NCT03452033

STATISTICAL ANALYSIS PLAN

PROTOCOL TITLE:	A Phase 1/2a Randomized, Double-masked, Placebo Controlled, Dose-ranging Study of the Safety and Efficacy of H-1337 in Subjects with Primary Open Angle Glaucoma (POAG) or Ocular Hypertension
PROTOCOL NUMBER:	ALY337-201
STUDY DRUG:	H-1337
IND NUMBER:	131440
DEVELOPMENT PHASE:	Phase 2a
SPONSOR:	Allysta Pharmaceuticals, Inc. 19 Davis Drive, Suite 102 Belmont, CA, 94002
DATE:	September 6, 2018

Confidentiality Statement

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse Event
AIDS	Acquired Immune Deficiency Syndrome
ALT	Argon Laser Trabeculoplasty
ALT	Alanine Aminotransferase
A_{max}	Peak Concentration
AMD	Age-related Macular Degeneration
ANCOVA	Analysis of Covariance
AP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BAK	Benzalkonium Chloride
BCVA	Best Corrected Visual Acuity
BID	Twice Daily
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CFB	Change From Baseline
CPK	Creatine Phosphokinase
ECG	Electrocardiogram
ET	Early Termination
ETDRS	Early Treatment Diabetic Retinopathy Study
GFR	Glomerular Filtration Rate
GGT	Gamma-Glutamyl Transferase
Hg	Mercury
HIV	Human Immunodeficiency Virus
Hrs	Hours
IC	Informed Consent Document
IND	Investigational New Drug
IOP	Intraocular Pressure
IP	Investigational Product
IWRS	Interactive Web Response System
LASIK	Laser-Assisted In Situ Keratomileusis
LDH	Lactate Dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
Mm	Millimeter
OTC	Over The Counter
OU	Both Eyes
PAS	Peripheral Anterior Synechiae
PK	Pharmacokinetic

POAG	Primary Open Angle Glaucoma
PRK	Photorefractive Keratectomy
PT	Preferred Term
RBC	Red Blood Cell
RGC	Retinal Ganglion Cells
ROCK	Rho Kinase
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SLT	Selective Laser Trabeculoplasty
TEAE	Treatment Emergent Adverse Event
TM	Trabecular Meshwork
T_{max}	Time to Peak Concentration
US	United States
VF	Visual Fields
WBC	White Blood Cell

This is version 1 of the statistical analysis plan (SAP) for study ALY337-201. If revisions are needed, the document will be amended. The SAP should be finalized prior to the database lock and final unmasking. All deviations from the analyses described in the final SAP will be noted in the clinical study report (CSR).

1. INTRODUCTION

Glaucoma is a slowly progressive optic neuropathy characterized by a loss of retinal ganglion cells (RGC) and optic nerve axons resulting in vision loss. Glaucoma is generally asymptomatic until advanced irreversible disease has occurred. It is estimated that close to 4 million Americans have glaucoma, 50% of whom are undiagnosed, and approximately 120,000 individuals are blind from glaucoma.

Current treatments for glaucoma aim to reduce IOP, which has been shown to slow visual field loss in clinical trials. Intraocular pressure (IOP) is the only modifiable risk factor, and current therapies aim to reduce IOP. Despite effective IOP lowering therapy, 30% of subjects still progress and lose vision and many subjects require multiple medications.

An area of unmet need in glaucoma is for effective agents that act through new mechanisms, particularly those that can promote outflow through the trabecular meshwork. While there are many classes of IOP-lowering drugs approved, no class acts to directly increase the conventional TM outflow other than pilocarpine. An IOP-lowering agent that could increase conventional TM outflow and be used as monotherapy or in combination with current therapies would provide useful additional pharmacologic options for patients with glaucoma or ocular hypertension.

H-1337 is a selective multi-kinase inhibitor, and the proposed mechanism of action of the compound is hypothesized to involve inhibition of kinases that play a role in controlling intracellular cytoskeletal dynamics and contractility.

It has been hypothesized that drugs that modulate the intracellular cytoskeleton of cells within the trabecular meshwork may beneficially affect aqueous outflow dynamics. This has been considered the primary mechanism of ROCK inhibitors. While H-1337 is less potent as an inhibitor of ROCK I and II, it may achieve concentrations in the aqueous humor following topical administration that have a biologic effect.

In general, the most frequent adverse event seen with topical administration of rho kinase inhibitors is conjunctival hyperemia and H1337 administration is associated with transient hyperemia in preclinical studies. The severity of this finding is variable, depending upon the compound, dose, and frequency of administration. The finding is typically transient and resolves with cessation of therapy. Systemic adverse drug effects are relatively uncommon following

topical administration, and kinase inhibitors have not generally shown class-related adverse effects.

2. STUDY DESCRIPTION

2.1 Study Objectives

Primary Objective:

Evaluate the local ocular and systemic safety of three concentrations of H-1337 in subjects with POAG or ocular hypertension treated daily for up to 28 days.

Secondary Objectives:

Evaluate the ocular hypotensive efficacy of H-1337.

Evaluate the systemic pharmacokinetics of H-1337.

2.2 Study Subjects

INCLUSION CRITERIA:

1. 18 years of age or older.
2. Diagnosis of bilateral primary open angle glaucoma or ocular hypertension.
3. One qualifying IOP criteria after washout:
4. Baseline (Day 0) at T_0 (T_0 = 8 am ± 30 min) IOP ≥ 23 mmHg in the study eye.
5. IOP criteria after washout ≤ 32 mmHg OU at all time points.
6. Best-corrected visual acuity (BCVA) in both eyes of 20/200 or better on Snellen, equivalent to + 1.0 log Mar.
7. Able and willing to sign informed consent, follow study instructions and complete all study visits.
8. As applicable, must be willing to discontinue the use of all ocular hypotensive medication(s) in both eyes prior to receiving the study medication and for the entire course of the study.
9. Able to self-administer or have a caretaker administer study eye drops.

