NCT03452033

CLINICAL STUDY PROTOCOL

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
Investigational Drug Product:	H-1337
IND Number:	107,895
Development Phase:	Phase 2a
Medical Monitor:	William C. Stewart, MD Telephone: (843) 327-0316
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Date of Protocol:	January 8, 2018
Version:	1.0

Confidentiality Statement

PROTOCOL APPROVAL SIGNATURE PAGE

Protocol Number: ALY337-201

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

No party involved in the conduct of this study will modify this protocol without obtaining Allysta Pharmaceutical's written agreement. Any modification will require appropriate written confirmation and any protocol amendment must follow written policies as stated in this protocol.

This trial will be conducted in accordance with current United States (US) Food and Drug Administration (FDA) regulations, International Conference on Harmonization (ICH) guidelines, Good Clinical Practice (GCP) standards, the Declaration of Helsinki, and local ethical and legal requirements.

Allysta Pharmaceuticals, Inc. Approval:

Signature: _____ Date: 06 Mar 2018

Name: Henry Hsu, MD
Chief Executive Officer
Allysta Pharmaceuticals, Inc.

INVESTIGATOR PROTOCOL SIGNATURE PAGE

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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

By signing below, the investigator agrees to adhere to the protocol as outlined and agrees that any changes to the protocol must be approved by Allysta Pharmaceuticals, Inc., prior to seeking approval from the Institutional Review Board/Ethics Committee.

The investigator also agrees to conduct the study in accordance with current United States (US) Food and Drug Administration (FDA) regulations, International Conference on Harmonization (ICH) guidelines, Good Clinical Practice (GCP) standards, the Declaration of Helsinki, and local ethical and legal requirements.

Investigator's Signature: _____

Printed Name: _____

Name of Institution: _____

Date: _____

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PROTOCOL SYNOPSIS

Protocol Number:	ALY337-201
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
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
Study Design:	The study will evaluate the safety, tolerability, and preliminary efficacy of three concentrations of H-1337 and vehicle administered twice daily (BID) in a parallel group, double-masked design for 28 days of dosing. Approximately 75-100 subjects will be enrolled. The study will include the following 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. Efficacy assessments will be based upon the 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. 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.

Eligibility Criteria:	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: • Baseline (Day 0) at T_0 (T_0 = 8 am ± 30 min) IOP ≥ 23 mmHg in the study eye. 4. IOP criteria after washout ≤ 32 mmHg OU at all time points. 5. Best-corrected visual acuity (BCVA) in both eyes of 20/200 or better on Snellen, equivalent to + 1.0 log Mar. 6. Able and willing to sign informed consent, follow study instructions and complete all study visits. 7. 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. 8. Able to self-administer or have a caretaker administer study eye drops.
	Exclusion criteria: Investigator will exclude any subject who in their medical opinion they consider unsafe for participation in this study or does not consider they can complete the requirement of the protocol for any reason. 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. <u>General/Systemic:</u> Exclude subjects with: 24. Participation in a clinical study with use of any investigational drug or treatment within 30 days prior to Baseline (Day 0). 25. 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. 26. 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. 27. 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. 28. Known history of Hepatitis B + C, HIV+, or AIDS and/or inadequate venous access. 29. 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. 30. History of drug or alcohol abuse within the last 5 years. 31. Related to site study staff and/or site employees.
Number of Subjects:	Approximately 75 – 100 subjects will be enrolled
Number of Sites:	Approximately 4-8 US sites
Duration of Study:	Screening Visit: 1 day Washout Period: up to approximately 6 weeks (+ 1 week) depending on pre-study ocular hypotensive therapy and subject/site availability Treatment Period: 28 days
Study Assessments:	The following study assessments and procedures will be performed: • Medical and ophthalmic history (including prior and concomitant medications) • Adverse events • IP Compliance • Heart rate and blood pressure • ECG • Physical examination • Best-corrected visual acuity (ETDRS) • Biomicroscopy • Pupillary Diameter • Conjunctival hyperemia grading • Intraocular pressure (Goldmann tonometry) • Pachymetry • Gonioscopy • Visual field testing (automated, threshold) • Ophthalmoscopy (dilated) • Blood collection for pharmacokinetic assessments (at selected sites) • Clinical laboratories (hematology, clinical chemistry, pregnancy (if applicable), and urinalysis [UA])
Statistical Considerations:	**Safety:** **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 (including 0.5). 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. **Null hypothesis**: 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 stage 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). The primary efficacy measure will be the mean change in IOP from Baseline (Day 0) IOP (mmHg) for each group on Days 14 and 28 at each matched time point. IOP will be measured as indicated in the Schedule of Assessments and Procedures Table. Secondary efficacy endpoints will include: (1) the observed IOP, mean change and percent change from Baseline IOP at each matched time point at each visit and (2) the mean observed, mean change from Baseline and mean percent change from Baseline for the mean diurnal IOP at each visit, and (3) the proportion of subjects reaching a target IOP ($\leq$ 18 mmHg) at each time point and with the mean diurnal IOP for each visit. Each concentration will be compared to vehicle and the difference in means between treatment arms will be presented.
Investigational Drug Product:	H-1337 (Topical Multi-Kinase Inhibitor), sterile and preserved
Route of Administration:	Topical eyedrop

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
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

[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)
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.

