ASSIGN DMB

Statistical Analysis Plan

Final Analysis

PP-001-1101

Protocol: A phase I safety and tolerability study of PP-001 eye drops in healthy adult volunteers

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Confidential

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Revision History		
Version	Date	Reason for Revision
FINAL 1.0	23-Sep-2021	First version;
FINAL 2.0	12-Oct-2021	Change of responsibilities of statistical activities; transfer to ADMB template; alignment with ADMB standards

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List of Abbreviations

ADMB	Assign Data Management and Biostatistics
AE	Adverse Event
CSP	Clinical Study Protocol
CV	Coefficient of variation
ITT	Intent-to-treat
PK	Pharmacokinetic
PP	Per-protocol
PT	Preferred Term
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class

1. OVERVIEW

1.1 Study Objectives

1.1.1 Primary Objective

The primary objective is to assess the safety and tolerability of 1 day (single dose) and 12 days (48 doses) dosing with ascending doses (0.05% [0.5 mg/mL], 0.15% [1.5 mg/mL] and 0.3% [3.0 mg/mL]) of PP-001 eye drops compared to placebo eye drops in healthy subjects (cohorts 1-3). In addition, safety and tolerability will be tested in a group of 21 patients with ocular surface inflammation (cohort 4).

1.1.2 Secondary Objectives

- To assess the pharmacokinetics of PP-001 eye drops following 1 day (single dose) and 12 days (48 doses) dosing in healthy subjects
- To assess the pharmacodynamics, in relation to the eye, of PP-001 eye drops following 1 day (single dose) and 12 days (48 doses) dosing in healthy subjects and following 12 days (24 doses) dosing in patients with ocular surface inflammation. The following procedures will be used to assess the pharmacodynamics with respect to the eye: Best corrected visual acuity, visual field, intraocular pressure, slit lamp examination, dilated fundoscopy, fundus photography (cohorts 1-3 only), amsler grid.

1.2 Study Design

In this prospective, single-centre, double-blind, placebo-controlled, randomised, parallel-group study will be conducted in accordance with the European Union Clinical Trial Directive 2001/20/EC and 2005/28/EC, the Declaration of Helsinki (revised version of Fortaleza, Brazil 2013), Good Manufacturing Practice, Good Clinical Practice and the current national regulations and guidelines. It will be approved by both the local ethics committee and regulatory authority prior to the first subject being screened.

A total of 24 healthy subjects aged between 18 and 64 years will be included into the first three cohorts of eight subjects each. Each cohort will be performed in two parts. In the first part, subjects will be randomized to receive a single dose of PP-001 or placebo. If safety and tolerability is acceptable, subjects will move on to Part II, where subjects will receive PP-001 or placebo according to the randomization in Part I four times a day for 12 consecutive days. If safety and tolerability is demonstrated in Part II, dose escalation will occur in the next cohort.

- In Cohort 1 six subjects will receive four drops of 0.05% (w/v) PP-001 in only one eye and two subjects will receive placebo in only one eye
- In Cohort 2 six subjects will receive four drops of 0.15% (w/v) PP-001 in only one eye and two subjects will receive placebo in only one eye
- In Cohort 3 six subjects will receive four drops of 0.30% (w/v) PP-001 in only one eye and two subjects will receive placebo in only one eye

Cohorts 1 to 3 will be performed in dose ascending order. On the first two study days in Part I of each cohort, one subject will be dosed per day. On the third study day up to two subjects can be dosed per day. On the following study

day(s) up to 4 subjects can be dosed per day. Randomization will be performed in two blocks (3:1, PP-001 vs. placebo) for each cohort. Part II will start at least 7 days after the last subject has been dosed in Part I and the Principal Investigator and the sponsor decide whether or not to continue dosing the cohort in Part II. Within each cohort neither subject nor investigator knows if the subject receives placebo or PP-001. A data safety monitoring board will decide if the safety and tolerability demonstrated in Parts I and II of the previous cohort warrant continuing the study at the next highest dose.

In Cohort 4, twenty-one (21) subjects aged between 18 and 64 years with diagnosed ocular surface inflammation will be included. Subjects will receive half of the highest dose that was considered safe by the DSMB based on the safety data of cohorts 1-3. This cohort will be performed in one part (multiple instillations). Subject will receive two daily instillations for 12 consecutive days (in total 24 administrations). Out of 21 subjects, 14 subjects will receive PP-001 eye drops and 7 subjects will receive placebo. A data safety monitoring board will be installed for safety monitoring of cohort 4.

