c. Per Protocol (PP) Population

The Per Protocol (PP) Population will include all mITT subjects who do not significantly violate the protocol. The PP population will be identified prior to locking the database. Subjects will be analyzed as treated.

d. Pharmacodynamics (PD) Population

All subjects who received study drug and had at least one evaluable, post-dose pupillometry assessment without protocol deviations or events deemed to affect the assessment of the pupils. Subjects excluded from the PD population will be reviewed and determined prior to database lock and unblinding.

e. Pharmacokinetics (PK) Population

All subjects who received study drug and had at least one evaluable, post-dose plasma concentration of carbachol or

brimonidine tartrate (i.e., without protocol deviations or events deemed to affect the PK).

G. Data Display Characteristics

Data displays produced for this study will include three types - summary tables, data listings, and figures. Unless stated otherwise, data listings will be produced for all recorded data. Summary tables will be produced as specified in following sections. Additional data listings will be produced for outcome measures that involve extensive procedures to derive the analyzed outcomes. Figures will be produced when specified in sections to follow.

Data listings will simply list the data recorded on the eCRF or derived for each subject. In general, they will be ordered by dosing regimen, subject number, visit and time of assessment. When expedient, additional levels of ordering hierarchy may reflect subsets of assessments within subject (e.g., further ordering by lab test names in the lab listings). For all listings unless otherwise stated, [redacted] The treatment associated with each visit will be included in all listings. For listings with monocular assessments, results of the right eye will be presented first.

Demographics, medical history, ocular history, disposition, and protocol deviations will be summarized for [redacted] Summary tables will display summary statistics calculated for each of the treatments and by timepoint, unless described otherwise in following sections.

VII. Subject Accountability

A. Baseline Characteristics

Subject Characteristics: Data collected about the following subject characteristics at the screening visit will be summarized:

- Age (years)
- Sex
- Race
- Ethnicity
- Weight (kg)
- Height (cm)

- Body Mass Index (BMI, kg/m²)

Ocular Characteristics: Data will be summarized by dose cohort for all subjects in the safety and mITT populations.
Data collected about the following ocular assessments at the screening visit will be summarized:

- Lens Status (Phakic vs. Pseudophakic)
- [redacted]
- Pupil Diameter
- Pupil Area
- Intraocular Pressure measurement
- Corneal stain total score

Medical/Ocular History. Medical/Ocular history will be summarized for all subjects in the safety population.
Medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA Version 23.1 September 2020) and summarized by system organ class (SOC) and preferred term (PT). Ocular history will be summarized for each eye. Ocular history involving both eyes will be counted for each eye separately.

B. Disposition

Subject disposition will be summarized for all screened subjects, the numbers of subjects who were screened, randomized, and either completed the study or prematurely withdrew from study participation. Study completion will be indicated by the response “Yes” on the study completion status form. Any other response on this form will be counted as a premature withdrawal.

Premature withdrawals will be further characterized as the number of subjects who withdrew prematurely for each of the following reasons listed on the study completion status form:

- Adverse Event
- Non-compliance with study drug
- Withdrawal by Subject
- Death
- Pregnancy
- Study terminated by sponsor
- Lack of Efficacy
- Protocol Deviation

- Physician Decision
- Lost to follow-up
- Other

Percentages of subjects who withdrew for each of these reasons will be calculated using all members of the relevant population for the denominator. Subjects in each analysis population will be summarized.

C. Protocol Deviations

The sponsor will review and determine major protocol deviations prior to database lock. A summary table will be used to report the number and percentage of subjects with major protocol deviations. All reported protocol deviations will be listed.

D. Subject Cohorts

The study protocol was initially implemented with all subjects receiving

[redacted]

Cohort.