LIST OF ABBREVIATIONS

AE	Adverse Event
AIDS	Acquired Immune Deficiency Syndrome
ALT	Argon Laser Trabeculoplasty
ALT	Alanine Aminotransferase
AMD	Age-related Macular Degeneration
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
CAI	Carbonic Anhydrase Inhibitor
°C	Degrees Celsius
CBC	Complete Blood Count
CFR	Code of Federal Regulations
A_{max}	Peak Concentration
CME	Cystoid Macular Edema
CPK	Creatine Phosphokinase
CRF	Case Report Form
ECG	Electrocardiogram
ET	Early Termination
ETDRS	Early Treatment Diabetic Retinopathy Study
°F	Degrees Fahrenheit
FDA	Food and Drug Administration
GCP	Good Clinical Practice(s)
GFR	Glomerular Filtration Rate
GGT	Gamma-Glutamyl Transferase
GLP	Good Laboratory Practice(s)
GTP	Guanosine-5'-Triphosphate
Hg	Mercury
HIV	Human Immunodeficiency Virus
HR	Heart Rate
IB	Investigator's Brochure
IC	Informed Consent Document
ICH	International Conference on Harmonization
IND	Investigational New Drug
IOP	Intraocular Pressure

IP	Investigational Product
IRB	Institutional Review Board
ITT	Intent-to-Treat
IUD	Intrauterine Device
IWRS	Interactive Web Response System
LASIK	Laser-Assisted In Situ Keratomileusis
LDH	Lactate Dehydrogenase
LLDPE	Linear Low Density Polyethylene
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
Mins	Minutes
mL	Milliliter
Mm	Millimeter
MOA	Mechanism of Action
NCS	Not Clinically Significant
NHP	Non-Human Primate
NZW	New Zealand White Rabbit
OD	Right Eye
OS	Left Eye
OHT	Ocular Hypertension
OTC	Over The Counter
OU	Both Eyes
PK	Pharmacokinetic
POAG	Primary Open Angle Glaucoma
PP	Per Protocol
PRK	Photorefractive Keratectomy
PT/PTT	Prothrombin/Partial Thromboplastin Time
QAM	Every Morning
QPM	Every Evening
q 12 hrs	Every 12 Hours
RBC	Red Blood Cell
RGC	Retinal Ganglion Cells
RhoA	Ras Homolog Gene Family, Member A
ROCKi	Rho Kinase Inhibitor
RPE	Retinal Pigment Epithelium
SAE	Serious Adverse Event
Ser/Thr	Serine/threonine
SLT	Selective Laser Trabeculoplasty
SPK	Superficial Punctate Keratitis
SUSAR	Suspected Unexpected Serious Adverse Reactions
TM	Trabecular Meshwork

T_{max}	Time to Peak Concentration
UA	Urinalysis
ULN	Upper Limit of Normal
US	United States
VF	Visual Fields
WBC	White Blood Cell

1 BACKGROUND

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 *(Prevent Blindness America www.preventblindness.org*; National Eye Health Program/National Institutes of Health *www.nei.nih.gov*).

Current treatments for glaucoma aim to reduce IOP, which has been shown to slow visual field loss in clinical trials (Musch et al., 2011). Intraocular pressure (IOP) is the only modifiable risk factor, and current therapies aim to reduce IOP. Drug therapies, classified by mechanism of action (MOA), include: beta-blockers, prostaglandin analogues, alpha-2 selective agonists, carbonic anhydrase inhibitors, sympathomimetics, miotics and hyperosmotic agents (Vetrugno, et al.,2008). Prostaglandins are now the most prescribed first-line agents to treat glaucoma worldwide and are used as a monotherapy and/or in conjunction with beta-blocker class agents (i.e., timolol). 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. While the regulation of intraocular pressure by the conventional (trabecular) outflow pathway is complicated, extracellular matrix expression and trabecular meshwork cytoskeletal shape and contractility appear to be important to control outflow resistance.

The IC_{50} values of kinases strongly inhibited by H-1337 include 7 kinases with values less than 0.1 µmol/L. Among the kinases that are potently inhibited by H-1337, LRRK2, PKA and PKD1 are kinases that are known to be involved in the modulation of intracellular cytoskeletal dynamics. 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 (Rao & Epstein, 2007). LRRK2 has been shown to affect actin polymerization *in vitro* and there is evidence for an association of LRRK2 with tubulin/actin, thus suggesting that cellular morphological changes may be consequences of LRRK2-modulation of cytoskeletal dynamics (Meixner, 2011, Gandhi, 2009). PKA phosphorylates many cytoskeletal proteins, including microtubules, intermediate filaments and actin and affects cell morphology and

migration through its ability to directly modulate the activity of LIM kinase and cofilin, a crucial modulator of actin dynamics (Nadella, 2009). Studies have also suggested an involvement of PKD1 in the regulation of cell shape, motility and adhesion, and phosphorylation of downstream substrates by PKD1 leads to altered cofilin activity and actin re-organization (Eiseler, et al, 2009). Finally, 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 (Stamer & Acott, 2012).

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.

1.1 Investigational Agent

H-1337 is a selective multi-kinase inhibitor. The Sponsor hypothesizes that inhibition of kinases that control intracellular cytoskeletal dynamics at the level of the trabecular meshwork and other cells that regulate flow is a mechanism that will improve outflow. As open-angle glaucoma is a disease of decreased trabecular outflow, the Sponsor believes that H-1337 beneficially enhance aqueous outflow by modulating the contractile state of TM cells to decrease resistance to fluid transport.

The H-1337 investigational drug product is a preserved, isotonic, sterile ophthalmic solution at three concentrations (w/v) (0.06%, 0.2%, and 0.6%), buffered at pH 6.5 (range 6.3 – 6.7). Sodium chloride is used to adjust the osmolarity to 280 - 320 mOsm/kg. The primary drug product packaging is a white, sterile low-density polyethylene (LDPE) multi-dose, 5 mL bottle. A sterile LDPE nozzle/dropper insert (with nominal drop volume of 43 mm^3) is used to dispense the ophthalmic solution. The container closure system is closed with a sterile white high-density polyethylene (HDPE) cap.

Table 1 Composition of H-1337 Drug Product

Component	Monograph	Quantity, w/v	Function
H-1337	N/A	Placebo, 0.06, 0.20, 0.60%	Active ingredient
WFI	USP	q.s. to 100%	Diluent
Sodium Phosphate, Dibasic, Anhydrous	USP	0.15	Buffer
Sodium Phosphate, Monobasic, Monohydrate	USP	0.05	Buffer
Sodium Chloride	USP	0.68-0.75	Tonicity
Edetate Disodium, Dihydrate	USP	0.10%	Preservative
50% Benzalkonium Chloride	NF	0.01%	Preservative
Sodium Hydroxide	NF	as needed	pH adjustment
Hydrochloric Acid	NF	as needed	pH adjustment

The placebo control (vehicle) will consist of the components of the ophthalmic formulation without H-1337.