EXCLUSION CRITERIA:

Ophthalmic:

Exclude subjects with:

1. Closed or very narrow angles (Grade 0-1) (*see Section 5, gonioscopy*) or those the investigator judges as occludable and/or with evidence of peripheral anterior synechiae (PAS) ≥ 180 degrees by gonioscopy within 6 months prior to Screening Visit in either eye. (Patent laser iridotomy with Grade 1-2 angles is acceptable in either eye, providing the PAS criteria are still met).

2. Previous glaucoma intraocular surgery in either eye. Prior laser trabeculoplasty (ALT or SLT) in either eye is allowed if performed more than 6 months prior to Screening Visit.
3. Any non-glaucoma intraocular surgery within 3 months prior to Screening Visit in either eye.
4. Intraocular laser surgery such as laser capsulotomy, laser iridotomy, and/or retinal laser within 1 month prior to Screening Visit in either eye.
5. Significant media opacity in either eye that would impede adequate posterior segment examination.
6. Contraindications to pupil dilation in either eye.
7. Other forms of glaucoma such as primary congenital, juvenile onset, chronic angle closure, and secondary glaucoma of any type including steroid-induced, inflammation-induced, or exfoliation glaucoma in either eye. Pigment dispersion syndrome/glaucoma is permitted in either eye.
8. Clinically significant corneal dystrophy, epithelial or endothelial disease, corneal irregularities or scarring that, in the investigator's judgment, would impede an accurate measurement of IOP or visualization of intraocular anatomy in the study eye.
9. History of refractive surgery in either eye (i.e., radial keratotomy, PRK, LASIK).
10. History of corneal cross-linking procedure in either eye.
11. Unwillingness to be contact lens free during study participation.
12. Any history of uveitis, keratitis, or scleritis in either eye.
13. Any history of penetrating ocular trauma in either eye.
14. History within 3 months prior to Screening Visit of clinically significant moderate or severe chronic or active blepharitis, ocular dermatitis, or recent ocular conjunctivitis and/or ocular inflammation in either eye. Mild blepharitis, hyperemia (due to prostaglandin use) and/or blepharitis, and/or mild inactive seasonal allergic conjunctivitis and non-infective dermatitis are acceptable.
15. Corneal thickness < 480 or > 620 μm in the study eye. Pachymetry measurement within 6 months prior to Screening Visit is acceptable.
16. Advanced or severe glaucoma with progressive visual field loss and/or optic nerve changes in either eye that, in the investigator's best judgment, prevent safe withdrawal from treatment for the time periods required in this protocol.
17. Progressive retinal (including, but not limited to worsening dry AMD, presence of active wet AMD, or unstable diabetic retinopathy) or optic nerve disease in either eye from any cause other than glaucoma.
18. Any prior intravitreal steroid injection in either eye.
19. Sub-tenon's, sub-conjunctival or periocular steroid injections within the 6 months prior to Screening Visit in either eye.

20. Any use of ocular topical corticosteroids in either eye within 7 days, or chronic (as determined by the investigator) topical steroids within 28 days prior to Baseline and ensuing trial participation.
21. Known hypersensitivity to any component of the H-1337 formulation, including BAK, or to topical anesthetics or diagnostic drops used during the study.
22. Any ocular, condition that, in the investigator's judgment, could prevent the subject from safe participation the study.
23. Planned ocular surgery or intraocular injection procedure in either eye during study participation.

General/Systemic:

Exclude subjects with:

1. Participation in a clinical study with use of any investigational drug or treatment within 30 days prior to Baseline (Day 0).
2. Clinically significant abnormalities in: laboratory tests, physical examination, vital signs and/or ECG at Screening Visit. If in the investigator's judgment a subject with clinically significant abnormalities is appropriate for enrollment in the study, a discussion between the investigator and the Medical Monitor must occur and be documented prior to enrollment of this subject in the study.
3. Clinically significant systemic, psychiatric or psychological disease (for example, renal, hepatic, uncontrolled diabetes, uncontrolled blood pressure, autoimmune disorders, psychiatric disorders, endocrine disorders, or any other disorders) or dependency which, in the investigator's judgment, would be unsafe and interfere with interpretation of the study results or the subject's ability to comply with the study requirements.
4. Anticipated changes or initiation of medications which might affect IOP and/or systemic blood pressure within 7 days prior to Baseline/Day 0 (e.g., oral anti-hypertensives such as sympathomimetic agents, beta-adrenergic blocking agents, alpha agonists, alpha adrenergic blocking agents, calcium channel blockers, angiotensin converting enzyme inhibitors; [diuretics are allowed]), and 2 months prior to Baseline/Day 0 for corticosteroids (i.e., oral, nasal, topical [dermal, mucosal], and/or inhaled corticosteroids). If there are no further anticipated changes in medications that could affect IOP and/or systemic blood pressure, then once the subject is stable on their new dose of medication for the required time period, the subject may complete the Baseline Visit, assuming that all other screening requirements are met. Medications used on an adjustable or sliding scale based on testing results are allowed.
5. Known history of Hepatitis B + C, HIV+, or AIDS and/or inadequate venous access.
6. Women of childbearing potential who are pregnant, nursing, planning a pregnancy, or not using a medically acceptable form of birth control. An adult woman is considered to be of childbearing potential unless she is one year post-menopausal or three months post-surgical sterilization. All females of childbearing potential must have a negative serum pregnancy test result at Screening Visit and a negative urine and serum pregnancy test at Baseline (Day 0) prior to randomization in the study and must not intend to become pregnant during the study.

