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Statistical Analysis Plan

AG-920-CS301

A Randomized, Double-Masked, Vehicle-Controlled, Parallel Evaluation of the Local Anesthetic Effect of [redacted] Sterile Topical Ophthalmic Solution

Protocol Number:	AG-920-CS301
IND Number:	[redacted]
Investigational Product:	[redacted] Sterile Topical Ophthalmic Solution (AG-920)
Indication:	Topical anesthesia for intravitreal injection
Phase:	Phase 3
Sponsor:	[redacted]
Plan Date:	17 Jun 2020
Plan Version:	Version 2.0

SIGNATURE PAGE

We, the undersigned, declare that we have thoroughly reviewed this analysis plan for completeness and accuracy with the Protocol Requirements, CRF details, Database, SOPs and ICH-GCP.

Prepared by Sign and Date: _____

[redacted]

Approved by Sign and Date: _____

[redacted]

Document Version History

Version	Date	List of changes
0.1	27/Jul/2020	Initial draft released for sponsor review.
0.2	15/Sep/2020	Changes made throughout the document to update different sections; clarify the primary and secondary datasets to be used for the analysis; clarify the analysis methods for the primary and secondary endpoints.
0.3	08/Oct/2020	Incorporated changes of mock-shells based on sponsor comments/feedback and amended protocol.
0.4	29/Oct/2020	Added more clarification of the secondary endpoints including examples of how individual subjects will be counted for different situations.
0.5	07/Dec/2020	Integrated finalized mock-shells
1.0	07/Dec/2020	Initial Final Version release
1.1	30/Mar/2021	Reason for amendement: An amdenment for aligning additional analsysis tables generated and included in the draft CSR after initial finalised SAP. Mock-shells have been updated. Following list of tables and listings has been added and/ or updated. [redacted]

Version	Date	List of changes
		[redacted]

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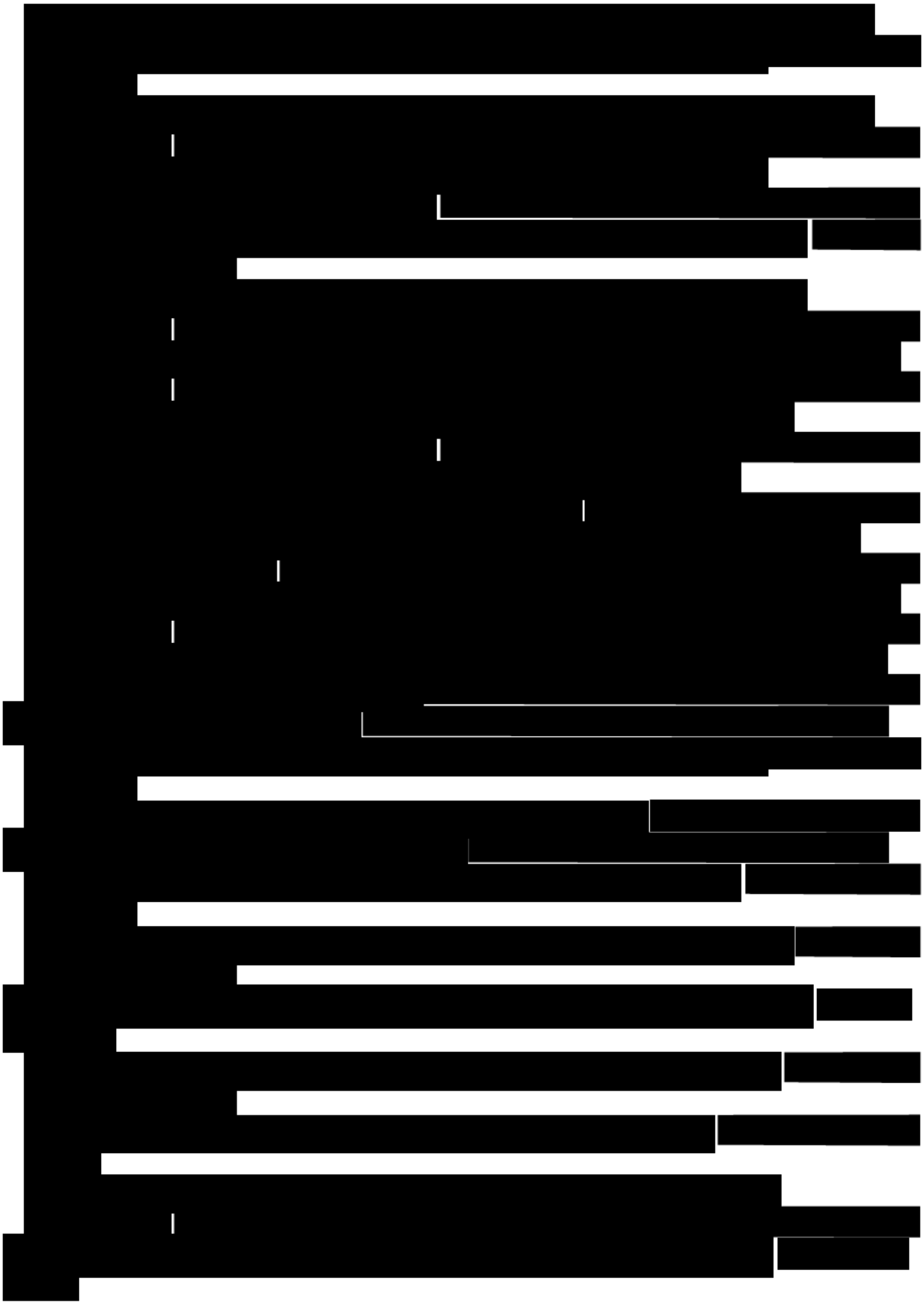

