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Study VVN559-CS201
Clinical protocol

A phase 2, double-masked, randomized, vehicle-controlled, dose-response study assessing the safety and ocular hypotensive efficacy of VVN539 in subjects with primary open angle glaucoma or ocular hypertension.

Dated 7 June 2022

Title Page

A phase 2, double-masked, randomized, vehicle-controlled, dose-response study assessing the safety and ocular hypotensive efficacy of VVN539 in subjects with primary open angle glaucoma or ocular hypertension.

Protocol No.:	VVN539-CS-201
Investigational New Drug (IND) No.:	158012
Study Phase:	2
Sponsor:	VivaVision Biotech, Inc. 304-866 Halei Rd, Pudong District, Shanghai, China
Medical Monitor:	
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Protocol Synopsis

Title:	A phase 2, double-masked, randomized, vehicle-controlled, dose-response study assessing the safety and ocular hypotensive efficacy of VVN539 in subjects with primary open angle glaucoma or ocular hypertension.
Phase:	2
Design/Conduct:	Prospective, parallel-comparison, multi-center, double-masked, randomized, vehicle-controlled dose-response study assessing the safety and ocular hypotensive efficacy of VVN539 in subjects with primary open angle glaucoma (POAG) or ocular hypertension (OHT) in the United States (US).
Objective(s):	Efficacy: To evaluate the ocular hypotensive efficacy of 2 concentrations of VVN539. Safety: To evaluate the ocular and systemic safety of 2 concentrations of VVN539
Endpoint(s):	Primary Efficacy: Mean intraocular pressure (IOP) at each diurnal time point (8AM, 10AM, and 4PM) at Visit 4 (Day 7), Visit 5 (Day 14) and Visit 6 (Day 21). Secondary Efficacy: Mean change in IOP from Visit 2 (Baseline) to Visit 4 (Day 7), Visit 5 (Day 14) and visit 6 (Day 21).
Population studied:	Approximately 60 (20 per cohort) evaluable adult males or females diagnosed with OHT or POAG. Inclusion criteria: 1. 18 years of age or older. 2. Diagnosis of POAG or OHT in both eyes that are untreated, or if treated, in the opinion of the investigator are well controlled on 2 or fewer ocular hypotensive medications prior to Visit 1. • Combination medications count as 2 medications 3. Unmedicated IOP of ≥ 22 mm Hg and ≤ 36 mm Hg in the study eye, with no more than 5 mm Hg inter-eye difference at 08:00AM and 10:00AM at Visit 2. 4. Corrected visual acuity in each eye +1.0 logMAR (Snellen equivalent to 20/200) or better by ETDRS in each eye. 5. Central corneal thickness of ≥ 400 and ≤ 620 μm in each eye. 6. Able and willing to give signed informed consent and follow study instructions. 7. In the opinion of the Investigator, the subject is able to safely discontinue current ocular hypotensive medication(s), when a washout is required. Exclusion criteria: <u>Ophthalmic:</u> 1. Known hypersensitivity to any kinase inhibitors, any excipient or preservative of the formulation or to topical anesthetics or fluorescein. 2. Unmedicated IOP of > 36 mm Hg in either eye at any time point at Visit 2.