1.3 Endpoints

1.3.1 Primary Endpoints

The primary endpoint will be safety and tolerability as assessed through:

- Adverse events
- Serious AEs (SAEs)
- Laboratory safety tests
- Vital signs
- Physical examination

1.3.2 Secondary Endpoints

- The concentration of PP-001 in plasma at predose, postdose (30 min and 1h ± 5 minutes after dosing), day 8 and day 19 (30 min ± 5 minutes after last dosing of the day) will be determined.
- Pharmacodynamic parameters relating to the eye as assessed through:
    - Best corrected visual acuity
    - Visual field
    - Intraocular pressure
    - Slit lamp examination
    - ad slit lamp examination: Conjunctival hyperemia according to the Efron scale (cohort 4 only)
    - Dilated fundoscopy
    - Fundus photography (cohorts 1-3 only)
    - Amsler grid
    - OSDI score (cohort 4 only)
    - Tear film osmolarity (cohort 4 only)
    - Tear film break up time (cohort 4 only)

- Corneal fluorescein staining (cohort 4 only)
- Conjunctival staining using lissamine green (cohort 4 only)

1.4 Sample Size Calculation

Twenty-four healthy female or male subjects will be enrolled in cohorts 1-3. Twenty-one subjects will be enrolled in cohort 4. No power calculations have been performed and the sample size is based on the requirements of the study design.

2. GENERAL CONSIDERATIONS

2.1 Conduct of Analysis

This document outlines the statistical methods to be implemented in the analysis of the data of Clinical Trial PP-001-1101. The purpose of this plan is to provide general guidelines from which the analysis will proceed. It contains a more detailed elaboration of the principal features of the analysis described in the protocol.

One final statistical analysis will be performed for this study that will analyze all four study cohorts. The analysis will be conducted after finalization of the Statistical Analysis Plan, database closure and general unblinding of the statistical team.

2.2 Statistical Software and Quality Control

All statistical analyses will be performed using SAS® version 9.3 or higher. Tables, figures and data listings will be generated in Microsoft® Word® as well as PDF® format.

Quality control of SAS® programs will include a review of the whole process of result generation:

- Review of all analysis SAS® programs
- Review of SAS® log for errors, warnings and other notes that could indicate mistakes in the programs
- Review of all tables, listings and figures for completeness and correctness

2.3 Blinding and Randomization

This is a double-blind study within each cohort. Subjects will receive either PP-001 or placebo. Randomization will be done in two blocks of 4 subjects per block (3:1, PP-001 vs. placebo) for each of the three cohorts (cohorts 1-3). For cohort 4, randomization will be performed in a 2:1 ratio (14 subjects will receive the PP-001 and 7 will receive placebo). Subjects within one cohort will not be aware whether they are receiving PP-001 or placebo. The investigators will not know whether a subject receives PP-001 or placebo within a cohort. The IP will be provided in pre-packaged eye drop bottles which will be labelled in a double-blind fashion.
General unblinding of ADMB personnel and the sponsor will occur after database closure, for final statistical analysis.

2.4 Descriptive Analyses and Columns in Summary Tables

Descriptive analyses of continuous variables (summary statistics) will be described with the number of non-missing observations, arithmetic mean, standard deviation (±SD), median, quartiles (Q1 and Q3) and range (minimum and maximum).

Categorical variables (frequency statistics) will be described with the number of non-missing observations and percentages (%). Percentages will be calculated within each stratum on the total number of non-missing observations, if not stated otherwise.

In general, tables will present the following columns:

- Cohort 1 PP-001, 0.05% [0.5 mg/mL], 4 drops per day, healthy subjects
- Cohort 2 PP-001, 0.15% [1.5 mg/mL], 4 drops per day, healthy subjects
- Cohort 3 PP-001, 0.3% [3.0 mg/mL] , 4 drops per day, healthy subjects
- Cohort 1-3 PP-001 pooled
- Cohort 4 PP-001, 0.15% [1.5 mg/mL], 2 drops per day, patients with diagnosed ocular surface inflammation
- Placebo subjects from all cohort 1-3
- Placebo subjects from cohort 4
- All subjects (only for overall study information and baseline tables)

The disposition table may also include a column for screening failures. For tables by time point the columns for Cohort 1-3 and Cohort 4 might be shown in a separate table, since Cohort 1-3 have a different visit schedule than Cohort 4.