[redacted]

VIII. Efficacy Analyses

All efficacy data will be summarized and analyzed [redacted]

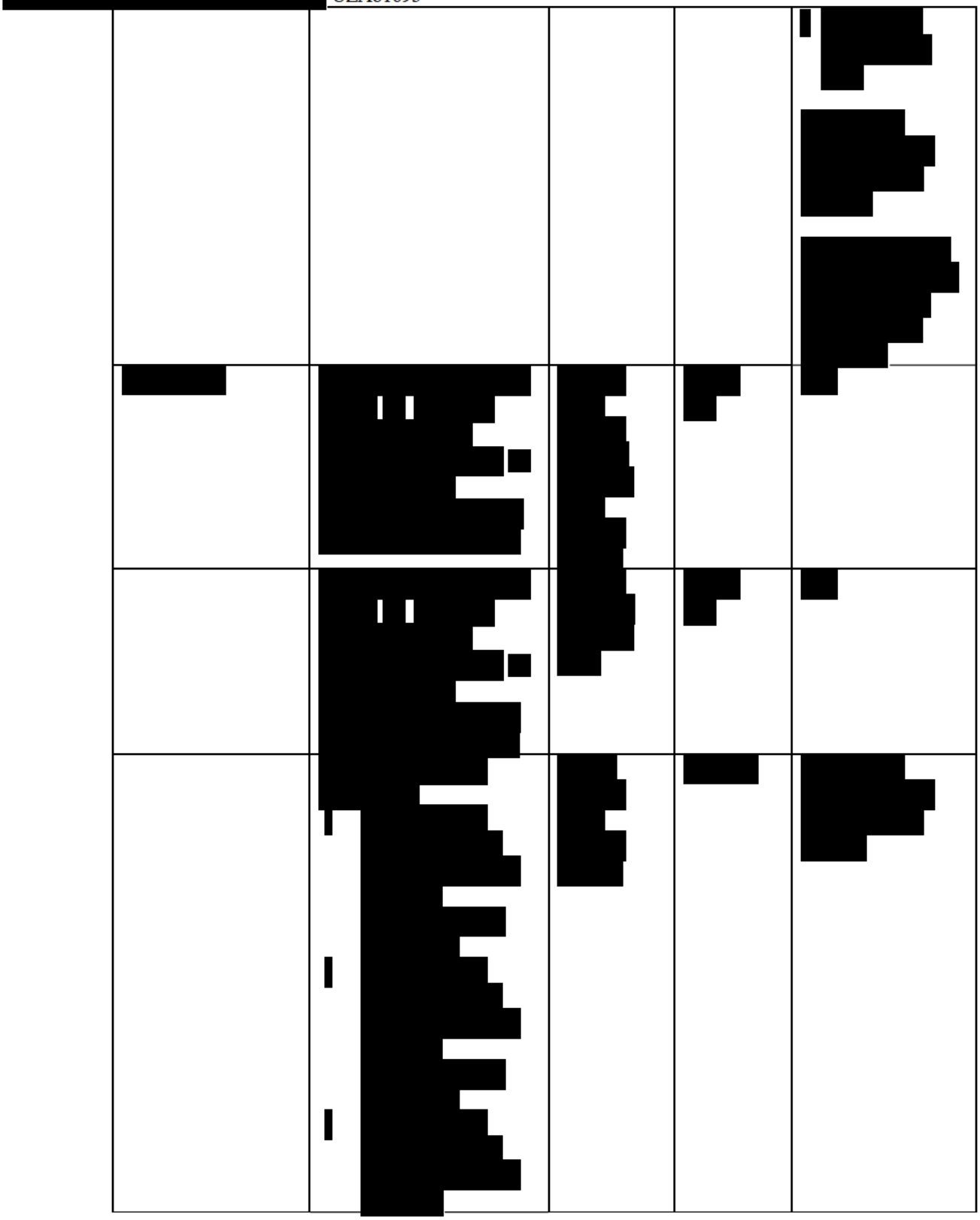
The primary analysis of the efficacy endpoints will be performed [redacted] in the PP population.

The efficacy analyses performed [redacted]

All planned efficacy analyses are summarized in the table below.