1.2 Preclinical Data

To support the topical ocular administration of H-1337 in humans, a series of nonclinical toxicology and safety pharmacology studies with topical and systemic dosing of H-1337 were conducted. These studies included GLP 4-week studies in rabbit and monkey using the topical/ocular mode of administration which evaluated the potential H-1337 to cause toxicity to the eye and surrounding ocular tissues as well as the potential for H-1337 to cause systemic toxicity. To more comprehensively characterize the systemic safety profile of H-1337 in the event of significant absorption from the ocular into the systemic or non- ocular peripheral tissue compartments, a comprehensive series of studies were conducted utilizing parenteral or oral routes of administration. The core battery of safety pharmacology studies included a neuropharmacology study and a pulmonary function study in rats as well as a cardiovascular study in conscious telemetered monkeys.

Overall, the concern for potential adverse effects is largely mitigated by the very large safety margins between the doses associated with systemic effects in animals and the proposed human clinical doses/concentrations to be administered topically.

The effects defined in nonclinical toxicology and safety pharmacology studies conducted with H-1337 following topical ocular administration in the proposed formulation support the doses proposed for clinical evaluation. Given the collective nonclinical data and the low systemic levels post topical ocular administration of H-1337, systemic effects are considered unlikely following topical ocular administration in humans.

For complete information on the preclinical studies performed, please see the Investigator's Brochure.

1.3 Risk/Benefits

H-1337 is a new drug and has not yet been evaluated in humans. While H-1337 is a new drug being tested for the first time in humans, other kinase inhibitors have been associated with conjunctival hyperemia and, in some instances, conjunctival hemorrhage. Transient conjunctival hyperemia has been noted in preclinical studies of H-1337. Additional adverse effects could include signs and symptoms associated with ocular irritation.

The potential benefit to humans will be the IOP-lowering capabilities via a new mechanism that affects the trabecular meshwork and conventional outflow system. Numerous studies, both *in vitro* and *in vivo,* have been conducted to characterize the systemic and ocular toxicology profile of H-1337. For complete information on the preclinical studies performed, please see the Investigator's Brochure.

1.4 Dose Rationale

Ocular PK data in rabbits and monkeys confirmed that H-1337 exhibits dose-related effects and can reduce IOP for upwards of 8-12 hours. The clinical doses, treatment period (28 days), and dosing regimen (BID) that are expected to have an acceptable safety profile have been selected based on preclinical toxicology data. The total daily administered dose of H-1337 in human subjects will be approximately 1 mg (or 0.014 mg/kg/day, calculated assuming a 70-kg subject) when administered twice daily in both eyes at the maximal concentration of 0.6%.

1.5 Trial Conduct

This study will be conducted in accordance with current US FDA regulations, International Conference on Harmonization (ICH) guidelines, Good Clinical Practice (GCP) standards, the Declaration of Helsinki, and local ethical and legal requirements.

1.6 Population

Study population will include subjects of either sex and of any race/ethnicity over 18 years of age with a diagnosis of bilateral primary open-angle glaucoma or ocular hypertension.

2 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.

3 STUDY DESIGN

3.1 Study Design

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.

3.2 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.

3.3 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. The mask should be broken only for safety purposes and only if knowing the drug allocation will have a direct impact on the subject's immediate medical management. If at all possible, the Medical Monitor should be contacted prior to emergency unmasking:

William C. Stewart, MD
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If this is not possible and unmasking occurs without prior Medical Monitor approval, the Medical Monitor must be notified within 24 hrs. The circumstances leading to unmasking of each subject are to be promptly communicated via telephone and/or in writing to the sponsor

(or sponsor's designee) and the IRB. Early termination procedures should be conducted for any subject for whom the randomization code is broken.

3.4 Trial Treatment

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.

3.5 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. The table below lists the applicable washout durations for various pre-study ocular hypotensive medications. (Note: This table may not be inclusive of all ocular hypotensive medications. The Medical Monitor should be contacted for further information regarding the washout period of an ocular hypotensive not included in the table.) If the Investigator assesses an acceptable risk, subjects may extend washout up to 49 days to accommodate scheduling.

Table 2 Ocular Hypotensive Medication Washout Schedule

Ocular Hypotensive Generic/Trade Names	Washout period* (between Screening and Baseline Visits)
Muscarinic agonists (e.g., pilocarpine) and Oral or Topical Carbonic Anhydrase Inhibitors (CAI)	≥ 5 days
Beta-adrenoceptor antagonists	≥ 6 weeks
Alpha adrenoceptor agonists	≥ 5 weeks
Prostaglandin analogues, Combination Drugs (use longest wash-out period of individual components) *	≥ 6 weeks

*The washout period of longest duration should be used when the subject is taking multiple ocular hypotensive medications from more than one class. Additional +1 week extended washout permitted to accommodate scheduling considerations.

Following the applicable washout period, the subject will return for applicable inclusion/exclusion evaluations on the Baseline Visit (Day 0). On Day 0, a subject meeting all inclusion/exclusion criteria will be randomized to IP. On Days 0 to 3, only the study eye will be dosed and evaluated for safety. For the remainder of treatment, both eyes will be dosed and evaluated for safety. The study eye is the eye that meets the entry 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.

Dosing will be initiated in the study eye on Day 0 in the office after the completion of all required examinations. The subject will be dosed for up to 28 days during which time they will be monitored for safety, tolerability, and efficacy assessments according to the Schedule of Study Assessments and Procedures.