2.5 Inferential Analyses

A comparison will be made between the cohorts with regard to safety and tolerability. For this aim a Fisher's exact test will be calculated to compare the following treatment groups in the summary table for adverse events:

- Cohort 1 PP-001 vs. Cohort 4 PP-001
- Cohort 2 PP-001 vs. Cohort 4 PP-001
- Cohort 3 PP-001 vs. Cohort 4 PP-001
- Cohort 1-3 PP-001 pooled vs. Placebo subjects from all Cohort 1-3
- Cohort 4 PP-001 vs. Placebo subjects from Cohort 4

NOTE: The study is not powered for p-values and the p-values are just calculated for exploratory reason.

2.6 Center and Country Effect

Not applicable. This is a single centre study.

2.7 Handling Missing Data

Missing values will not be imputed.

2.8 Medical Coding

Adverse Events will be coded according to MedDRA. MedDRA preferred terms and system organ class will be used for AE summaries. Concomitant medications and medical and ophthalmic histories will not be coded.

2.9 Analysis Populations

2.9.1 Intent-to-Treat Analysis Set

The Intent-to-treat (ITT) Analysis Set will consist of all randomized subjects regardless of whether or not the subject received study drug.

The ITT analysis set will be used for baseline summaries and as secondary analysis set for pharmacodynamics analysis.

2.9.2 Per-protocol Analysis Set

The Per-protocol (PP) Analysis Set will consist of all ITT Analysis Set subjects who have no major protocol deviations and who complete the study up to the end of the post-study assessments (i.e. attended the follow-up visit).
Major protocol deviations will be identified by the sponsor and provided to the statistician in order to create the per-protocol analysis set.
The PP analysis set will be used as primary analysis set for pharmacodynamics analysis.

2.9.3 Safety Analysis Set

The Safety Analysis Set will consist of all subjects who receive any amount of study drug (including placebo). All safety analyses will be conducted in this population. Safety analysis will be performed according to actual treatment.

2.9.4 Pharmacokinetic Analysis Set

All subjects who receive any amount of study drug will be included in the formal analysis of pharmacokinetic parameters providing they have at least one evaluable pharmacokinetic sample.
All pharmacokinetic analyses will be conducted in this population.

2.10 Subject Data Listings

Listings will be sorted by treatment group / cohort, subject ID, and/or parameter and/or visit (in this order), where applicable. Treatment group / cohort will be shown in all listings.

2.11 Changes in the Conduct of the Study or Planned Analysis

In CSP Version 4.0 (March 26, 2021), which is the latest protocol version at the time when writing this SAP, concomitant medication is mentioned erroneously as primary endpoint. This is not included as a primary endpoint in the SAP, since it is not considered a relevant safety endpoint.

The recruitment of the first healthy subject in Cohort 1-3 was in November 2018. In June 2019, the study was placed on-hold while Cohort 3 was in progress, and Cohort 3 was stopped prematurely. The study was re-started with addition of a 4th Cohort which recruits patients with ocular surface inflammation starting in May 2021.

3. OVERALL STUDY INFORMATION

The following information will be analyzed descriptively and corresponding details on the subject level will be provided in data listings:

- Subject overview
- Inclusion/exclusion criteria (will be listed only)
- Protocol deviations
- Attended visits with dates
- Study completion
- Study drug administration

4. BASELINE EVALUATION

The following information will be analyzed descriptively and corresponding details on the subject level will be provided in data listings:

- Demographic information
- Medical and ophthalmic histories
- Prior/concomitant medication
- Non-pharmacologic treatments and procedures

5. PHARMACOKINETICS / PHARMACODYNAMICS ANALYSIS

Pharmacokinetics:

The PP-001 concentrations in plasma will be listed.