Table 3: Summary of Efficacy Analyses

Analysis	Efficacy Endpoint	Dose Cohort	Primary Analysis	Sensitivity Analysis
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]



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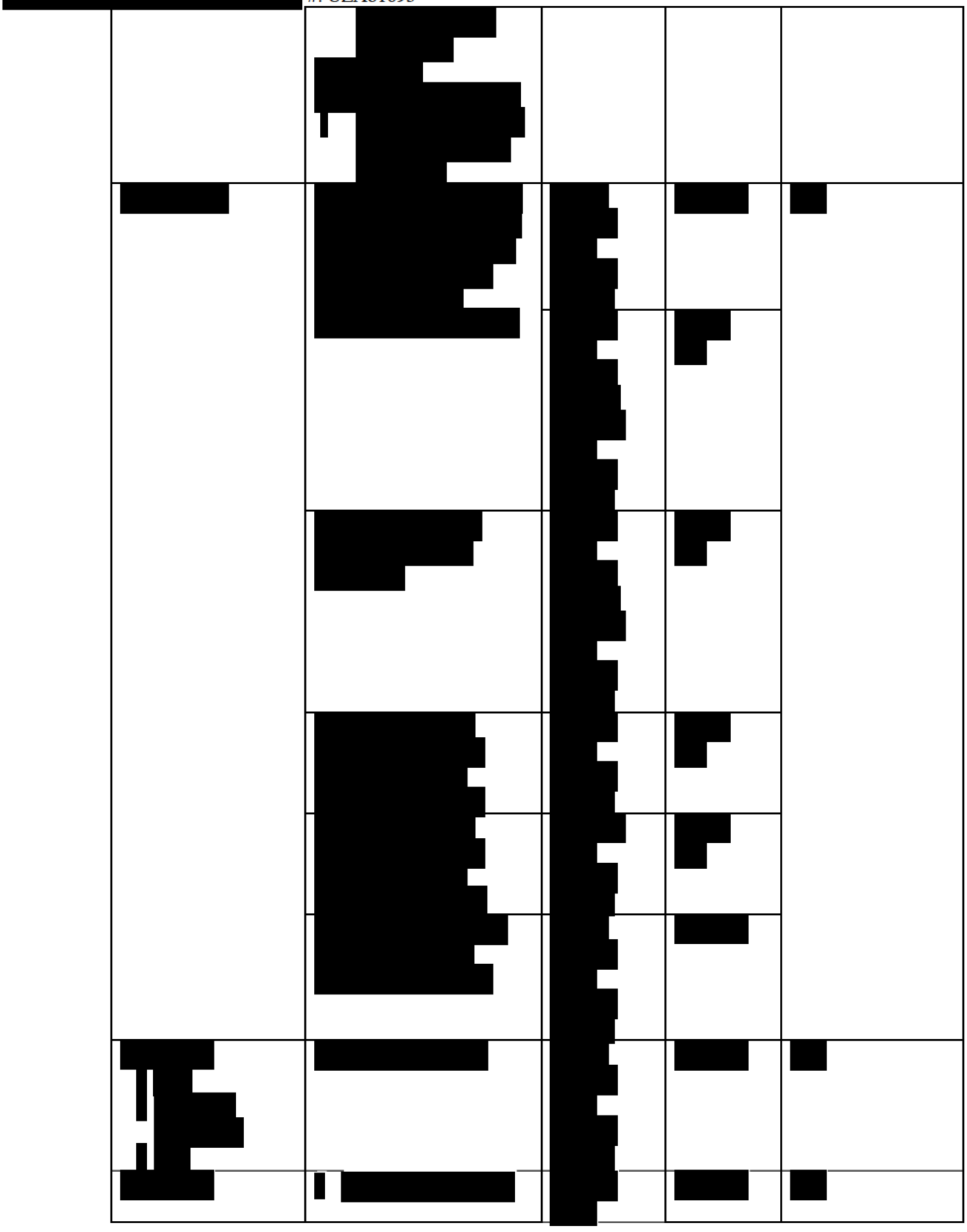


[redacted]

A. Efficacy Outcomes

Visual acuity outcomes assessed by ETDRS will be calculated as the change from screening visit baseline in the ETDRS letter score at each post-dose timepoint. A positive change will be denoted as a letter gain while a negative change will be denoted as a letter loss.