	3. History of intraocular implant for IOP treatment, glaucoma filtering surgery, placement, or removal of minimally invasive glaucoma implant (MIGS) in the study eye. 4. History of laser IOP lowering surgery within 6 months of Visit 1 in the study eye. • Note: Laser peripheral iridotomy for narrow angle is allowed if performed 3 months or more prior to Visit 1. 5. Ocular trauma within the past six months, or surgery involving the ocular surface (including cataract surgery) or non-IOP-lowering laser treatment within the past three months of Visit 1 in the study eye. 6. Refractive surgery in the study eye (including but not limited to radial keratotomy, Photorefractive Keratectomy [PRK], Laser-Assisted *in situ* Keratomileusis [LASIK], etc.). • Refractive surgery planned during study participation in either eye. 7. Have received intravitreal corticosteroid injection within 6 months or subtenon/subconjunctival steroid injection within 3 months of Visit 1 in either eye. 8. History or evidence of ocular infection, inflammation, clinically significant blepharitis, conjunctivitis, or of herpes simplex keratitis within 2 months of Visit 1 in either eye. 9. Unable or unwilling to avoid wearing of contact lenses from Visit 1 through the study. 10. Currently using 3 or more ocular hypotensive medications prior to screening in either eye (combination medications count as 2 medications). 11. Ocular medication of any kind within 28 days of Visit 2 in either eye other than: • Ocular hypotensive medications which must be washed out according to the provided schedule • Ocular Medication as part of an eye exam • Lubricating drops for dry eye 12. Clinically significant ocular disease in either eye (e.g. corneal edema, uveitis, severe keratoconjunctivitis sicca) which might interfere with the study. 13. Pseudoexfoliative, pigmentary, congenital, developmental or secondary glaucoma (e.g. neovascular, uveitic, pigmentary, lens-induced, steroid-induced, trauma induced or glaucoma associated with increased episcleral venous pressure) in either eye. 14. Closed angle forms of glaucoma in either eye • Shaffer grade < 3 (in 1 or more quadrants) • Gonioscopy within 3 months prior to Visit 1 is acceptable. 15. Cup-disc ratio of 0.7 or greater in either eye. 16. Nerve fiber layer thickness assessment by Optical Coherence Tomography (OCT) documents thinning of the NFL into the abnormal range for either eye. • If, in the opinion of the investigator, the thinning of the NFL may be attributed to other ocular features (including but not limited to

	optic nerve drusen, myopia, chorioretinal scars), then the subject is allowed. 17. Subjects with visual fields with substantial glaucomatous loss (e.g., Bjerrum scotoma, blind spot enlargement) for whom washout and potential randomization would be unsafe. 18. In addition to the above criteria, glaucomatous damage in either eye so severe that washout of ocular hypotensive medications for up to 5 weeks and treatment for 3 weeks (for subjects randomized to vehicle control) is not judged safe in the opinion of the Investigator. • Note: Subjects may be changed to a another IOP lowering therapy with a shorter washout period as long as the appropriate washout is met prior to Visit 2. 19. Any abnormality preventing reliable applanation tonometry of either eye. <u>General/Systemic:</u> 20. Laboratory tests indicative of any clinically significant disease in the opinion of the Investigator. 21. Clinically significant systemic disease (e.g., uncontrolled diabetes, myasthenia gravis, hepatic, renal, cardiovascular or endocrine disorders) which might interfere with the study in the opinion of the Investigator. 22. Participation in any study involving an investigational product within the past 30 days of Visit 1. 23. Changes of systemic beta blockers during the study that in the opinion of the Investigator could have a substantial effect on IOP within 30 days prior to Visit 1 or anticipated during the study. 24. Have received systemic corticosteroids (oral, injectable, inhaled) within 30 days of Visit 1 or planned use during the study. • Note: Topical dermatologic steroids are allowed if used for less than 3 consecutive days on no more than 3 separate areas of the body within 30 days of Visit 1 and the dosage is not expected to increase during the study. • Intranasal steroids are allowed if used less than or 4 times per week within 30 days of Visit 1 and the dosage is not expected to increase during the study. 25. History of substance abuse within 1 year of Visit 1 26. Subjects that are employees or relatives of employees at the clinical site. 27. Women of childbearing potential who are pregnant, nursing, planning a pregnancy, or not using a medically acceptable form of birth control. • Females of childbearing potential with a positive urine pregnancy test (UPT) result at Visit 1 or Visit 2 are excluded. • An adult woman is considered to be of childbearing potential unless she is one year post-menopausal or three months post-surgical sterilization.
Investigational product(s):	VVN539 ophthalmic solution 0.02% (3mL in 5 mL bottles) VVN539 ophthalmic solution 0.04% (3mL in 5 mL bottles) VVN539 ophthalmic solution vehicle (3mL in 5 mL bottles)