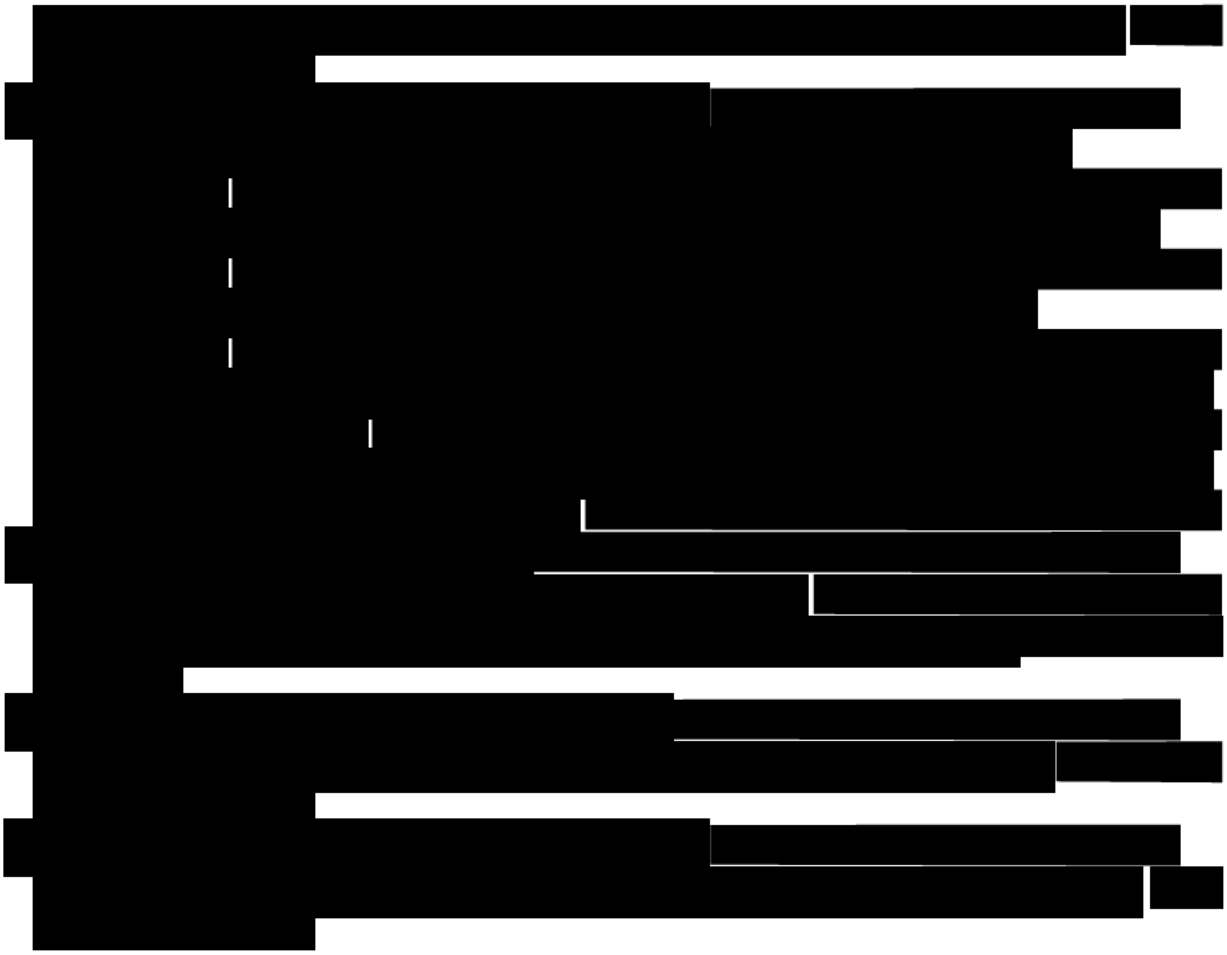

List of Abbreviations

ADaM	Analysis Data Model
AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
BCVA	Best Corrected Visual Acuity
CI	Confidence Interval
CRF	Case Report Form
CSR	Clinical Study Report
DDD	Defined Daily Dose
EDC	Electronic Data Capture
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IMP	Investigational Medicinal Product
IOP	Intraocular Pressure
ITT	Intent to Treat
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
OD	Right Eye
OS	Left Eye
OTC	Over The Counter
PP	Per Protocol
PT	Preferred Term
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SOC	System Organ Class
SOP	Standard Operating Procedure
TEAE	Treatment Emergent Adverse Event
TLFs	Tables, Listings, and Figures
WHOCC	World Health Organization Collaborating Center

1. Administrative Structure

1.1. Study Sponsor

1.2. Introduction and Scope

This Statistical Analysis Plan (SAP) provides detailed statistical methodology for the analysis of data from protocol number AG-920-CS301, Version 1.0 (09-Jun-2020) that will be presented in the Clinical Study Report (CSR). The variables and methods described in this plan supersede those described in the protocol. Any deviations from the analyses described in the final SAP will be noted in the CSR.

The SAP may also serve as a reference for the creation of any outputs required outside of the CSR, e.g., abstracts, posters, presentations, manuscripts and management updates. Data used for these analyses will have a status aligned to the database lock guidance.

The statistical analyses will be done in accordance with the ICH-E9 guidelines "Statistical Principles for Clinical Trials" and applicable local guidelines.

1.3. Responsibility of Data Management Team

The responsibility of providing clean data for the statistical analysis lies with TAB Clinical Data Management team. The Data Management team has designed the eCRF in the Castor EDC system, version 2020.2.8, in order to perform data capture, data review and data cleaning including a query management process. The data review, cleaning and locking are described in detail in the Data Management Plan and the related SOPs.

1.4. Responsibility of SAS Programmer or Statistician

SAS programmers or statisticians will be responsible for programming the statistical tables, listings and figures (TLFs), as well as the ADaM/STDM datasets as per the approved SAP for the study clinical data.