The duration of participation for subjects in each stage will be as follows:

Screening Visit: 1 day
Washout Period: up to 6 weeks (+ 1 week) depending on pre-study ocular hypotensive therapy
Treatment Period: 28 days

3.6 Drug Packaging and Labeling

H-1337 and its vehicle will be packaged in a 5 mL low density polyethylene (LDPE) clear bottle with a dropper nozzle insert delivering a drop volume of 43 µL. The bottles will be packaged in cartons in a format appropriate for the study; both bottles and cartons will be labeled in a format appropriate for the study. Representative labels are as follows:

Bottle label:

Placebo or H-1337 Ophthalmic Solution, 5.0 mL
Use as directed. Refrigerate at 2-8°C until opened.
Caution: New Drug-Limited by Federal (US) Law to Investigational Use. Allysta Pharmaceuticals, Inc.
Protocol: ALY337-201 Number: ######

Carton label:

Placebo or H-1337 Ophthalmic Solution, 5.0 mL
Protocol Number: ALY337-201
Subject Number: _____
Assignment Number: #####
Store refrigerated at 2-8 °C (36-46°F). Protect from Freezing. "Caution: New Drug-Limited by Federal (United States) Law to Investigational Use". Sponsor: Allysta Pharmaceuticals, Inc.

3.7 Drug Accountability and Storage

All IP required for this study will be provided by Allysta Pharmaceuticals, Inc., or its designee. The recipient will acknowledge receipt of IP, indicating shipment content and condition. Damaged supplies may be replaced upon notification to Allysta Pharmaceuticals, Inc., or its designee. Accurate records of all IP dispensed from and returned to the study site should be maintained and recorded. A study monitor will periodically check the supplies of IP held at the site to verify accountability of all IP. All used and unused IP (non-dispensed) will be returned to Allysta Pharmaceuticals, Inc., or its designee.

IP must be kept in a safe storage area with limited access (e.g., in a locked cabinet). The storage area should be temperature monitored. IP should be stored at controlled refrigerated temperatures from 2°C to 8°C and protected from light. After opening, IP bottles may be stored at room temperature, i.e., 15°C to 25°C. Subjects should be instructed not to leave the IP in their car, near a window, or outdoors where it may be exposed to direct sunlight and/or heat for a prolonged period.

The IP must not be used outside the context of this protocol. Under no circumstances should the investigator or site personnel supply IP to other clinical sites, investigators or subjects, or allow the IP to be used other than as directed by this protocol without prior authorization from Allysta Pharmaceuticals, Inc.

4 SELECTION AND WITHDRAWAL OF SUBJECTS

Subjects must fulfill all the inclusion and exclusion criteria and the investigator must obtain written approval from Allysta Pharmaceuticals, Inc., to confirm any deviation from these ***prior to*** randomization of the subject.

4.1 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:
   - Baseline (Day 0) at T_0 (T_0 = 8 am ± 30 min) IOP ≥ 23 mmHg in the study eye.
4. IOP criteria after washout ≤ 32 mmHg OU at all time points.
5. Best-corrected visual acuity (BCVA) in both eyes of 20/200 or better on Snellen, equivalent to + 1.0 log Mar.
6. Able and willing to sign informed consent, follow study instructions and complete all study visits.
7. 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.
8. Able to self-administer or have a caretaker administer study eye drops.

4.2 Exclusion Criteria

Exclusion criteria:

Investigator will exclude any subject who in their medical opinion they consider unsafe for participation in this study or does not consider they can complete the requirement of the protocol for any reason.

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.

<u>General/Systemic:</u>

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.

4.3 Subject Withdrawal and/or Discontinuation

Subjects are free to discontinue their participation in the study at any time without prejudice to further treatment. The investigator must withdraw any subject from the study at their request. Subjects should provide the reason for withdrawal and confirm whether they are withdrawing consent or withdrawing from IP treatment and will continue to participate in the study. The investigator or Allysta Pharmaceuticals, Inc., may withdraw the subject from IP treatment for any reason or if the subject meets any of the following criteria.

- Experiences a serious or intolerable AE that is judged by the investigator to preclude further study participation.
- Develops a clinically significant laboratory or other abnormality that is judged by the investigator to be potentially related to IP.
- Requires a medication that is prohibited by the protocol. Contact the Medical Monitor to discuss exiting a subject due to use of a prohibited medication before exiting the subject.
- Does not follow the protocol procedures, including noncompliance.
- Is lost to follow-up; every effort should be made to contact the subject.
- Administrative reasons, including termination of the trial by the Sponsor, unavailable for follow-up procedures.

- Experiences glaucomatous progression and/or unacceptable IOP elevation, per the discretion of the investigator.

Should a subject's medical or mental condition change or become unstable during the study, such that the ability of the subject to continue in this study becomes compromised or, in the investigator's judgment, their health is jeopardized, or further continuation is unadvisable, the subject may be withdrawn from study treatment and/or withdrawn from the study.

If a subject prematurely withdraws from the study, either at her/his request or at the discretion of the investigator or Allysta Pharmaceuticals, Inc. the reason for withdrawal should be recorded. All subjects prematurely discontinuing study dosing, regardless of cause, will return within 14 days from their last dose of IP to complete Early Termination visit assessments; consult the Medical Monitor to determine which diurnal measurements, if any, are required for the ET visit. Study subjects who withdraw from the study may be replaced at the Sponsor's discretion.

It is vital to obtain follow-up data for any subject withdrawn due to an AE or abnormal laboratory test. In any case, every effort must be made to undertake safety follow-up procedures.

If a subject does not return for a scheduled visit, every effort should be made to contact the subject. If every effort is made to contact the subject (including two telephone calls and a traceable letter sent to the subject) and these attempts are unsuccessful, the subject is considered lost to follow-up.

4.4 Medication

During the washout period from prior IOP-lowering monotherapy, topical carbonic anhydrase inhibitors (CAI) (i.e., Dorzolamide (Trusopt®) or Brinzolamide (Azopt®) will be permitted, but will not be provided by the Sponsor. Should a topical CAI be employed during the washout period, the applicable washout for CAIs should be implemented and the CAI should be discontinued 5 days prior to Baseline (Day 0).