Primary visual acuity endpoints are proportion of subjects with at least 15 ETDRS letter gain from screening visit baseline in BUCNVA without a ≥ 5 ETDRS letter loss in BUCDVA using both eyes under mesopic conditions at:

- 1-hour post study drug administration

[redacted]

B. Primary Efficacy Outcome Analysis.

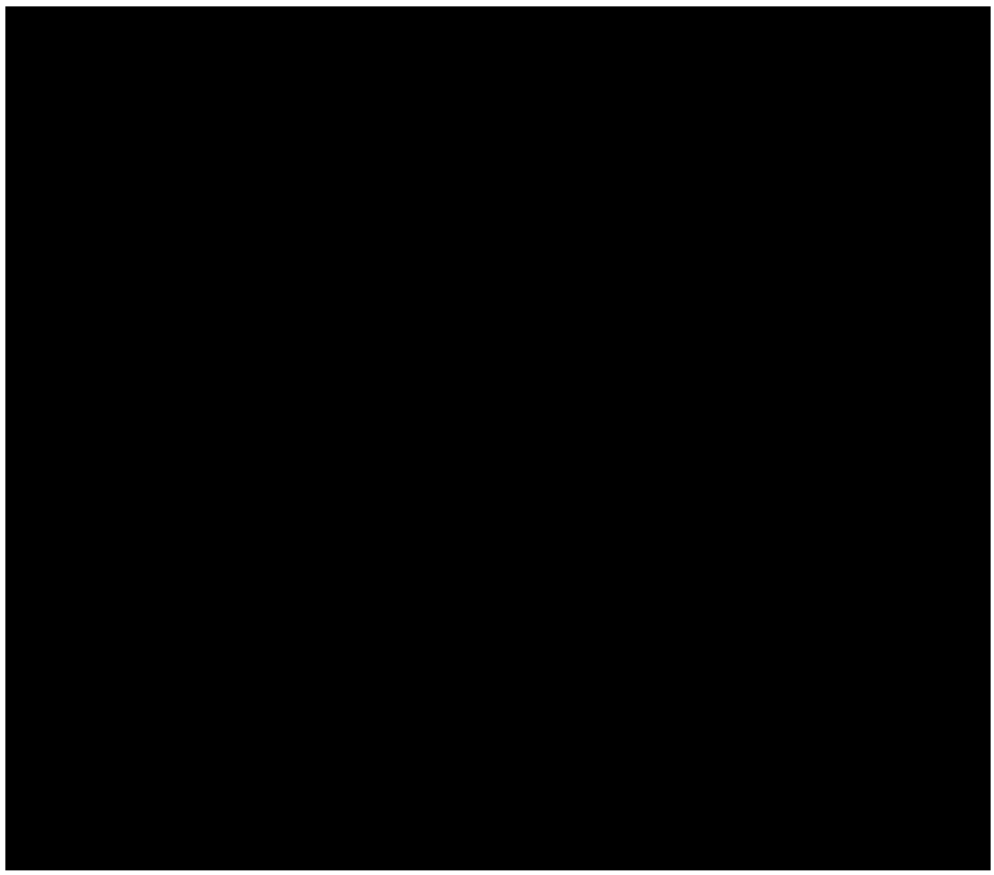
The primary analysis of the primary efficacy outcome will be a Generalized Estimating Equations (GEE) model for repeated measures

with an alternating logistic regression (ALR) method to model associations by using the log odds ratios (Carey et al, 1993).

[redacted]

[redacted]

Figure 1: Order of Testing



[redacted]

Sensitivity Analyses

1. [redacted]

2. [redacted]

3. [redacted]

4. [redacted]

5. [redacted]

C. [redacted]

[redacted]

D. [redacted]

a. [redacted]

b. [redacted]

c. [redacted]

d. [redacted]

e. [redacted]

E. Efficacy Analysis on Subgroups of Subjects

[redacted]

1. [redacted]
2. [redacted]
3. Sex (Female, Male)

[redacted]

F. Other Efficacy-related Summaries

[redacted]

Descriptive statistics will be used to summarize the following:

- [redacted]
- [redacted]
- [redacted]
- [redacted]

IX. Pharmacodynamic Analyses

[redacted]

[redacted]