2. Study Objectives and Endpoints

2.1. Study Objectives

Primary Objective:

- To evaluate anesthetic efficacy of AG-920

Secondary Objectives:

- To evaluate how long it takes one dose of AG-920 to anesthetize the eye
- To evaluate how long one dose of AG-920 anesthetizes the eye
- To evaluate the safety and tolerability of AG-920

2.2. Endpoints

Primary Endpoint

- The proportion of patients with no pain at 5 minutes

Secondary Endpoints

- The proportion of patients with no pain within 5 minutes
- Mean time to no pain score (onset)
- Mean duration of anesthetic effect
- Visual acuity, biomicroscopy, adverse events (AEs), treatment-emergent adverse events (TEAEs), serious AEs (SAEs), withdrawals due to TEAEs

3. Study Design

3.1. Overall

A brief description of the study design and activities is provided below. For detailed information, refer the study design section of the protocol.

This is a randomized, placebo-controlled, double-masked, parallel-group study to evaluate the local anesthetic effect of [redacted] Sterile Topical Ophthalmic Solution in healthy volunteers. The study will consist of one or two clinic visits and one follow-up phone call. Subjects will be screened for entry at Visit 1 and randomized/treated at Visit 2. Visit 1 and 2 may be on the same day. The subjects will be randomized in a 1:1 ratio to receive a single dose of either AG-920 or Placebo (vehicle) in one (study) eye. The study eye will be randomized as either the right eye (OD) or the left eye (OS). The single dose will be administered by the clinic staff as two drops in the study eye 30 seconds apart. All ocular assessments at Visits 1 and 2 will be performed on both eyes. Subjects will then have a safety follow-up phone call 1-4 days following Visit 2 (Study Day 2-5).

Approximarely 120 subjects are planned to be enrolled to ensure 60 in each treatment group.

3.2. Sample Size

With 60 subjects per treatment group and assuming a 15% response in the vehicle group for the primary endpoint, this study will have 88% power to detect a treatment effect of at least 25% (40% vs. 15%) between AG-920 and placebo. This is based on a two-sample chi-square test and assuming a two-sided Type I error rate (α) of 0.05.

3.3. Investigational Products

[redacted] Sterile Topical Ophthalmic Solution (AG-920) is a sterile, isotonic, non-preserved aqueous solution containing the active ingredient [redacted] Boric Acid, Mannitol, Sodium Acetate Trihydrate, Glacial Acetic Acid, and Edetate Disodium Dihydrate. [redacted]

Placebo ophthalmic solution is identical to the active product, with the exception of the active ingredient.

3.4. Randomization

A randomization code for allocating the treatments will be prepared by an independent biostatistician, who is not involved in the day-to-day conduct of the study. Subjects will be randomized in a 1:1 ratio to receive AG-920 or Placebo.

Treatment assignments will be masked to the Investigator, the clinical study team (Sponsor, personnel involved in day to day study management, Monitors, Data Managers, and Statisticians), and the subjects. Only in the case of medical emergency or occurrence of adverse events that warrant unmasking in the opinion of the Investigator, will the treatment assignment(s) be unmasked and made available to the Investigator and the Sponsor Safety Officer. In the absence of medical need, the randomization code will not be available to the above personnel until after the study is completed and the database is locked.

3.5. Post Treatment and Pinch Test

A [redacted] orceps will be used to "pinch" the inferior bulbar conjunctiva of the study eye at the [redacted]

As soon as the subject does not experience pain [redacted] pinching will stop until the 5-minute timepoint [redacted]

Within 15-60 minutes after the last pinch test, the following examinations will be performed:

- Best corrected visual acuity (BCVA)
- Slit lamp biomicroscopy and external eye exam
- Assessment for adverse events

[redacted]