7. History of drug or alcohol abuse within the last 5 years.
8. Related to site study staff and/or site employees.

2.3 Study Design/ Procedures

The study will evaluate the safety, tolerability, and preliminary efficacy of 3 concentrations of H-1337 and vehicle administered twice daily for 28 days. Up to 100 subjects, naïve to H-1337, will be randomized and dosed for 28 days. The parallel group, double-masked, placebo-controlled study will include the following dosing cohorts:

H-1337 0.06% (n=20)

H-1337 0.2% (n = 20

H-1337 0.6% (n = 20)

Control (Vehicle) (n = 20)

Dosing will be BID unilaterally in the study eye for the first 3 days, then BID OU from Day 4 to Day 28.

2.4 Randomization

Randomization will be stratified by Baseline IOP (23 to 26 mmHg and > 26 to 32 mmHg at 8 am). H-1337 and its vehicle will be packaged in identical bottles. An interactive web response system (IWRS) will be used for randomization.

2.5 Study Treatments

Subjects will receive one of four dosing arms: H-1337 at concentrations of 0.06%, 0.2%, or 0.6%, or a control vehicle. IP will be administered BID unilaterally in the study eye for the first 3 days, then BID OU from Day 4 to Day 28.

The IWRS will assign a number to subjects who qualify for randomization. The first IP bottle will be dispensed on Day 0 (Baseline) and the second IP bottle will be dispensed to the subject on Day 14. The third IP bottle will serve as a back-up if the subject needs a replacement IP bottle.

2.6 Duration

Each subject's participation will consist of two phases: a screening phase (Screening and Baseline Visits) where subjects will wash out any current ocular hypotensive therapy and a dosing phase, starting on Day 0. If a subject is not on ocular hypotensive therapy, there is no

applicable washout period and the Baseline Visit should occur after clearance of clinical laboratories.

A subject could participate in the study for a total of up to approximately 11 weeks. Subjects who meet the applicable inclusion/exclusion criteria at the Screening Visit will discontinue use of any current ocular hypotensive therapy during the washout period. The washout duration will be dependent on the subject's pre-study ocular hypotensive therapy.

2.7 Maintenance of Randomization Codes

The study will be double-masked to reduce potential bias in clinical assessments. Upon approval of Allysta Pharmaceuticals, Inc., and prior to initiation of the study, an unmasked statistician will generate and maintain the randomization codes which are consistent with the study design. Other study statisticians will be masked to the identity of the treatments until all data have been entered into the database and locked.

In the case of an emergency only, if it is necessary for the investigator (or a treating physician) to know the treatment a subject is receiving, site personnel can complete the subject treatment breaking code procedure in IWRS.

3. STUDY ASSESSMENTS/ENDPOINTS

Informed Consent: Properly executed informed consent (written and verbal) is to be obtained prior to completion of any study-related procedures. Subjects must review, sign, and date the informed consent document and receive a copy.

Medical and Ophthalmic History: Relevant and clinically significant medical and ophthalmic history must be recorded.

Concomitant Medications: All prescription and over-the-counter (OTC) medications and nutritional supplements taken during the study and 30 days prior to the Screening Visit will be recorded. Diagnostic eye drops do not need to be recorded.

Randomization: Randomization will be accomplished using an Interactive Web Response System (IWRS). Site personnel will receive instruction about IWRS access and use.

Adverse Events: Any events occurring after the first dose of IP will be recorded as an adverse event. At each visit, the subject should be asked questions such as, "How are you feeling? How are your eyes feeling today? Have you had any changes since the last exam?"

IP Administration and Compliance: The first dose of study IP will be administered to the study eye by study staff after the IOP measurement at T0 + 12 hrs. At Day 1/Visit 3 the AM dose of

study IP will be administered to the study eye by study staff after the IOP measurement at T0. Subjects will self-dose unilaterally until the Day 4 visit. At Day 4/Visit 4 the AM dose of study IP will be administered OU by study staff after the IOP measurement at T0. Subjects will self-dose OU for the remainder of the trial except on Day 14/Visit 5 (AM and PM doses) and Day 28/Visit 6 (AM dose) when study IP will be administered OU by study staff after the related IOP measurements. Compliance will be assessed at applicable visits by asking the subject about any missed doses. If the subject reports missing more than 4 doses since the prior visit, the subject will be counseled on IP compliance.

Heart Rate and Blood Pressure (BP) Measurements: Both systolic and diastolic blood pressure should be recorded.

Heart rate measurements will be measured by radial pulse. The subject should be in a seated and relaxed state. An automated blood pressure unit may be used to measure the heart rate.

Electrocardiogram (ECG): 12-lead ECGs should be performed according to the site's standard procedures. ECG interpretation must be documented and reviewed by the investigator and or another qualified medical doctor for clinically significant findings that might affect the subject's safe participation in this trial.

Physical Examination: The following systems should be examined: cardiopulmonary, endocrine, gastrointestinal, musculoskeletal, rheumatic, neurologic, psychiatric, dermatologic, hepatic, renal, and head, ears, nose, and throat. Other body systems may be deferred at the discretion of the investigator. Abnormalities should be recorded in the medical history.