Dosing regimen:	Both eyes (OU), q.d. (morning) for 7 days, then q.d. (evening) for 7 days, and then b.i.d. for 7 days.
Assessments/Evaluations:	Efficacy: • IOP for efficacy will be measured by Goldmann applanation tonometry at 8:00AM, 10:00AM and 4:00PM at Visits 2, 4, 5 and 6. Safety: • Adverse event monitoring • Best Corrected Visual Acuity (ETDRS) • IOP • Biomicroscopy of anterior segment • Central corneal thickness • Cup-disc ratio • Ophthalmoscopy (dilated) • Heart rate and blood pressure (BP) • Blood chemistry, hematology
Duration of study:	There are a total of 6 clinical visits over 8 weeks (up to 5 weeks of washout and 3 weeks of study treatment)
Statistical methods:	With a sample size in each group of 20, the study will have 80% power to detect a difference of 3.0 mm Hg between an VVN539 dose compared to vehicle at each diurnal time point assuming a common standard deviation of 3.3 mm Hg, $\alpha = 0.05$ (two-tailed). There is no correction for multiplicity for multiple time points or comparisons for high or low VVN539 doses. The primary endpoint for this study is to compare mean IOP at each diurnal time point (8AM, 10AM, and 4PM) at Visit 4 (Day 7), Visit 5 (Day 14) and Visit 6 (Day 21) for two doses of VVN539 compared to vehicle using data from the study eye. Time-matched longitudinal models adjusting for baseline and including a random effect for center will be used to test time-specific comparisons. Data from Days 7, 14, and 21 will be analyzed using mixed-model repeated measures (MMRM) with an unstructured covariance assumed for each treatment with treatment, visit, and visit by treatment interaction as a fixed effects, baseline measurement as a covariate, and a random effect for site. Sensitivity analyses include a time-matched last observation carried forward (LOCF) of the last available IOP measurement as well as analysis of covariance (ANCOVA) analyses at each visit. Additional sensitivity analyses will be performed on the PP Analysis Set as necessary. Analyses of change from baseline and observed and change from baseline for diurnal IOP will be performed in a manner similar to the primary efficacy analysis. Safety analyses will be performed on all subjects in the Safety Analysis Set. The assessment of safety will be based on the summary of ocular and non-ocular treatment emergent adverse events (TEAEs), Best Corrected Visual Acuity (BCVA), central corneal thickness, vital signs, clinical laboratory tests, and ophthalmic exams using slit-lamp biomicroscopy and dilated ophthalmoscopy. Summaries will be provided by treatment group, and for ocular assessments separately by study-eye and non-study-eye.

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1 Introduction

1.1 Background

A relatively new class of topical agents being evaluated for the treatment of glaucoma are Rho kinase (ROCK) inhibitors (Kopczynski 2014). The most commonly prescribed ocular hypotensive medications reduce intraocular pressure (IOP) by increasing uveoscleral aqueous outflow (prostaglandin analogues) or decreasing aqueous humor production (ß-adrenoceptor antagonists, α-adrenoceptor agonists, and carbonic anhydrase inhibitors). ROCK inhibitors lower IOP through a different mechanism of action, increasing aqueous outflow through the trabecular outflow pathway by decreasing actomyosin-driven cellular contraction and reducing production of fibrotic extracellular matrix proteins (Tian 2005; Tokushige 2007; Honjo 2001; Wang 2015; Rao 2017).

In this class of ROCK inhibitors are netarsudil (approved in the U.S. as Rhopressa® (NDA 2085254) in 2017, and as a component of the fixed dose product, Rocklatan® (NDA 201259) in 2019 (Serle 2018), and ripasudil (approved in Japan in 2014 as Glanatec®, Tanihara 2015).