3.6. Study Schedule

	Visit 1	Visit 2							Phone Follow-Up
	Screening	*Screening & Baseline*							
	Day -2 to 0/1	Day 1[1]							Day 2-5
	Pre-dose	Dose		[redacted]			5 m	15-60 m post last pinch	
Procedures		0 sec	30 sec						
Written Informed Consent	X								
Inclusion/Exclusion Criteria	X	X							
Demographics, Systemic and Ocular Medical History	X								
Concomitant Medication Query	X	X	X						X
OTC Tear Tolerability	X								
BCVA	X							X	
Urine Pregnancy Test (if applicable)	X								
Biomicroscopy and External Eye Exam	X							X	
IOP Measurement	X								
Randomization		X							
IMP Administration[2]		X	X						
Conjunctival pinch[3]				X	X	X	X		
Assessment of Pinch Pain				X	X	X	X		
Adverse Event Assessment		X	X	X	X	X	X	X	X

BCVA = Best corrected visual acuity, IOP = Intraocular pressure, OTC = over the counter, IMP = investigational medicinal product.

[1] Screening may occur on the same day as Visit 2 (≥60 minutes) or up to 3 days previously. If on separate days, inclusion/exclusion criteria will be re-evaluated prior to dosing subject to ensure subject still qualifies.

[2] One dose is 2 drops. First drop administered at 0 seconds and the second drop administered at 30 seconds (2 drops 30 seconds apart).

[redacted]

4. Analysis Sets

4.1. Randomized Population

The randomized population will include all subjects who are randomized to treatment. Baseline (screening) variables and demographic characteristics will be summarized for this population.

4.2. Intent-to-Treat (ITT) Population

The ITT population will include all randomized subjects who have received at least one dose (drop) of study medication. This population will be the primary population for the efficacy analyses and all efficacy variables will be summarized using this set of subjects. The ITT population will include all subjects as randomized, even if there are subjects who are given the wrong study medication.

4.3. Per-Protocol (PP) Population

The PP population is a subset of the ITT population, which will include those subjects (and their visits) who do not have major protocol violations likely to seriously affect the primary outcome of the study as judged by a masked evaluation prior to the unmasking of the study treatment. This population will be the secondary population for the efficacy analyses and all efficacy variables will be summarized using this set of subjects. If the PP and ITT populations are exactly the same and there are no missing data, then additional efficacy analyses on the PP population will not be performed. The PP population will include subjects as treated, so if there are subjects who are given the wrong study medication, then they will be included in the treatment group based on what they received.

4.4. Safety Population

The Safety population will include all randomized subjects who have received at least one dose (drop) of study medication. This population will be used to summarize the safety variables and will include all subjects as treated.

5. General Aspects of Statistical Analysis

All TLFs will be independently checked for consistency, integrity and in accordance with the applicable SOP(s). The shells for the TLFs are presented in a separate document.

5.1. Presentation of Summaries and Analyses

In general, continuous data will be summarized by descriptive statistics, i.e., n, mean, standard deviation (SD), median, minimum and maximum values, and categorical data will be summarized using counts (N: number of subjects per treatment group, n: number of subjects with non-missing values) and percentages. Percentages will be suppressed when the count is zero, however the category will still be displayed. The denominator for all percentages will be the number of subjects per treatment in the analysis set of interest (%: n/N*100), unless otherwise indicated.

In general, and unless specified otherwise, all data summaries will be sorted by the parameter under consideration and then by visit. Listings will be sorted as per subject ID, the parameter under consideration and then by visit.

Unless otherwise stated, baseline will be defined as the last value of the assessment recorded prior to first study drug administration.

5.2. Precision of Display

Means and medians will be rounded off to one decimal place more than the reported data values. Standard deviations will be rounded off to two decimal places more than the reported data values. Minimum and maximum values will be of the same precision as that of the reported data.

The precision of display for the variables will be up to one decimal place for percentage values, e.g., xx (xx.x), where the value inside the parentheses represents the percentage value. Counts (n) will be displayed as integers.

All *P* values will be reported based on two-sided significance tests and all *P* values less than or equal to 0.05 (≤0.05) will be considered statistically significant. *P* values will be reported to four decimal places or as <0.0001.