Best-Corrected Visual Acuity (ETDRS): Visual acuity will be performed before implementing any procedure that can affect vision (i.e., pupil dilation, tonometry, and gonioscopy). The most current refraction within 6 months prior to the Screening Visit will be recorded unless changes in refraction are suspected. If changes are suspected since the most current refraction, refraction should be repeated at the Screening Visit. The same optotype should be used throughout the study for a specific subject, and the right eye should be tested first. Best corrected visual acuity (BCVA) should be recorded using the ETDRS chart (or a modified ETDRS chart). If a change in BCVA is noted, pinhole may be used to test for the BCVA, but a formal refraction prior to dilation should be performed during that visit and/or on the next visit. The LogMAR scores will be calculated according to the standard scoring methodology. A change of ≥ 10 letters may indicate an adverse event and should be investigated further by the investigator and the refraction should be rechecked.

Slit Lamp Biomicroscopy: This will be performed by the investigator's usual clinical technique. Biomicroscopy will be performed prior to pupil dilation OU. Changes and/or abnormalities of the anterior segment in the lids, conjunctiva, cornea, anterior segment, and lens will be graded.

Iris color will be recorded at screening. Findings will be graded according to the following scales:

LID

Erythema

None (0) =	Normal, without any redness, or less than mild
Mild (+1) =	A low grade flushed reddish color
Moderate (+2) =	Diffused redness encompassing the entire lid margin
Severe (+3) =	Deep diffused reddish color of lid margins and superior or inferior eyelid

Edema

None (0) =	Normal, no swelling of the lid tissue, or less than mild
Mild (+1) =	Slight diffuse swelling above normal
Moderate (+2) =	General swelling
Severe (+3) =	Extensive swelling of the eyelid(s), with or without eversion of upper and/or lower lids.

CONJUNCTIVA

Hyperemia

None (0) =	Normal: few vessels of bulbar conjunctiva easily observed
Minimal (+0.5) =	Trace flush, reddish-pink color of the bulbar conjunctiva
Mild (+1) =	Mild flush – reddish pink of the bulbar conjunctiva
Moderate (+2) =	Moderate, bright reddening of the bulbar conjunctiva
Severe (+3) =	Deep, severe, bright and diffuse reddening of the bulbar conjunctiva

Edema

None (0) =	Normal, no swelling of the conjunctiva or less than mild
Mild (+1) =	Slight diffuse or regional swelling of the conjunctiva
Moderate (+2) =	General swelling of the conjunctiva
Severe (+3) =	Extensive swelling of the conjunctiva

CORNEA

Edema

None (0) =	Transparent and clear or less than mild
Mild (+1) =	Dull glassy appearance
Moderate (+2) =	Dull glassy appearance of epithelium with large number of vacuoles
Severe (+3) =	Epithelial bullae and/or stromal edema, localized or diffuse, with or without stromal striae

ANTERIOR CHAMBER

Cells

None (0) =	No cells seen or less than mild
Mild (+ 1) =	1-5 cells
Moderate (+2) =	6-10 cells
Severe (+3) =	11-20 cells
Hypopyon (+4) =	> 20 cells, Hypopyon Formation (indicate size of hypopyon)

Flare

None (0) =	No Tyndall effect or less than mild
Mild (+1) =	Tyndall beam in the anterior chamber has a mild intensity
Moderate (+2) =	Tyndall beam in the anterior chamber is of strong intensity
Severe (+3) =	Tyndall beam is very intense. The aqueous has a white, milky appearance

LENS

Phakic	Yes/No for each eye
Aphakic	Yes/No for each eye
Pseudophakic	Yes/No for each eye

Lens Opacity (for phakic only)

None (0) =	None present or less than mild
Mild (+ 1) =	Subtle
Moderate (+2) =	Moderate
Severe (+3) =	Dense

Pupillary Diameter: Measurement of pupil size should occur under normal lighting conditions to the nearest 0.5 mm using a millimeter ruler or pupillary gauge while the patient fixates on a distant, non-accommodative target.

Conjunctival Hyperemia: Hyperemia will be assessed and recorded separately from biomicroscopy through direct visual observation prior to fluorescein instillation and IOP measurement. The hyperemia will be graded on a 0 to 3 scale using the photographic reference scale provided by the Sponsor for use in this protocol.

0 Normal: few vessels of bulbar conjunctiva easily observed

0.5 Trace flush, reddish-pink color of the bulbar conjunctiva

1 Mild flush – Reddish pink of the bulbar conjunctiva

2 Moderate, Bright reddening of the bulbar conjunctiva

3 Deep, severe, bright and diffuse reddening of the bulbar conjunctiva

Intraocular Pressure: All IOP measurements must be measured by Goldmann applanation tonometry.

Two consecutive IOP measurements should be taken for each IOP time point, with the right eye being measured first. The applanation probe should be withdrawn between measurements. Intraocular pressure will be measured 2 times and the average will be used for analysis. If the two measurements differ by more than 4 mmHg, a third measurement will be taken and the median value will be used for analysis. The same procedure will be repeated on the contralateral eye.

Pachymetry: Pachymetry will be performed OU at the Screening Visit; results obtained within the prior 6 months may be used if no change is suspected. Central corneal thickness (central zone) will be measured with a calibrated ultrasound pachymeter in both eyes after topical anesthetic has been applied. Three measurements will be taken, first in the right eye, and the average will be recorded in micrometers (μm). The subject will be in a seated position and fixating on a target straight ahead. The same procedure is then performed on the left eye once the measurements have been completed in the right eye.

Gonioscopy: The gonioscopy examination will be performed OU at the Screening Visit; results obtained within the prior 6 months may be used if no change is suspected. Gonioscopy should be performed prior to instillation of dilating or miotic drops, with high magnification, and with dim illumination to potentiate visualization of the angle in its natural configuration. The angular width of the angle recess will be graded as an average score representative of all 4 quadrants using the grading criteria below.