VivaVision is developing VVN539, a ROCK inhibitor with nanomolar potency. Upon contact with tissue, it releases nitric oxide (NO) from the nitrate (ONO_2) functional group, and is metabolized to VIP-5156, a ROCK inhibitor with subnanomolar potency. The release of NO from VVN539 is a characteristic like latanoprostene bunod (approved in the U.S. as Vyzulta® in 2017, Medeiros 2016). It has been demonstrated “NO” alone can lower the IOP by 10-20% (2-4 mmHg) by increasing outflow facility of aqueous humor (Cavet 2018).

Nitric oxide released by organic nitrates such as VVN539 stimulates soluble guanylate cyclase (GC), leading to an increase of cGMP in TM cells (Torfard 1994). This leads to the relaxation of trabecular meshwork, a smooth muscle like tissue. In addition, NO can also alter calcium-dependent potassium channel conductance, which leads to channel membrane activation and hyperpolarization with lower calcium ions resulting in vascular smooth muscle relaxation (Garcia-Calvo 1994).

The IOP lowering mechanisms of action by NO is different from the IOP lowering MOA by ROCK kinase inhibition. The sponsor believes that there may be synergy or additivity of NO releasing capacity and ROCK inhibition. Thus, the Sponsor suggests this molecule may provide a favorable benefit/risk profile in the lowering of elevated IOP in patients with open angle glaucoma (OAG) and ocular hypertension (OHT).

VVN539 ophthalmic solution is a preserved multi-dose product, intended for topical ocular instillation. As a new chemical entity (NCE), the intended regulatory route is 505(b)(1).

1.2 Study Rationale

Please refer to the Investigator's Brochure (IB) for a summary of findings from the VVN539 non-clinical studies.

This first-in-human clinical trial with VVN539 is a phase 2 study intended to assess the efficacy as well as the ocular and systemic safety of two doses of VVN539 and two dosing regimens as compared to vehicle control in subjects with OHT or primary open angle glaucoma (POAG).

1.3 Risk/Benefit Assessment

1.3.1 Known Potential Risks

The risks associated with VVN539 are not well understood at this time as this is a first-in-human clinical trial. Clinical trials of other ROCK inhibitors found the most common side effect to be transient, mild ocular hyperemia (Kopczynski, 2014). Other frequent adverse events reported include conjunctival hemorrhage (Serle et al 2018, Kahook et al 2019).

1.3.2 Known Potential Benefits

There are no known direct benefits as this is the first-in-human study for VVN539. Currently approved treatments for OHT and POAG are often not well tolerated and / or are not sufficient for disease progression. As VVN539 has a different mechanism of action, it may provide an alternative for subjects replacing other therapeutics or acting synergistically. Subjects in this study contribute to the development of this new drug that may help others with OHT and POAG.

1.3.3 Assessment of Benefits and Risks

Topical ocular VVN539 has demonstrated an excellent safety and tolerability profile during pre-clinical testing. Based on clinical experience with other ROCK inhibitors and based on preclinical data, it is expected that transient conjunctival hyperemia will be observed. No other potential risks have been identified in association with VVN539 during its development and clinical trials with netarsudil have shown a similar pattern of systemic adverse events (AEs) as timolol, minimal treatment related serious AEs overall, and manageable ocular AEs (Singh, 2020). Even though blood levels of VVN539 after ocular instillation in animals have been minimal in the nonclinical studies to date, the Sponsor intends to conduct clinical laboratory and hematology testing at Visit 1 (screening) and Visit 6 (end of treatment).

Given the experience with this product in pre-clinical studies as well as with similar approved products used in a dry eye population, there is a favorable benefit-risk ratio. Further the risks of this trial will be mitigated via routine monitoring and reporting of potential new adverse events.

2 Study Objectives and Endpoints

2.1 Study Objectives

The primary objective of this study is to evaluate the ocular hypotensive efficacy of 2 concentrations of VVN539 in subjects with OHT or POAG.

The secondary objective is to evaluate the ocular and systemic safety of the 2 concentrations in this subject population.