5.3. Strategy of Pooling the Data Across Centers

Not applicable for this single-site study.

5.4. Coding Dictionaries

The Medical Dictionary for Regulatory Activities (MedDRA) version 23.0 or higher will be used to code the adverse events and medical history. The World Health Organization Collaborating Center (WHOCC) Anatomical Therapeutic Chemical/Defined Daily Dose (ATC/DDD) Index 2020 classification system will be used to code the concomitant medications.

5.5. Handling of Missing/Partial Dates for Adverse Events

Because of the short duration of the study and only a single dose of study drug being given, missing or partial start or end dates for AEs are not expected. Therefore, the duration of most AEs should be able to be calculated. If there is a missing or partial start or end date for an AE, then the duration of that event will be missing.

Missing or partial dates will be listed in the subject data listing as they appear in the database.

5.6. Handling of Data from Unscheduled Visits

Data from unscheduled visits will only be displayed in subject data listings and will not be used in calculating summary statistics. However, if AEs occur or are reported during an unscheduled visit then those will be summarized.

6. Subject Disposition

The following subject disposition details will be summarized by treatment group and overall and listed to include the number and percentage of subjects:

- Informed Consent signed
- Screened
- Screened Failure
- Dosed or did not initiate treatment
- Completed the study as per protocol
- Discontinued study
- Withdrew from the study
- Included in each analysis set

These data will also be listed. Individual reasons for withdrawal will be presented in listings for all enrolled subjects.

7. Demographic and Baseline Measurements

7.1. Demographics

The following demographic variables and subject characteristics will be summarized for the enrolled (randomized) population (based upon screening visit data):

- Age (Completed years)
- Sex
- Ethnicity
- Race
- Iris color

Age will be summarized using descriptive statistics (n, mean, SD, median, minimum and maximum), and sex, ethnicity, race and iris color will be summarized using counts and percentages. All summary data will be presented by treatment group and overall. A subject-wise data listing of all demographic characteristics will also be presented.

7.2. Inclusion/Exclusion Criteria

The inclusion and exclusion criteria will be listed together with the overall eligibility for each subject.

7.3. Protocol Deviations

Protocol deviations are considered any deviation from the clinical study protocol relating to a subject, and include the following:

- Inclusion/exclusion criteria deviations.
- Dosing deviations (e.g., incorrect treatment received or administered to the incorrect eye).

- Time window deviations for safety assessments.
- Subjects receiving prohibited concomitant medications.
- Other procedural and study conduct deviations recorded by the site on the protocol deviation log.

All protocol deviations will be reviewed and finalized prior to database lock in order to define the per-protocol analysis population for the study.

All protocol deviations will be listed by subject.

7.4. Medical History

Medical history will be coded using MedDRA version 23.0 or higher. All medical history events will be listed by system organ class (SOC) and preferred term (PT) and will be summarized by treatment group and overall using the Safety population.

7.5. Prior and Concomitant Medication

All medications which the subject has taken within 30 days prior to screening and during the study will be recorded on the CRF. Concomitant medications are defined as those with a start date on or after the date of first IMP use and those with a start date prior to the first IMP use but with an end date on or after the date of first IMP use. If the medication dates are incomplete and it is not clear whether the medication was concomitant, it will be assumed to be concomitant.

Concomitant medication drug names will be coded using the WHOCC ATC/DDD Index 2020. All concomitant medications will be listed by reported generic name and coded medication term, indication, dose, dose unit, frequency, route of administration, start date and end date or 'ongoing' and duration of use.

7.6. Investigational Product Administration

Listings will be provided for the date of administration, kit number, study eye, time of each drop administration, treatment given, etc. for each enrolled subject.

8. Evaluation of Efficacy

The primary analysis population for efficacy will be the ITT population as defined in Section 4.2, and the secondary analysis population for efficacy will be the PP population as defined in Section 4.3. The PP population will be used to determine the robustness of the results, and all efficacy variables will be analyzed using both sets of subjects.