Grade Description (Shaffer System, based on the angular width of the angle recess [Marsh & Cantor, 2005])

4 45 to 35° angular approach, wide open

3 35 to 20° angular approach, wide open

2 20° angular approach, narrow

1 ≤ 10° angular approach, extremely narrow

Slit 0° angle, narrowed to slit

Visual Field Examination: A visual field will be performed OU at the Screening Visit; reliable results obtained within the prior 6 months may be used if no change is suspected. Visual fields must be automated threshold visual fields (i.e., Humphrey [30-2 or 24-2] or Octopus equivalent). SITA Standard (24-2 or 30-2) is preferred, SITA fast is also allowed. The subject's best

correction at 33 cm is to be used. Visual fields must be reliable, defined as those with a) fixation losses less than or equal to 20%, b) false positives less than or equal to 33% and c) false negatives less than or equal to 33%.

Dilated Ophthalmoscopy: Posterior segment exam will be performed according to the investigator's preferred procedure. Magnification, lighting, and examiner should be consistent for each subject throughout the study. Ophthalmoscopy should be performed after pupil dilation (i.e., 1% tropicamide or cyclopentolate and 2.5% phenylephrine). The examination should include an evaluation of the vitreous, retina, macula, choroid, and optic nerve (including the absence or presence of notches and/or disc or peripapillary hemorrhages). Abnormalities and pathologic findings will be graded. Ocular structures will be graded according to the following scale.

Vitreous, Retina, Macula and Choroid: Examination emphasizes the visual axis.

0 Normal

1 Abnormal, describe.

Optic Nerve

0 Normal. No damage

1 Mild Optic nerve damage, secondary to glaucoma including any rim loss (sloping or thinning)

2 Moderate Optic nerve damage, including cupping to disc margin at one or more points

3 Severe Optic nerve damage, nearly total cupping, only nasal rim or less present

Any new findings (i.e., notch and/or peripapillary hemorrhage) or deterioration from Screening Visit findings will be reported as an adverse event. The cup/disc ratio will be recorded horizontally and vertically for each examination, and reported in 0.1 increments.

Blood Collection for PK assessment: Will be conducted at selected sites. See separate blood collection manual.

Frequency	Day 0 and Day 28.
Time Points	Pre-dose at Days 0 and 28 and approximately 2 and 8 hours (± 10 min) post- AM dose on Day 28

Analysis	Plasma analysis for the test article and/or metabolites, if applicable, will be conducted. Some collected samples may not be assayed if the expected concentration of test article and/or metabolites, based on initial assay results, is below the lower quantitation limit of the assay. Pharmacokinetic analysis will be conducted and will include (if appropriate), but not be limited to, peak concentration (C_{max}), time to peak concentration (T_{max}), and area under the concentration-time curve (AUC).
Reporting	Plasma analysis (including principle investigator compliance statements and Quality Assurance statements) and pharmacokinetic determination reports will be provided for inclusion in the clinical study report.

Clinical Laboratories: See separate blood collection manual.

Hematology: White blood cells (WBC) (neutrophils, eosinophils, basophils, lymphocytes, monocytes), red blood cells (RBC), hematocrit, hemoglobin, platelets, MCV, MCH, MCHC, RDW

Clinical Chemistry: Total protein, albumin, blood urea nitrogen (BUN), total bilirubin, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (AP), gamma-glutamyl transferase (GGT), total cholesterol, calcium, sodium, potassium, chloride, glucose, uric acid, triglycerides, direct bilirubin, creatine phosphokinase (CPK), and GFR estimate.

Urinalysis: Specific gravity, pH, ketones, protein, glucose, bilirubin, urobilinogen, blood, leukocyte esterase (urinalysis abnormalities in protein, blood, and leukocyte esterase will reflex a microscopic examination including white blood count, red blood count, epithelial cells, bacteria, mucus, casts, and crystals).

Lab results must be reviewed by the investigator or another medical doctor (with documentation) to assure the subject's safety.

Pregnancy Test:

Serum pregnancy will be required at the Screening Visit for women of child bearing potential.

Urine pregnancy and serum pregnancy tests will be performed in women of child bearing potential at Baseline (Day 0). Pregnancy test results must be negative for subjects to continue in the study. On Day 28, a urine pregnancy test will be administered.

4. SAMPLE SIZE AND POWER CONSIDERATIONS

The sample size is driven by interest in the IOP lowering effect of H-1337. The study is designed to test whether one or more concentrations of H-1337 are superior to the vehicle control in the reduction of IOP. With n = 20/group this protocol is powered at 94% to detect a difference in IOP reduction of 4.0 mmHg between groups against a standard deviation of 3.5 mmHg (2-sided t-test, alpha=0.05).

Adverse events: With 20 subjects per group there is a 95% certainty that an event that is not observed would have a true incidence rate of no more than 15%.

Hyperemia: Scored on a 5-point scale from 0 to 3. Assume the SD is 20% of the scale range or 1 unit. Then, with n = 20/group there is 86% power to detect a mean difference of 1 unit between pairs of groups (2 group two-sided t-test, alpha=0.05). If a response is defined as a hyperemia score ≥ 3 and the response rate in the control group is 10%, then with n = 20/group there is 85% power to detect a difference in response rates of 46% (Fisher's exact test).

In general, with 20 subjects per group there is 86% power to detect an effect size of 1.0.

If a concentration is chosen that is less than the maximum, the safety data from the higher doses can be combined to increase power.

5. ANALYSIS POPULATIONS

The Safety Population will include all randomized subjects who receive at least one dose of study medication.

All subjects who receive study medication and who complete at least one on-therapy study visit will comprise the Intent to Treat Population.