2.2 Study Endpoints

2.2.1 Primary Endpoint(s)

The primary endpoint for this study is to compare mean IOP at each diurnal time point (8AM, 10AM, and 4PM) at Visit 4 (Day 7), Visit 5 (Day 14) and Visit 6 (Day 21) for two doses of VVN539 compared to vehicle. The primary statistical analysis will be performed using the data from the study eye.

The study eye is defined as meeting all inclusion criteria and no exclusion criteria, and with the higher IOP at 8:00AM at Visit 2. Should both eyes have the same IOP and meet all inclusion criteria and no exclusion criteria, then the right eye will be the study eye.

2.2.2 Secondary Endpoint(s)

Secondary efficacy endpoints include:

- Mean change from baseline (Visit 2) in IOP at each diurnal time point to Visit 4 (Day 7), Visit 5 (Day 14) and Visit 6 (Day 21);
- Mean change from baseline (Visit 2) in diurnal IOP to Visit 4 (Day 7), Visit 5 (Day 14) and Visit 6 (Day 21). Diurnal IOP is the average of 8AM, 10AM, and 4PM measurements at a given visit.

2.2.3 Safety Assessments

The safety assessments include:

- Frequency of treatment emergent ocular and systemic adverse events and serious adverse events (SAEs).
- Clinically relevant changes in:
    - Best Corrected Visual Acuity (by Early Treatment Diabetic Retinopathy Study [ETDRS])
    - Slit-lamp Biomicroscopy

- Central corneal thickness
- Ophthalmoscopy (dilated)
- Vital signs (heart rate (HR) and blood pressure (BP))
- Clinical laboratory tests (blood chemistry, hematology)

Data from both eyes will be used for the safety analysis.

3 Study Design

3.1 Overall Design of the Study

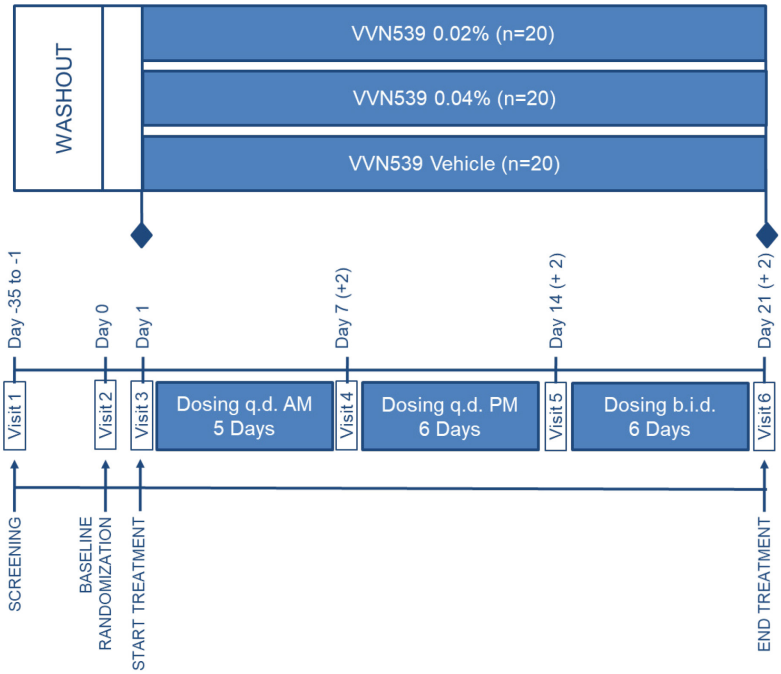
This is a prospective, parallel-comparison, multi-center, double-masked, randomized, vehicle-controlled dose-response study assessing the safety and ocular hypotensive efficacy of VVN539 in subjects with POAG or OHT. Approximately 60 subjects will be randomized into one of two active treatment arms or a control arm (20 subjects per cohort):

- Arm 1: 0.02% VVN539 ophthalmic solution
- Arm 2: 0.04% VVN539 ophthalmic solution
- Arm 3: VVN539 ophthalmic solution vehicle

Treatment assignments will be masked to VivaVision, study subjects, Investigators, and site staff.