A minimal amount of missing data is expected in this study because of the short duration and the subjects being in the clinic on Day 1. For the ITT population, any missing efficacy data on Day 1 will be imputed using the method of last observation carried forward (LOCF), where the closest non-missing value prior to the missing value will be carried forward and imputed for the missing value. The PP population will be based on observed data only (without imputation). Any missing, unused, or spurious data will be noted and explained in the final clinical study report.

Subgroup analyses based upon pre-study characteristics such demographics (age, sex, race and iris color) may be completed to further investigate the efficacy measures.

8.1. Primary Endpoint

The primary efficacy endpoint in this study is the proportion of subjects with no pain at 5 minutes.

[redacted]

8.2. Secondary Endpoints

The secondary efficacy endpoints in this study include the proportion of subjects with no pain within 5 minutes, the mean time to no pain score (onset of anesthesia Effect in Minutes), and the duration of anesthetic effect.

[redacted]

[redacted]

Comparisons between the two treatment groups for the mean duration of anesthetic effect will be made using a two-sample t-test. The estimated difference in mean duration will be presented as AG-920 minus placebo, together with its 95% confidence interval and *P* value.

8.3. Interim Analyses

There are no interim analyses of efficacy planned for this study.

9. Evaluation of Safety

The data from subjects included in the Safety population will be used for summarizing the safety endpoints.

Listings of the safety data will be generated for all the subjects in the study.

9.1. Adverse Events

Adverse events will be reported starting from the time of signing the informed consent until the end of the study assessment, as is required by protocol. Adverse events with a start date and start time before the start of study medication will be considered non-treatment emergent adverse events. Such events will only be displayed in the individual subject data listing. Adverse events with a start date and start time on or after the start of study medication will be considered treatment emergent adverse events (TEAE). All summaries of adverse events will only include TEAEs.

For the following cases of a partial or missing start date or time, the adverse event will be considered treatment emergent:

- The event started on Day 1 but has a missing start time
- The event has a partial start date and the month and/or year are the same as the month and/or year of Visit 2 or later
- The start date is missing

Severity of event

For AEs, the following guidelines will be used to describe severity:

Mild	An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
Moderate	An event that causes sufficient discomfort and interferes with normal everyday activities.
Severe	An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.

Relationship of event to study drug

All AEs will have their relationship to study drug assessed by the Principal Investigator (PI) who will examine and evaluate the subject based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the following two categories:

Unrelated	• Clinical event with an incompatible time relationship to IMP administration, and that could be explained by underlying disease or other drugs or chemicals or is incontrovertibly not related to the IMP, **or** • Clinical event whose time relationship to IMP makes a causal connection improbable, but that could plausibly be explained by underlying disease or other drugs or chemicals.
Related	• Clinical event with a reasonable time relationship to IMP, but that could also be explained by concurrent disease or other drugs or chemicals, **or** • Clinical event with a reasonable time relationship to IMP and is unlikely to be attributed to concurrent disease or other drugs or chemicals, **or** • Clinical event with plausible time relationship to IMP, and that cannot be explained by concurrent disease or other drugs or chemicals.

Abbreviations: IMP=investigational medicinal product

If the PI is unable to determine the relationship between the adverse event and study drug administration, such a case will be classified as related to study drug administration, and the whole episode will be assessed in terms of expedited reporting to the regulatory authorities until the PI determines the final causality.

In general, events will be summarized by counts and percentages.

The following information will be summarized for TEAEs. All summaries will be presented by treatment group and overall:

- Overall Summary of TEAEs.
- TEAEs by SOC and preferred term (PT).
- TEAEs by SOC, PT, and causality.
- TEAEs by SOC, PT, and severity.
- TEAEs by SOC, PT, and verbatim terms.
- Listing of Serious AEs.
- Listing of AEs leading to discontinuation.