Current systemic mediations which may influence intraocular pressure should be stable and not expected to change over the course of the trial. Should a subject's medical condition change or become unstable during the study, such that the change in non-ocular medication that can or may affect IOP are added or altered (i.e., steroids, systemic beta blockers, etc.), the subject's condition should be reviewed with the Medical Monitor prior to possible withdrawal from the study. If the subject is withdrawn, the subject will continue to be followed until he/she returns within 14 days from the last dose of IP to complete the Early Termination visit.

If, during the study, an investigator determines the IOP is inadequately controlled such that the safety of the subject is compromised, the PI may withdraw the subject from study treatment and institute IOP treatment.

4.5 Monitoring for Subject Compliance

Subjects or their caretakers will administer study medications according to the IP dosing instructions and training provided. Compliance will be assessed at each visit 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.

4.6 Lifestyle Guidance

During the study, female subjects of childbearing potential (females who are menarchal) must use reliable precautions to prevent pregnancy. These restrictions should be followed throughout the duration of the study and maintained for 30 days after trial completion.

Acceptable contraceptive methods for females of child-bearing potential:

- Abstinence
- Hormonal methods of contraception (including oral and transdermal contraceptives, injectable progesterone, progestin sub-dermal implants, progesterone-releasing IUDs)
- Placement of a copper-containing intrauterine device (IUD)
- Condom with spermicidal foam/gel/film/cream/suppository
- Male partner who has had a vasectomy for at least 4 months
- Tubal ligation
- Hysterectomy

5 STUDY VISIT PROCEDURES

All study visit procedures should be conducted in the order listed Section 5.1.

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. The same type of device and cuff must be used throughout the study on the same subject. An appropriate cuff size with a bladder encircling at least 80% of the upper arm should be used to ensure accurate measurements.

Blood pressure will be measured using the most ideal conditions as possible given the subject's medical condition and age. If possible, the same arm should be used throughout the study. Blood pressure should be measured after sitting in a seated position for 5 minutes, prior to the collection of blood samples for PK evaluations.

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 $\geq$ 10 letters may indicate an adverse event and should be investigated further by the investigator and the refraction should be rechecked.

ETDRS Scoring Methodology: The best-corrected visual acuity will be measured using an ETDRS (Early Treatment Diabetic Retinopathy Study) chart. The test distance must be at the distance specified on the ETDRS chart.

Position the ETDRS chart at the approximate eye level of an average-height seated subject. Mark a spot on the floor (e.g., with tape) that is the appropriate test distance from the ETDRS chart. The test distance for the chart must be kept constant throughout the study.
The subject should be seated comfortably, directly in front of the chart, so that the eyes remain at the appropriate distance as determined by the ETDRS chart. Testing always begins with the right eye, followed by the left eye. The fellow eye should be occluded.

Do not point to specific letters on the chart during the test. Ask the subject to read each letter slowly, line by line, left to right, beginning with Line 1 at the top. Tell the subject that the chart has letters only, no numbers. If the subject reads a number, remind him/her that the chart contains no numbers, and request a letter in lieu of the number. The subject should be told that only one chance is given to read each letter, but he/she may change his/her mind before moving to the next letter. Do not allow the subject to proceed to the next letter until he/she has given a finite answer. If the subject is unsure about the identity of the letter, then the subject should be encouraged to guess. The subject should be encouraged to continue reading even if making mistakes. When a subject reaches a level where he/she cannot guess, the examiner may stop the test.

Each letter read correctly is counted. The examiner will total each line and the whole column (0 if no letters are incorrect) on the data collection form and record in the format specified.

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. Magnification, lighting, and examiner should be consistent for each subject throughout the study. 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 conjunctiva1
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

CONJUNCTIVA (continued)

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 conjunctiva1
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. The tonometer calibration should be checked for accuracy within one month before screening the first subject for the study. A Tonometer Calibration Form must be completed and filed before the first subject is screened and calibration must be checked monthly throughout the study. Variation within ± 2 mm Hg is acceptable. The fluorescein and anesthetic agents should remain consistent throughout the study. IOP will be measured following slit lamp biomicroscopy.

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. Mean IOP values should be rounded up to the next whole number if the value is equal to or greater than 0.5 mmHg, and rounded down if less than 0.5 mmHg (e.g., 24.5 should be rounded to 25, 24.4 should be rounded to 24). The same procedure will be repeated on the contralateral eye.

In order to minimize confounding variables, every effort should be made to measure all IOP measurements for each subject by the same examiner using the same tonometer and approximately at the same time at all visits.

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%. Visual fields are to be performed with a non-dilated pupil unless, in the opinion of the investigator, the pupil is so miotic that dilation is required (i.e., < 3 mm). If the visual field examination was conducted using dilation at the Screening Visit, dilation should be used for any subsequent visual field examinations (i.e., if performed at an unscheduled visit; visual field testing should be performed during the trial if glaucomatous progression is suspected). Visual field interpretation must be documented and reviewed by the investigator, including an assessment of whether the results are normal or abnormal and the clinical significance of abnormalities.

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.

5.1 Study Visits

Perform study procedures as referenced in the Schedule of Study Assessments and Procedures (see protocol synopsis) in the order specified below.

5.1.1 Screening

- Informed consent
- Medical/ophthalmic and concomitant medication history, with review of inclusion/exclusion criteria
- BCVA
- Biomicroscopy
- Goldmann tonometry
- Gonioscopy
- Pachymetry
- Visual field testing
- Dilated ophthalmoscopy
- At any time during the visit after informed consent:
    - Heart rate/blood pressure
    - ECG
    - Physical examination
    - Phlebotomy and urine sample for clinical laboratories

5.1.2 Day 0/Baseline

- Medical/ophthalmic and concomitant medication history, with review of inclusion/exclusion criteria
- BCVA
- Biomicroscopy at all required timepoints
- Pupillary diameter
- Conjunctival hyperemia grading
- Goldmann tonometry at all required timepoints

- Phlebotomy for pK (any time prior to IP dosing)
- IP administration

5.1.3 Days 1 and 4

- Concomitant medications
- Adverse events
- IP compliance (Day 4 only)
- BCVA
- Biomicroscopy
- Conjunctival hyperemia grading (Day 4 only)
- Goldmann tonometry
- IP administration

5.1.4 Days 14 and 28

- Phlebotomy and urine sample for clinical laboratories (Day 28 only at any time during the visit)
- Concomitant medications
- Adverse events
- IP compliance
- Heart rate/blood pressure at all required timepoints
- BCVA
- Biomicroscopy at all required timepoints
- Pupillary diameter (Day 28 only)
- Conjunctival hyperemia grading at all required timepoints
- Goldmann tonometry
- Phlebotomy for pK at all required timepoints (Day 28 only)
- IP administration (Day 14 AM and PM, Day 28 AM only)
- Dilated ophthalmoscopy after final Goldmann tonometry (Day 28 only) – may be performed on following day for scheduling convenience.