The study involves 6 clinic visits, including screening, baseline, and treatment visits. The study is open to subjects with OHT and POAG who are either treatment-naïve or are already on ocular hypotensive medication. Subjects on ocular hypotensive who meet screening inclusion and do not meet screening exclusion criteria at Visit 1 will discontinue their prior ocular hypotensive medication for up to 35 days before returning to the site for Visit 2 (baseline). Treatment naïve subjects can return for the baseline visit as early as the day after Visit 1. Subjects meeting all baseline inclusion criteria and none of the baseline exclusion criteria will be randomized into one of the study arms and will return to the study site the next day for the first treatment. Three different dosing regimens (once a day [q.d.] in the morning, q.d. in the evening and twice a day [b.i.d.]) will be tested for 7-9 days, each.

Figure 1: Study Treatment



3.2 Rationale for the Study Design

The design of this study, including choice of control, is similar to that of the first-in-human study of netarsudil, a ROCK inhibitor currently approved in the US.

3.3 Dose Justification

The concentrations of VVN539 ophthalmic solution and dosing schedule were chosen based on data from non-clinical ocular hypotensive effect, pharmacokinetic (PK) and toxicology studies. For details, please refer to the Investigator's Brochure.

Dosing will be q.d. in the morning for 7 days, followed by q.d. dosing in the evening for 7 days and b.i.d. dosing for 7 days. The route of administration for this study is ocular topical as this is the intended route for VVN539.

3.4 End of Study Definition

A subject is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Procedures and Assessments. The end of the study is defined as completion of the last visit or procedure shown in the schedule in the study

4 Study Population

The study population will consist of adult subjects who have/are diagnosed with OHT or POAG. Subjects can be treatment naïve subjects or on ocular hypotensive medication. Ocular eligibility criteria in general apply only to the study eye, unless specified otherwise.

Treatment during the study will be both eyes (OU), only data from the study eye will be used for efficacy analysis. Data from both eyes will be used for safety analysis. The study eye will be defined as meeting all inclusion criteria and no exclusion criteria, and with the higher IOP at 8:00AM at Visit 2. Should both eyes have the same IOP and meet all inclusion criteria and no exclusion criteria, then the right eye will be the study eye.

4.1 Inclusion Criteria

Subjects must meet all of the following in order to be enrolled into the study:

1. 18 years of age or older.
2. Diagnosis of POAG or OHT in both eyes that are untreated, or if treated, well controlled with a stable regimen of 2 or fewer ocular hypotensive medications 30 days prior to Visit 1.
   - Combination medications count as 2 medications
3. Unmedicated IOP of ≥ 22 mm Hg and ≤ 36 mm Hg in the study eye, with no more than 5 mm Hg inter-eye difference at 08:00AM and 10:00AM at Visit 2.
4. Corrected visual acuity in each eye +1.0 logMAR (Snellen equivalent to 20/200) or better by ETDRS in each eye.
5. Central corneal thickness of ≥ 400 and ≤ 620 μm in each eye.
6. Able and willing to give signed informed consent and follow study instructions.
7. In the opinion of the Investigator, the subject is able to safely discontinue current ocular hypotensive medication(s), when a washout is required.

4.2 Exclusion Criteria

Subjects with any of the following will not be allowed to participate in this study:

<u>Ophthalmic:</u>

1. Known hypersensitivity to any kinase inhibitors, any excipient or preservative of the formulation or to topical anesthetics or fluorescein.
2. Unmedicated IOP of > 36 mm Hg in either eye at any time point at Visit 2.
3. History of intraocular implant for IOP treatment, glaucoma filtering surgery, placement, or removal of minimally invasive glaucoma implant (MIGS) in the study eye.