Pharmacodynamics:

The following information will be analyzed descriptively and corresponding details on the subject level will be provided in data listings (Note: Some parameters are just available for either Cohort 1-3 or Cohort 4):

- Best corrected visual acuity
- Visual field
- Intraocular pressure
- Dilated fundoscopy
- Fundus photography
- Amsler grid
- Ocular Surface Disease Index
- Tear film osmolarity
- Slit lamp examination
- Tear film break up time
- Corneal staining with fluorescein

- Conjunctival staining using lissamine green
- Evaluation of ocular discomfort

6. SAFETY ANALYSIS

6.1 Adverse Events

The following information will be analyzed descriptively and corresponding details on the subject level will be provided in data listings:

- Adverse Event (AE)
- AEs leading to premature discontinuation from the study drug
- Serious AEs
- AEs by severity
- AEs by causality
- AEs by SOC and PT

Definitions:

- In tables showing subjects with AEs by maximum severity, subjects will be counted only in the highest grading category, but events will be counted in each reported grading category.
- In tables showing AEs by maximum causality, subjects will be counted only in the strongest relationship category, but events will be counted in each category.

6.2 Laboratory Parameters

The following information will be analyzed descriptively and corresponding details on the subject level will be provided in data listings:

- Laboratory results

Laboratory results for the different laboratory parameters will be shown by time point.

6.3 Other Safety Parameters

The following information will be provided in data listings:

- Vital signs
- Physical examination
- Electrocardiogram
- Pregnancy Test

7. LIST OF TABLES AND DATA LISTINGS

Some additional notes:

- Every table number has as prefix 14.
- Every listing number has as prefix 16.2.
- In some cases numbering and labels may be adjusted in the analysis. Here are some examples:
  - Laboratory result table may be subdivided into several tables for the different laboratory parameters
  - Tables are split up by cohort, e.g. show table only for Cohort 1-3 and repeat it for Cohort 4
  - Listings do not fit on one page in the output and have to split up into several parts.

7.1 List of Tables

No.	Legend	Comment
Overall study information		
1.1.1	Subject overview (all subjects)	
1.1.2	Protocol deviations (safety analysis set)	
1.1.3	Attended visits with dates (safety analysis set)	
1.1.4	Study completion (safety analysis set)	
1.1.5	Study drug administration (safety analysis set)	
1.2.2 – 1.2.5	Repeat tables for ITT analysis set, if not the same as safety analysis set.	
Baseline evaluation		
2.1.1	Summary table of demographic information (safety analysis set)	
2.1.2	Summary table for medical and ophthalmic histories and prior and concomitant medication (safety analysis set)	
2.1.3	Non-pharmacologic treatments and procedures (safety analysis set)	
2.2.1 – 2.2.3	Repeat tables for ITT analysis set, if not the same as safety analysis set.	
Pharmacodynamics Analysis		
3.1.1	Pharmacodynamics by time point (ITT analysis set)	
3.2.1	Repeat table for PP analysis set.	
Safety Analysis		
4.1	Summary table for adverse events (safety analysis set)	Including tests described in section 2.5.
4.2	Adverse events by SOC and PT (safety analysis set)	
4.3	Adverse events by severity (safety analysis set)	
4.4	Adverse events by maximum severity (safety analysis set)	"By maximum severity" is explained in section 6.1.
4.5	Adverse events by causality (safety analysis set)	
4.6	Adverse events by maximum causality (safety analysis set)	"By maximum causality" is explained in section 6.1.
4.7	Laboratory results by time point (safety analysis set)	

7.2 List of Data Listings

No.	Legend	Comment
Overall study information		
1.1	Subject overview (all subjects)	
1.2	Inclusion/exclusion criteria (all subjects)	
1.3	Protocol deviations (safety analysis set)	
1.4	Attended visits with dates (safety analysis set)	
1.5	Study completion (safety analysis set)	
1.6	Study drug administration (safety analysis set)	
Baseline evaluation		
2.1	Demographic information (safety analysis set)	
2.2	Medical and ophthalmic histories (safety analysis set)	
2.3	Prior/concomitant medication (safety analysis set)	
2.4	Non-pharmacologic treatments and procedures (safety analysis set)	
Pharmacokinetics / Pharmacodynamics Analysis		
3.1	Pharmacokinetics information listing (Pharmacokinetic Analysis Set)	Note: PP analysis set yes/no column will be added in the listing.
3.2	Pharmacodynamics information listing (ITT analysis set)	Note: PP analysis set yes/no column will be added in the listing.
Safety Analysis		
4.1	Adverse events (safety analysis set)	
4.2	Adverse events with missing assessments in severity, causality or seriousness (safety analysis set)	
4.3	Laboratory results (safety analysis set)	
4.4	Vital signs (safety analysis set)	
4.5	Physical examination (safety analysis set)	
4.6	Electrocardiogram (safety analysis set)	
4.7	Pregnancy test (safety analysis set)	