6 ASSESSMENT OF EFFICACY

6.1 Efficacy Parameters

Primary Efficacy Endpoints:

The primary efficacy measure will be the mean change in IOP from Baseline (Day 0) IOP (mmHg) for each group on Days 14 and 28 at each matched time point as compared to vehicle. IOP will be measured according to the Schedule of Assessments and Procedures Table.

Secondary efficacy endpoints:

1. The observed IOP, mean change from Baseline, and % change from Baseline IOP at each matched time point at each visit, and
2. The mean observed, mean change from Baseline and mean % change from Baseline for the mean diurnal IOP at each visit, and
3. The proportion of subjects reaching a target IOP ($\leq$ 18 mmHg) at each time point and with the mean diurnal IOP for each visit. Each concentration will be compared to vehicle and the difference in means between treatment arms will be presented.

6.2 Method and Timing

The efficacy parameter for this study is IOP as measured by Goldman applanation tonometry and recorded on the CRF. The timing of the IOP measurements are based on preclinical IOP data that was observed both in NHP and rabbits. The time points of diurnal IOP measurements are based on the expected peak and trough of H-1337 efficacy, the timing and frequency of dosing of H-1337 during 24 hours, and the circadian IOP diurnal curve.

7 ASSESSMENT OF SAFETY

7.1 Adverse Event Reporting

The investigator is responsible for monitoring the safety of subjects who have entered the study. Subjects should be asked at each visit about any changes in their health status. Any changes in health status after informed consent has been obtained, but prior to the first dose of IP, will be reported as medical history. AEs will be reported after the subject receives the first dose of IP (Day 0). Ongoing AEs at early termination or study completion (Day 28) will be followed until the event resolves, stabilizes, or for 30 days, whichever comes first.

All adverse events (serious and non-serious) must be documented regardless of the causal relationship to the study medication. The severity, frequency, seriousness, duration, relationship to IP(s), treatment of event, action taken with IP, and outcome for every adverse event should be documented in the source documents and CRFs.

Non-clinically significant incremental < 2 grade changes in conjunctival hyperemia (according to the photographic reference scale provided for the trial) are not required to be reported as adverse events.

To capture the most potentially relevant safety information during a clinical study, it is important that investigators record accurate AE terms on CRFs. Wherever possible, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the investigator and recorded on the CRF. However, if an observed or reported sign or symptom is not considered a component of a specific disease or syndrome by the investigator, it should be recorded as a separate AE on the CRF.

Investigators are required to report to Allysta Pharmaceuticals, Inc. or its representative all observed and/or volunteered AEs occurring during the clinical trial (21 CFR §312.64[b]) regardless of treatment group or suspected causality to the investigational drug.

7.2 Definition of Adverse Event

An AE is any unfavorable medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product, which does not necessarily have to have a causal relationship with this treatment. An AE can, therefore, be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product. All AEs, including observed or volunteered problems, complaints, or symptoms, must be recorded. This definition also includes AEs which are reported up to 30 days after a subject has completed a clinical study. Therapeutic failures during clinical trials are not considered to be AEs.

Examples of adverse events include, but are not limited to:

- Abnormal test finding
- Clinically significant symptoms and signs

- Changes in physical examination findings
- Hypersensitivity
- Progression/worsening of underlying disease
- Change in BCVA > 10 letters
- Clinically significant change in pachymetry

Additionally, they may include the signs and symptoms resulting from:

- Drug overdose
- Drug withdrawal
- Drug abuse
- Drug misuse
- Drug interactions
- Exposure in utero (i.e., pregnancy within 30 days of study completion)

7.3 Abnormal Test Findings

The criteria for determining whether an abnormal objective test finding should be reported as an adverse event are as follows:

- Test result is associated with accompanying symptoms

  and/or

- Test result requires additional diagnostic testing or medical/surgical intervention

  and/or

- Test result leads to a change in study dosing or discontinuation from the study, significant additional concomitant drug treatment, or other therapy

  and/or

- Test result is considered to be an adverse event by the investigator or Allysta Pharmaceuticals, Inc.

Reporting an abnormal test, in absence of any of the above conditions, does not constitute an adverse event. Any abnormal test result that is determined to be an error or is felt to be not clinically significant (NCS) by the investigator does not require reporting as an adverse event.

Abnormal laboratory test results reported to the investigator from the time the subject has taken at least one dose of the study treatment through last subject visit should be recorded as an adverse event if the above criteria are met.

7.4 Definitions of Non-Serious Adverse Events

Adverse events that do not meet the definitions for serious adverse events (SAEs) are non-serious AEs. Subjects should be instructed to report any AE that they experience to the investigator.

7.5 Definition of Serious Adverse Event

An SAE is any experience that suggests a significant hazard, contraindication, side effect, or precaution. This includes any experience that:

- Results in death
- Is acutely life-threatening
- Requires in-subject hospitalization or prolongs the existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Requires medical or surgical intervention to prevent one of the outcomes listed above

Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-subject hospitalization, or the development of drug dependency or drug abuse. This definition includes concurrent illness or injuries, exacerbation of pre-existing conditions and AEs occurring as a result of drug withdrawal, abuse or overdose. Serious adverse events observed during all periods of a clinical study are to be recorded, including those occurring during a period without study medication and whether or not the AE is judged to be related to the study medication.