Also, a listing of all adverse events will be provided and will include the reported term, PT, SOC, start and end dates, seriousness, frequency of occurrence, severity, action taken regarding investigational drug, outcome, and relationship to investigational drug.

For each AE and SAE, the frequency of the event in the two arms will be summarized. The incidence of each event will be calculated as the percentage of subjects experiencing a particular AE, out of the total number of subjects at risk (Safety population) in that treatment arm and overall.

9.2. Other Safety Parameters

Best corrected visual acuity and slit lamp biomicroscopy will be measured at Visit 1 (screening) and at the end of Visit 2. BCVA will be measured in logMAR units and results will be summarized using descriptive statistics for the Visit 1 and Visit 2 values and for the changes from Visit 1 to Visit 2. Results will be presented separately for the study eye and non-study eye.

The biomicroscopy grading scales are given in Appendix 1 of the protocol; the following assessments will be done on four anterior locations of each eye:

- Lid (erythema and edema)
- Conjunctiva (hyperemia and edema)
- Cornea (edema)
- Anterior chamber (cells and flare)

The data for each assessment will be summarized using counts and percentages of eyes within each category and will be summarized for the Visit 1 and Visit 2 values and for the changes from Visit 1 to Visit 2. Results will be presented separately for the study eye and non-study eye.

10. Software Used for Analysis

SAS Software Version 9.4 or higher (SAS Institute Inc., Cary, NC), will be used for the statistical analysis and statistical reporting.

11. Changes from the Protocol

The variables and methods described in this plan will supersede those described in the protocol.

12. Requirements and Specifications (Mock-shells) for Programming and Presentation of Tables, Listings and Figures (TLFs)

This document is intended to be used only as a guide in the development of TLFs and may be adjusted to meet study and Sponsor specific needs.

The specifications for the TLFs must follow the description in the associated Statistical Analysis Plan (SAP).

The following details the requirements and specifications for the programming and presentation of the TLFs, as set out in the TLF shells.

- A separate RTF document will be created for each TLF.
- All TLFs will be produced in a landscape format, as far as is feasible.
- The margin, page size specifications are stipulated below and will used for the presentation of all TLFs:

Item:	**Specification:**
Page Layout	Landscape
Paper Size	8.5 x 11 inch

- The standard font size and font type are "9 point", "Courier New" for all TLFs.
- Every page of each output will have a header indicating the Sponsor name and protocol number (to be presented as set out in the example below).
- Every page of each output will contain a footer indicating the program name and the date and time when the output was produced (to be presented as set out in the example below).
- Every page of each output will contain a page number written in the format "Page X of Y" (to be presented as set out in the example below).
- The following treatment group labels will be used for all TLFs in the following order:
  - Treatment A: AG-920
  - Treatment B: Placebo

- The ordering of visits should be chronological (unless otherwise specified):
- Footnotes should be ordered as follows:
    1. Non-standard abbreviations separated by semi-colons
    2. n = Number of subjects … N = Total number of subjects … % = Percentage of subjects.
    3. Definitions.
    4. Footnotes pertaining to statistical methodology
    5. Version of the medical coding dictionary used for programming
    6. Study-specific footnotes further clarifying data presentation
- Each of the footnote types enumerated above will flow continuously instead of starting on a new line and end with a period.
- Footnotes are left justified.
    - Where applicable, each table should provide the listing supporting the table.
    - All listings should be sorted by site name/country, subject identifier, visit date/event date (in the case of multiple observations per visit date/event date, the observations should be sorted alphabetically within visit date/event date).
        - An example of country/subject identifier field: SGP/4000101
    - Calendar dates in the listings will be displayed in the format DD-MMM-YYYY, e.g. 15-SEP-2015. Where dates are partial, only the partial data will be listed.
    - Where a listing or table has been planned, but no data meet the criteria, then a single line stating 'No data meet the criteria for this report' will be provided in the output.
- All tables will be decimal aligned as specified in the study SAP

For all outputs containing calculated changes from baseline, the reference point will be specified in a footnote.