Subjects who receive study medication, satisfy pre-randomization inclusion/exclusion criteria, and who complete at least one on-therapy study visit will be evaluable for a Per Protocol Population analysis. Individual subject visits and data points that do not satisfy protocol criteria may be excluded from per protocol analyses. Evaluability will be determined prior to breaking the code for masked treatment assignment.

All analyses will be based on the actual treatment each subject received.

6. HANDLING OF MISSING DATA

Missing data will not be imputed for efficacy assessments.

If intensity of an adverse event is missing the event will be considered severe. If relationship of adverse event to study drug is missing the event will be counted as related.

Adverse events will be considered treatment emergent unless it can be determined from the onset or end date information that the event cannot have been treatment emergent.

Other adverse events will be assumed to occur during the screening period.

7. STATISTICAL ANALYSIS

All statistical analyses and reporting will be performed using the SAS® System Version 9.3 or later.

Unless otherwise specified, continuous variables will be summarized with descriptive statistics (n, mean, median, standard deviation, standard error, minimum, and maximum), and categorical variables will be summarized with counts and percentages.

All data collected in the study database will be presented in the listings.

Definition of Study Eye: The study eye will be the eye that meets the entry intraocular pressure (IOP) criteria after washout. Should both eyes meet the criteria, then the study eye will be defined as the eye with the higher 8 am IOP on Baseline (Day 0). If both eyes have the same 8 am IOP on Baseline (Day 0), the study eye will be the right eye. The study eye will be designated by the investigator. The designation may take into account ocular history as well as IOP criteria.

Definition of Baseline:

For vital signs, PK, BCVA and biomicroscopy baseline is the pre-T0 time point on Day 1. For clinical laboratories and pupil diameter baseline is the Day 0 collection. For IOP and hyperemia baseline is the set of repeated measures at different times during the Day 0 visit. For ophthalmoscopy baseline is the Screening Day.

7.1 Subject Disposition

Subject disposition, including the number of subjects randomized, treated, and completing the study (and completing each study visit), will be tabulated by treatment group. The percentage of subjects treated and completing the study will be based on the total number randomized.

Eligibility criteria exemptions and major protocol deviations will be summarized by treatment group and presented in a listing. Major protocol deviations may include the following: violation of inclusion or exclusion criteria, using prohibited medications, non-adherence to study treatment schedule.

The total number and percentage of subjects included in each of the analysis datasets described in Section 5 will be summarized by treatment group, with percentages based on the total number randomized subjects.

Discontinuations and the reasons for discontinuation will be summarized for all randomized subjects. Summaries will include the number and percentage of subjects within each treatment group. Reasons for discontinuation following the receipt of study drug will include the following:

1. Protocol violation
2. Lost to follow-up
3. Adverse event
4. Non-compliance
5. Subject withdrew consent
6. Investigator decision
7. Uncontrolled IOP
8. Other

7.2 Demographic and Baseline Characteristics

The demographic and baseline characteristics will be summarized for all randomized subjects by treatment group.

Continuous variables such as age will be summarized using descriptive statistics (n, mean, standard deviation, median, minimum, and maximum), and categorical variables such as gender, race, and ethnicity will be summarized using counts and percentages of subjects.

Age will be determined as the whole integer number of years from the date of birth (DOB) to the date of the screening visit, i.e., the truncated integer difference between the DOB and Visit 1.

Baseline comparability between treatment groups will be assessed via descriptive statistics.

Prior medications will be tabulated by preferred term (WHO drug dictionary).

Medical and surgical history classified using MedDRA dictionary will be presented in a listing.

7.3 Treatment Compliance and Exposure

The number of subjects by number of doses missed will be summarized by eye, visit and treatment group.

Duration of drug exposure will be calculated for each subject as the number of days they received any study drug. The number and percentage of subjects by treatment group will be summarized according to the following duration-of-exposure categories: '<7 days', '7 to <14 days', '14 to <21 days', '21 to <31 days', and '≥31 days'.

7.4 Intraocular pressure

The comparisons among treatment groups in IOP will be done for the various endpoints and time points. The outline of the analyses and groups is presented in Appendix 2.

For each visit in which IOP is measured descriptive statistics at each time point will be presented for the observed, change from baseline and % change from baseline values. In addition, at visits where five assessments are made descriptive statistics will be presented for the average of the observed values and the change from baseline and percent change from baseline where the values will be computed using average of five corresponding values at Day 0 as a baseline.

Descriptive statistics of IOP measures described above will be presented for the study eye, non-study eye and average of both eyes by time point and treatment group.

The 95% confidence intervals of the mean IOP by treatment group and time point and for diurnal IOP as well as for corresponding change from baseline will be presented.

For the IOP, diurnal IOP, and their change from baseline, each of the active treatment groups will be compared to the placebo group using the analysis of covariance (ANCOVA) model, separately for each time point. Baseline value will be used as a covariate. The difference between treatment groups in adjusted least-square means will be presented. Mean diurnal IOP will be presented as the mean of all values (including the 8 pm IOP value) (12-hour diurnal) and mean of all values up to the 4 pm IOP value (8 hour diurnal). If a subject is missing any time point for the calculation of the diurnal IOP, that subject will be excluded from the affected diurnal IOP analysis in the per protocol population.

The proportion of subjects reaching a target IOP (≤ 18 mmHg) at each time point and with the mean diurnal IOP for each visit will be presented by treatment group.

Within each dose level the change from baseline IOP will also be compared (a within subject comparison) with descriptive statistics.

Exploratory summaries may be presented to examine if treatment effects differ depending on iris color, age and baseline IOP.

Each comparison will be tested at the significance level of 0.05. No adjustments for multiple comparisons will be made.