7.6 Hospitalization

Reported adverse events associated with hospitalization or prolonged hospitalizations are considered serious. Any initial admission (even if less than 24 hours) to a healthcare facility meets these criteria. Admission also includes transfer within the hospital to an acute/intensive care facility.

Hospitalization does not include the following:

- Rehabilitation facility
- Hospice facility

- Respite care (caregiver relief)
- Skilled nursing facility
- Nursing homes
- Routine emergency admission
- Same day surgeries

Hospitalization or prolongation in the absence of a precipitating, clinical adverse event (e.g., for work-up of persistent pre-treatment lab abnormality) is not in itself a serious adverse event.

Diagnostic and therapeutic noninvasive procedures, such as surgery, should not be reported as adverse events. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an adverse event. For example, an acute appendicitis that begins during the adverse event reporting period should be reported as an adverse event and the resulting surgical intervention should be recorded as a treatment of that adverse event.

7.7 Pregnancy

If a subject becomes pregnant during the study or up until 30 days after the last dose of IP, the Investigator must notify Allysta Pharmaceuticals, Inc. or its representative immediately and within 24 hours of learning of its occurrence by completing a Pregnancy Report Form and forwarding to Allysta Pharmaceuticals, Inc. or its representative. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. Any complications during pregnancy should be recorded as an AE and may constitute an SAE if they fulfill any of the specified criteria for an SAE. If upon outcome, the pregnancy meets one of the serious criteria (e.g. spontaneous miscarriage, congenital anomaly, or birth defect), it will then be considered an SAE and full details will be requested. These events will not be included in the clinical study report, but will be maintained in the clinical study files.

7.8 Definition of Unexpected Adverse Events

An AE is considered an unexpected AE if it is not mentioned in the Investigator's Brochure (IB), or if it is of greater frequency and/or severity than that mentioned in the IB, in the judgment of the Medical Monitor.

7.9 Definition of Relationship of Adverse Event to IP

The investigator's assessment of causality must be provided for all adverse events (serious and non-serious). The investigator must record the relationship in the CRF, as appropriate, and report such an assessment in accordance with the serious AE reporting requirements, if

applicable. An investigator's causality assessment is the determination of whether there exists a reasonable possibility that the investigational product caused or contributed to the adverse event. If the investigator does not know whether or not the investigational product caused the event, then the event will be handled as "related to investigational product" for reporting purposes, as defined by Allysta Pharmaceuticals, Inc. If the investigator's causality assessment is "unknown but not related to investigational product," this should be clearly documented in study records.

In addition, if the investigator determines a serious adverse event is associated with study procedures, the investigator must record this causal relationship in the source documents and CRF, as appropriate, and report such an assessment in accordance with the serious adverse event reporting requirements, if applicable. The categories are as follows:

0=Unrelated:	No temporal association, or the cause of the event has been identified, or the drug, biological, or device cannot be implicated.
1=Possibly Related:	Temporal association, but other etiologies are likely to be the cause; however, involvement of the drug, biological, or device cannot be excluded.
2=Probably Related:	Temporal association, other etiologies are possible, but unlikely.
3=Definitely Related:	Temporal association and no other etiologies possible.

7.10 Documentation of Severity of Adverse Events to IP

All adverse events will be examined to determine severity. The categories are as follows:

Mild:	Awareness of sign, symptom, or event, but easily tolerated. Does not interfere with subject's usual function.
Moderate:	Discomfort enough to cause some interference with usual activity and may warrant intervention.
Severe:	Incapacitating with inability to do usual activities or significantly affects clinical status, and warrants intervention.
Life-threatening:	Immediate risk of death.

7.11 Reporting Serious Adverse Events

SAEs (this refers to any AE that meets one or more of the aforementioned serious criteria) occurring after the subject receives the first dose of IP (Day 0) will be reported to Allysta Pharmaceuticals or its representative, followed until the event resolves or stabilizes, the subject's early termination or study completion (Day 28), or within 30 days of the last administration of IP, whichever comes first.

SAEs, and all deaths occurring within the study population, whether considered to be drug-related or not, will be reported on the SAE Report Form within 24 hours of knowledge of the occurrence to Allysta Pharmaceuticals, Inc. or its representative.

This can be done by emailing a completed SAE Report Form to:

Name: Ashley Hehr
Title: Drug Safety Specialist Consultant
Company: Safety Sphere, LLC (www.safety-sphere.com)
Email: aly337-201sae@safety-sphere.com
Office Phone: 844-965-1070
Office Fax: 855-805-2406

Where the initial report is made verbally or by telephone to Allysta Pharmaceuticals, Inc. or the study monitor, the SAE Report Form must be emailed within 24 hours according to the instructions above.

The investigator will be requested to supply detailed information regarding the event at the time of the initial contact. All SAEs (initial and follow-up information) must be reported to the reviewing Institutional Review Board/Ethics Committee, according to their reporting requirements, and a copy of that report must be forwarded to Allysta Pharmaceuticals, Inc. or its representative. Allysta Pharmaceuticals (or designee) will report all Suspected Unexpected Serious Adverse Reactions (SUSARs) to Regulatory Authorities within 7/15 days of receipt depending on reporting requirements.

7.12 Serious Adverse Event Follow-Up

For all SAEs, the investigator must submit follow-up reports to Allysta Pharmaceuticals, Inc., regarding the subject's subsequent course until the SAE has subsided, or until the condition stabilizes (in the case of persistent impairment), the subject dies, or within 30 days of the last administration of IP, whichever comes first.

SAEs that come to the attention of the investigator within 30 days following early termination or Day 28 will be reported to Allysta Pharmaceuticals, Inc. or its representative within 24 hours of knowledge of the occurrence.

8 STATISTICAL PLAN

8.1 Sample Size

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 stage 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.