X. Pharmacokinetic Analyses

A. Pharmacokinetic Concentrations

A subject listing of PK blood sample collection times, derived sampling time

[redacted]

[redacted]

B. Pharmacokinetic Parameters

[redacted]

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[redacted]

[redacted]

XI. Safety Analyses

Safety analyses will be performed based on the Safety population. Safety analyses will be performed separately for each dosing cohort [redacted]

A. Adverse Events

Adverse events will be coded using the MedDRA dictionary version 23.1. AE summaries will include treatment-emergent AEs (TEAEs), that is, AEs with an onset date or worsening in severity on or after the date of any study treatment administration.

Missing AE start date will be imputed using the following algorithm:

- If completely blank, assume January 01 and year of the stop date (if available) or the year of screening visit date (otherwise).
- If only year is recorded, assume January 01 for the missing month and day.
- If only month and year are recorded, assume 01 for the missing day.

Missing AE stop date will be imputed using the following approach:

- If completely blank, assume the day of the last study visit.
- When only year is recorded, assume the 31st December for the missing date (if the year is before the year of the last study visit) or the date of the last study visit (otherwise).
- If only month and year are recorded, assume the last day of the month.

If the relationship to study drug is missing, the event will be conservatively summarized as being related to the study drug. If severity is missing, a

separate category of missing severity will be included in the summary table, and no imputation of severity will be performed. Through the data cleaning process, all attempts will be made to avoid missing values for relationship and severity.

TEAEs occur on or after the administration of BRIMOCHOL F, BRIMOCHOL and carbachol will be summarized separately. An AE starting after the administration of the study drug at a treatment visit and continuing into the next treatment visit will only be categorized under the treatment given at the onset visit if the severity is not worsened in the next period. On the other hand, if an AE continues into the next treatment visit and the severity is worsened during the next treatment period, the AE will be attributed twice: once to the first treatment for the onset and the other time to the second treatment for worsening. Similarly, if an AE occurs and stops during one treatment period but recurs in the next treatment period, the AE will be attributed to both treatments.

AEs will be summarized by treatment and overall, as incidence rates of:

- All AEs
- All TEAEs
- All Ocular TEAEs
- All Non-ocular TEAEs
- Serious TEAEs
- Deaths
- Ocular Events of Special Interest
- Treatment-related TEAEs
- TEAEs leading to study drug withdrawal
- TEAEs by maximum severity

In addition, the following AE summaries will be produced:

- All ocular TEAEs by preferred term (PT) in descending order of overall frequency
- All non-ocular TEAEs by system organ class (SOC) in alphabetical order and preferred term (PT) in descending order of overall frequency
- Serious TEAEs by SOC and PT
- Ocular events of special interest by PT
- TEAEs leading to study drug withdrawal by SOC and PT. This subset includes TEAEs with an Action Taken of “Permanent Discontinuation.”
- Treatment-related TEAEs by SOC and PT. This table will include TEAEs with a drug relationship of “Possible,” “Probable,” and

"Definite". It will also include TEAEs with missing drug relationships as the missing relationship will be regarded as related. An AE reported by a subject more than once will be included in this table if at least one of the drug association grades is one of the grades listed here.

- TEAEs by SOC, PT and maximum severity. On this table, treatment groups will be subdivided into four potential grades of AE severity—Mild, Moderate, Severe, Life threatening. TEAEs missing a severity grade will be included in a separate "Missing" category. An AE reported by a subject more than once will be represented in the most severe category.

At each level of summarization, a subject will be counted once if he/she reported one or more events.
No hypothesis test will accompany these AE summary tables.

All AEs will be listed by subject, detailing the verbatim term given by the investigator, PT, SOC, onset date and time, end date and time, severity, relationship to study drug, outcome, action taken with study drug, other action taken to treat the event, seriousness and criteria for seriousness, sight-threatening AE (Y/N), most recent study treatment administered prior to onset along with the dosing date and time.