7.5 Adverse Events

Treatment emergent adverse events (TEAE) are those with onset after randomization or if occurring prior to randomization worsened after randomization. Only treatment emergent events will be summarized. All events in the clinical database regardless of when they occurred will be provided in data listings. Adverse events will be classified according to the MedDRA system to the levels of System Organ Class (SOC) and primary Preferred Term (PT).

An overall summary will be presented which gives the number and percentage of subjects within each treatment group that experienced any TEAE, experienced any treatment related TEAE, permanently discontinued treatment due to a TEAE, experienced a treatment emergent SAE, and that died.

In summary tables, AEs occurring in both eyes will be counted once at the greater intensity and relationship to study drug.

Events that are possibly, probably or definitely related will be counted as an event related to study drug.

The number and percentage of subjects experiencing one or more events within a MedDRA system organ class and preferred term class without regard to intensity, relationship, or seriousness will be tabulated by treatment group. In addition, tables will display events by SOC, PT, and maximum intensity or closest relationship to treatment.

The number of deaths and SAEs will also be presented, and AEs leading to premature discontinuation from the study will be listed and tabulated.

A glossary listing that shows the verbatim terms assigned to each SOC and PT will be provided.

A listing of TEAEs by treatment group ordered by subject, SOC, PT, and onset date will be provided.

A listing of serious TEAEs by treatment group ordered by subject, SOC, PT, and onset date will be provided.

7.6 Concomitant Medications

All concomitant medications listed on the case report form will be provided in data listings in the clinical study report. Each medication will be mapped to their corresponding Preferred Term from the WHO Drug Dictionary. A frequency distribution of all concomitant medications used during the study sorted by Preferred Term will be provided for each treatment group.

7.7 Ocular and Other Medical History

Ocular and other medical history reported at the Screening Visit and on Day 0 prior to the first dose of study drug, will be summarized by System Organ Class and Preferred Term.

7.8 Clinical Laboratory Assessment

Clinical laboratory data (hematology, serum chemistry, and urine) at each scheduled visit will be summarized with descriptive statistics by treatment group, as will the change from baseline to each post-baseline visit. Each subject's data will also be classified to low, normal, and high according to the normal ranges. These data will be summarized in shift tables comparing each post-baseline visit to baseline.

7.9 Physical examination

Physical examination findings at the Screening Visit will be summarized by System Organ Class and Preferred Term.

7.10 Vital Signs

Descriptive statistics of vital signs and their change from baseline will be tabulated by time point and treatment group.

7.11 Electrocardiogram

The interpretation of the ECG with respect to normal/abnormal will be tabulated.

7.12 Best-Corrected Visual Acuity

Descriptive statistics of the observed and change from baseline BCVA will be tabulated for each eye by visit and treatment group.

7.13 Ocular Hyperemia, Biomicroscopy, Ophthalmoscopy and Ocular Symptoms

The observations of ocular hyperemia, biomicroscopy, ophthalmoscopy, and ocular symptoms will be summarized in frequency tables based on the ordinal scales for each measure for each eye by time point and treatment group. In addition, the photographic assessment of ocular hyperemia will be summarized by descriptive statistics (See Appendix 2).

7.14 Pupil Diameter

Descriptive statistics of pupil diameter and its CFB will be tabulated for each eye by time point and treatment group.

7.15 Pachymetry

Descriptive statistics of the observed corneal thickness will be tabulated for each eye by treatment group.

7.16 Gonioscopy at Screening Visit

Anterior chamber angle classified by the Shaffer's classification will be summarized in frequency tables for each eye by treatment group.

7.17 Visual field

The observation of visual field examination will be summarized in a frequency table for each eye by treatment group.

8. INTERIM ANALYSIS

No interim analyses are planned.

9. APPENDICES

9.1 Appendix 1: Schedule of Study Assessments and Procedures

Assessments/Procedures[1]	Screening (-42[2] to -1 days)	Day 0 Baseline/ Randomization	Day 1	Day 4 (± 1 day)	Day 14 (± 1 day)	Day 28 Exit/ET (± 2 days)
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6
Informed Consent	X					
Medical and Ophthalmic History	X	X				
Concomitant Medications	X	X	X	X	X	X
Adverse Events		X	X	X	X	X
Randomization		X				
IP Administration in Office		X Unilateral post T_0 + 12 hrs IOP	X Unilateral post IOP	X OU post IOP	X OU post T_0 and T_0 + 12 hrs IOP	X OU post T_0 IOP (final dose)
Assess IP Compliance				X	X	X
Heart Rate/Blood Pressure *(± 30 min)*	X	X Pre-T_0 T_0 + 1 hr T_0 + 2 hrs T_0 + 4 hrs				X Pre-T_0 T_0 + 1 hr T_0 + 2 hrs T_0 + 4 hrs
ECG	X					
Physical Examination	X					
Best-corrected Visual Acuity (ETDRS)	X	X Pre-T_0	X	X Pre-T_0	X Pre-T_0	X Pre-T_0
Biomicroscopy[3] *(± 30 min)*	X	X T_0 T_0 + 2 hrs T_0 + 4 hrs T_0 + 8 hrs T_0 + 12 hrs	X Pre-T_0	X Pre-T_0	X Pre-T_0	X Pre-T_0 T_0 + 2 hrs T_0 + 4 hrs T_0 + 8 hrs T_0 + 12 hrs
Pupillary Diameter *(± 30 min)*		X Pre-T_0				X Pre-T_0

[1] When possible, one examiner should conduct all ocular assessments for a subject during a single visit. If possible, the same examiner should conduct all ocular assessments for a subject throughout the duration of the study.

[2] +1 week permitted to accommodate scheduling considerations

[3] If any adverse findings are noted after IP administration, the subject should continue to be monitored per the investigator's judgment.