8.2 Statistical Methods

Continuous and ordinal measures will be summarized by descriptive statistics (n, mean, standard deviation, median, minimum and maximum values). Qualitative measures will be tabulated by counts and percentages. Selected safety measures may be summarized by the use of shift tables. Summary tables will show the data for each time point (visit and/or time of day as appropriate) and will display the observed and change (and for IOP the % change) from baseline value.

A statistical analysis plan will be prepared prior to unmasking the treatment assignments.

An analysis of results will be conducted when the database for each stage has been locked and the treatment assignments have been revealed. No within-stage interim analyses are planned.

8.3 Subject Population(s) for Analysis

Up to approximately 4 to 8 US sites will be active.

All subjects who receive IP and at least one dose will be evaluable for the safety analysis. For efficacy considerations, the primary analysis population will be the intent-to-treat (ITT) population. All subjects who receive study medication and who complete at least one on-therapy study visit will be evaluable for the ITT analyses. 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.

8.4 Significance

For superiority testing, a 2-sided test with a significance level of $p \leq 0.05$ will be used.

8.5 Accountability Procedure

It is expected that in this small trial of brief duration, the incidence of missing data will be kept to a minimum. Sites, subjects and Allysta Pharmaceuticals, Inc., or its representative will be trained in methods to encourage continued participation to maximize the completion rate and overall quality of the trial. As this is a small Phase 1/2a exploratory trial, there are no plans to impute values for missing data. With regard to the primary efficacy variable, IOP, there are steps taken in the procedure for collecting these data that minimize the likelihood of spurious data.

8.6 Deviation Reporting

Any deviations from the statistical plan enumerated in the protocol or final statistical plan will be described and justified in the clinical study report.

9 DIRECT ACCESS TO SOURCE DATA/DOCUMENTATION

Study visits will be conducted by an authorized Allysta Pharmaceuticals, Inc., representative to inspect study data, subjects' medical records, and CRFs in accordance with current US Good Clinical Practices (GCP) and the respective local, national government and international regulations and guidelines.

The investigator will permit authorized representatives of Allysta Pharmaceuticals, Inc., or its representative, the Food and Drug Administration (FDA), and the appropriate health authorities to inspect facilities and records relevant to this study.

10 DATA REVIEW AND ANALYSIS

There will be no formal external Data Monitoring Committee. The masked data will be continually monitored on an ongoing basis, according to the safety plan, as subjects are enrolled and complete the study. During the study, investigators, site personnel, and Allysta Pharmaceuticals personnel responsible for the daily conduct of the study will remained masked to the results to maintain the scientific rigor and credibility of the trial. Allysta Pharmaceuticals personnel and selected external consultants will be unmasked once data is locked. Data will then be reviewed, the safety and efficacy for each dose determined.

11 QUALITY CONTROL AND QUALITY ASSURANCE

Each investigator must adhere to the protocol as detailed in this document and agrees that any changes to the protocol must be approved by Allysta Pharmaceuticals, Inc., prior to seeking approval from the IRB/Ethics Committee. Each investigator will be responsible for enrolling only those subjects who have met protocol eligibility criteria. During study conduct, Allysta Pharmaceuticals and/or its representative will conduct periodic monitoring visits to ensure that the protocol and Good Clinical Practices (GCPs) are being followed. The monitors may review source documents to confirm that the data recorded on CRFs is accurate. The investigator and institution will allow Allysta Pharmaceuticals, Inc., monitors or its agents and appropriate regulatory authorities direct access to source documents and CRFs to perform this verification.

It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

12 ETHICAL CONSIDERATIONS

This study will be conducted in accordance with current US FDA regulations, International Conference on Harmonization (ICH) guidelines, Good Clinical Practice (GCP) standards, the Declaration of Helsinki, and local ethical and legal requirements. The Principal Investigator must sign the protocol signature page to indicate acceptance of the protocol.

12.1 Informed Consent

The subject must sign the Informed Consent Document before his or her participation in the study. A copy of the Informed Consent Document must be provided to the subject or the subject's legal guardian. If applicable, it will be provided in a certified translation of the subject's first or native language. The original signed informed consent document for each participating subject shall be filed with records kept by the investigator and must be available for verification by study monitors at any time, and a copy will be given to each subject.

12.2 Institutional Review Board/Ethics Committee

This protocol, the informed consent document, relevant supporting information and all types of subject recruitment or advertisement information must be submitted to the IRB/Ethics Committee for review and must be approved before the study is initiated. Any amendments to the protocol must also be approved by the IRB/Ethics Committee prior to implementing changes in the study. The investigator is responsible for keeping the IRB/Ethics Committee apprised of the progress of the study, any SAEs, and any changes made to the protocol according to the requirements of the site's IRB.

12.3 Confidentiality

The information obtained during the conduct of this clinical study is confidential, and disclosure to third parties other than those noted below is strictly prohibited. Information obtained during the conduct of this study will be used by Allysta Pharmaceuticals, Inc., in connection with the development of the IP. The study investigator is obliged to provide Allysta Pharmaceuticals, Inc., or designee with complete test results and all data developed in this study. This information may be disclosed to other physicians who are conducting similar studies and to the FDA as deemed necessary by Allysta Pharmaceuticals, Inc. Subject-specific information may be provided to other appropriate medical personnel only with the subject's permission.

12.4 Early Sponsor Discontinuation of the Trial

All clinical investigational data will be reviewed by the Medical Monitor on a regular basis. Reports of all data will be made available to the appropriate IRB/Ethics Committee and to the FDA. The clinical investigation may be suspended if the Medical Monitor, upon review and evaluation of the clinical data, finds the severity or incidence of single or total complications unacceptable for continuation of the investigation.

13 DATA HANDLING AND RECORD KEEPING

The investigator must retain all study records according to ICH guidelines and according to the record retention policies of the country where the study is being conducted. FDA requires that records are retained for at least 2 years after a marketing application is approved for the drug, or if an application is not approved, until 2 years after FDA has been notified of the discontinuation of the investigational use of the drug (21CFR312.57).

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