B. Ocular Assessments

Visual acuity Assessments: [redacted]

Ocular Health Assessments: The findings of slit-lamp biomicroscopy and ophthalmoscopy assessments (Conjunctiva, Cornea, Anterior Chamber, Iris/Pupil, Lens, Macula, Retina, Choroid, Vitreous and Optic nerve) will be summarized for each visit. Changes from baseline will be summarized using shift tables. Counts and percentages will be used to summarize Cell grade and Shaffer grade categories at the screening visit.
Descriptive statistics will be reported for IOP measurements and change in IOP.

C. Clinical Laboratory Results

Central labs are performed at the screening visit only. Laboratory test results (including hematology, coagulation, serum chemistry, and urinalysis) and abnormal laboratory values will be summarized for all subjects in the safety population and presented in data listings.

D. Vital Signs

Vital signs measurements including pulse rate, temperature, systolic blood pressure, and diastolic blood pressure, at each scheduled visit and changes from the Screening visit will be summarized by treatment. All vital signs measurements will be listed by subject.

E. Physical Examination

Physical examination are performed at the screening visit only. Categories of findings (normal, abnormal, not done) on physical examination will be presented in a by-subject listing.

F. Prior and Concomitant Medications

Prior and concomitant medications will be coded using the WHODrug Version 01 September 2020 (Global, B3 format)
Missing medications start date will be imputed using the following approach:

- If completely blank, assume 01Janyyyy, where yyyy is the year of the Stop date (if available) or the year of Screening visit date (otherwise).
- If only year is recorded, assume 01Jan for the missing month and day.
- If only month and year are recorded, assume 01 for the missing day.

Missing medications stop date will be imputed using the following approach:

- If completely blank, assume the day of the last study visit.
- When only year is recorded, assume the 31Dec for the missing date (if the year is before the year of the last study visit) or the date of the last study visit (otherwise).
- If only month and year are recorded, assume the last day of the month.

Prior medication: Any medication with a start date prior to the first dose of study drug is classified as a prior medication.

Concomitant medication: Any medication is classified as a concomitant medication if the start date is on or after the first dose of study drug or any

prior medication (whose start date is before the first dose of study drug) which is ongoing after the first dose of study drug and then has an increase in either the dosage or the frequency. Thus, a prior medication ongoing after the first dose of study drug is NOT classified as a concomitant medication if it does not increase either the dosage or the frequency during the study.

Concomitant medications will be further attributed to study treatment depending on which treatment was taken when the medication was administered. For example, concomitant medication attributed to treatment A will be any medications with start date on or after initiation of treatment A but before the initiation of the next study treatment or any medications started prior to the instillation of treatment A but had dose or frequency changes after the instillation of treatment A.

Concomitant medications/therapies for will be summarized by treatment using WHO Drug Dictionary (WHO-DD) Anatomical-Therapeutic-Chemical (ATC) classification and preferred term (PT). The highest available level of ATC among ATC 1-4 will be presented. For example, if ATC 1, ATC 2 and ATC 3 are available, the summary table and subject listing will present ATC 3.

G. 12 Lead Electrocardiogram

Electrocardiogram (ECG) are performed at the screening visit only. Overall ECG interpretation categorized as Normal, Abnormal and Not Evaluable will be presented in a by-subject listing.

XII. [redacted]

XIII. [redacted]

XIV. References

1. Carpenter, J. R. and Kenward, M. G. (2013), Multiple Imputation and Its Application, New York: John Wiley & Sons.
2. Carey, V., Zeger, S. L., and Diggle, P. J. (1993). “Modelling Multivariate Binary Data with Alternating Logistic Regressions.” *Biometrika* 80:517–526.
3. Dmitrienko A, Tamhane AC. (2011). Mixtures of multiple testing procedures for gatekeeping applications in clinical trials. Statistics in Medicine. 30, 1473–1488.Rubin DB. Multiple imputation for nonresponse in surveys. New York, NY: John Wiley and Sons; 1987.SAS Institute Inc: SAS® 9.4 Statements: Reference, Third Edition. Cary, NC: SAS Institute Inc; 2014.
4. Rubin DB. Multiple imputation for nonresponse in surveys. New York, NY: John Wiley and Sons; 1987.

Appendix A Example SAS codes

[redacted]

[redacted]