Assessments/Procedures[1]	**Screening (-42[2] to -1 days)**	**Day 0 Baseline/ Randomization**	**Day 1**	**Day 4 (± 1 day)**	**Day 14 (± 1 day)**	**Day 28 Exit/ET (± 2 days)**
Conjunctival Hyperemia Grading *(± 30 min)*		X T_0 T_0 + 2 hrs T_0 + 4 hrs T_0 + 8 hrs T_0 + 12 hrs		X Pre-T_0	X Pre-T_0	X Pre-T_0 T_0 + 2 hrs T_0 + 4 hrs T_0 + 8 hrs T_0 + 12 hrs
Intraocular Pressure[4] (Goldmann Tonometry) *(± 30 min)*	X	X T_0 T_0 + 1 hrs T_0 + 2 hrs T_0 + 4 hrs T_0 + 8 hrs T_0 + 12 hrs	X T_0	X T_0	X T_0 T_0 + 1 hr T_0 + 2 hrs T_0 + 4 hrs T_0 + 8 hrs T_0 + 12 hrs	X T_0 T_0 + 1 hr T_0 + 2 hrs T_0+ 4 hrs T_0 + 8 hrs T_0 + 12 hrs
Pachymetry[5]	X					
Gonioscopy[6]	X					
Visual Fields (automated, threshold)	X[7]					
Dilated ophthalmoscopy	X					X[8]
Blood Collection for PK of H-1337 *(± 10 min at 2 & 8 hrs)*		X				X Pre-T_0 T_0 + 2 hrs T_0 + 8 hrs
Clinical Laboratories (hematology, clinical chemistry, pregnancy (if applicable), and urinalysis)	X[9]	X[10]				X[11]

[4] Timing of IOP measurements for each subject should be consistent throughout the study.

[5] Pachymetry performed at screening will be used for inclusion/exclusion criteria only. Pachymetry within 6 months of Screening acceptable unless suspected change.

[6] Gonioscopy within 6 months of Screening acceptable unless suspected change.

[7] Historical VF may be used if within 6 months of screening visit unless suspected change.

[8] After T_0 + 12 hrs IOP; may be deferred to following day for scheduling convenience.

[9] At Screening, if applicable, a serum pregnancy test should be performed.

[10] At Baseline, if applicable, a urine and serum pregnancy test should be conducted. Urine pregnancy test (UPT) must be negative to proceed to randomization; serum pregnancy test results should be reviewed as soon as available to confirm negative UPT result.

[11] On Day 28, if applicable, a urine pregnancy test should be conducted.

9.2 Appendix 2. Efficacy Endpoints and Time Points

IOP

Category	Day 0 Visit	Day 1 Visit*	Day 4 Visit*	Days 14 & 28 Visits
Eye subgroups	Study eye Mean of both eyes	Study eye	Study eye	Study eye Mean of both eyes
Analysis variables	IOP & Diurnal IOP	• IOP • Change in IOP from baseline (Day 0 Visit)* • % change in IOP from baseline (Day 0 Visit)* • Proportion of subjects achieving IOP ≤ 18 mmHg	• IOP • Change in IOP from baseline (Day 0 Visit) • % change in IOP from baseline (Day 0 Visit) • Proportion of subjects achieving IOP ≤ 18 mmHg	• IOP & Diurnal IOP • Change in IOP & Diurnal IOP (Day 0 Visit) • % change in IOP & Diurnal IOP (Day 0 Visit) • Proportion of subjects achieving IOP/Diurnal IOP ≤ 18 mmHg
Analysis Time points	• T_0 T_0 + 1 hrs T_0 + 2 hrs T_0 + 4 hrs T_0 + 8 hrs T_0 + 12 hrs • Diurnal (mean of 5 measurements & mean of 4 measurements excluding T_0 + 12 hrs)	T_0	T_0	• T_0 T_0 + 1 hrs T_0 + 2 hrs T_0 + 4 hrs T_0 + 8 hrs T_0 + 12 hrs • Diurnal (mean of 5 measurements & mean of 4 measurements excluding T_0 + 12 hrs)
Treatment Groups/ Comparisons	H-1337 vs. Vehicle	H-1337 vs. Vehicle	H-1337 vs. Vehicle	H-1337 vs. Vehicle

*Note: the non-study eye will not be treated until after T_0 on the Day 4 visit. Day 0 measures will be used as baseline for all time points for the non-study eye.

Conjunctival Hyperemia by Photographic Assessment

Category	Day 0 Visit	Day 4 Visit*	Day 14 Visit	Days 28 Visit
Eye subgroups	Study eye Mean of both eyes	Study eye	Study eye	Study eye Mean of both eyes
Analysis variables	Conjunctival Hyperemia by Photographic Assessment	• Hyperemia • Change in Hyperemia from baseline (Day 0 Visit)*	• Hyperemia • Change in Hyperemia from baseline (Day 0 Visit)	• Hyperemia • Change in Hyperemia from baseline (Day 0 Visit)
Analysis Time points	• Pre-T_0 T_0 + 2 hrs T_0 + 4 hrs T_0 + 8 hrs T_0 + 12 hrs	• Pre-T_0	Pre-T_0	• Pre-T_0 T_0 + 2 hrs T_0 + 4 hrs T_0 + 8 hrs T_0 + 12 hrs
Treatment Groups/ Comparisons	H-1337 vs. Vehicle	H-1337 vs. Vehicle	H-1337 vs. Vehicle	H-1337 vs. Vehicle

*Note: the non-study eye will not be treated until after T_0 on the Day 4 visit. Day 0 measures will be used as baseline for all time points for the non-study eye.