4. History of laser IOP lowering surgery within 6 months of Visit 1 in the study eye.
    - Note: Laser peripheral iridotomy for narrow angle is allowed if performed 3 months or more prior to Visit 1.
5. Ocular trauma within the past six months, or surgery involving the ocular surface (including cataract surgery) or non-IOP-lowering laser treatment within the past three months of Visit 1 in the study eye.
    - Refractive surgery in the study eye (including but not limited to radial keratotomy, Photorefractive Keratectomy [PRK], Laser-Assisted *in situ* Keratomileusis [LASIK], etc.).
6. Refractive surgery planned during study participation in either eye.
7. Have received intravitreal corticosteroid injection within 6 months or subtenon/subconjunctival steroid injection within 3 months of Visit 1 in either eye.
8. History or evidence of ocular infection, inflammation, clinically significant blepharitis, conjunctivitis, or of herpes simplex keratitis within 2 months of Visit 1 in either eye.
9. Unable or unwilling to avoid wearing of contact lenses from Visit 1 through the study.
10. Currently using 3 or more ocular hypotensive medications prior to screening in either eye (combination medications count as 2 medications).
11. Ocular medication of any kind within 28 days of Visit 2 in either eye other than:
    - Ocular hypotensive medications which must be washed out according to the provided schedule
    - Ocular Medication as part of an eye exam
    - Lubricating drops for dry eye
12. Clinically significant ocular disease in either eye (e.g. corneal edema, uveitis, severe keratoconjunctivitis sicca) which might interfere with the study.
13. Pseudoexfoliative, pigmentary, congenital, developmental or secondary glaucoma (e.g. neovascular, uveitic, pigmentary, lens-induced, steroid-induced, trauma induced or glaucoma associated with increased episcleral venous pressure) in either eye.
14. Closed angle forms of glaucoma in either eye
    - Shaffer grade < 3 (in 1 or more quadrants)
    - Gonioscopy within 3 months prior to Visit 1 is acceptable.
15. Cup-disc ratio of 0.7 or greater in either eye.
16. Nerve fiber layer thickness assessment by Optical Coherence Tomography (OCT) documents thinning of the NFL into the abnormal range for either eye.
    - If, in the opinion of the investigator, the thinning of the NFL may be attributed to other ocular features (including but not limited to optic nerve drusen, myopia, chorioretinal scars), then the subject is allowed.
17. Subjects with visual fields with substantial glaucomatous loss (e.g., Bjerrum scotoma, blind spot enlargement) for whom washout and potential randomization would be unsafe.

18. In addition to the above criteria, glaucomatous damage in either eye so severe that washout of ocular hypotensive medications for up to 5 weeks and treatment for 3 weeks (for subjects randomized to vehicle control) is not judged safe in the opinion of the Investigator.
    - Note: Subjects may be changed to a another IOP lowering therapy with a shorter washout period as long as the appropriate washout is met prior to Visit 2.
19. Any abnormality preventing reliable applanation tonometry of either eye.

General/Systemic:

20. Laboratory tests indicative of any clinically significant disease in the opinion of the Investigator.
21. Clinically significant systemic disease (e.g., uncontrolled diabetes, myasthenia gravis, hepatic, renal, cardiovascular or endocrine disorders) which might interfere with the study in the opinion of the Investigator.
22. Participation in any study involving an investigational product (IP) within the past 30 days of Visit 1.
23. Changes of systemic beta blockers during the study that in the opinion of the Investigator could have a substantial effect on IOP within 30 days prior to Visit 1 or anticipated during the study.
24. Have received systemic corticosteroids (oral, injectable, inhaled) within 30 days of Visit 1 or planned use during the study.
    - Note: Topical dermatologic steroids are allowed if used for less than 3 consecutive days on no more than 3 separate areas of the body within 30 days of Visit 1 and the dosage is not expected to increase during the study.
    - Intranasal steroids are allowed if used less than or 4 times per week within 30 days of Visit 1 and the dosage is not expected to increase during the study.
25. History of substance abuse within 1 year of Visit 1
26. Subjects that are employees or relatives of employees at the clinical site.
27. Women of childbearing potential who are pregnant, nursing, planning a pregnancy, or not using a medically acceptable form of birth control.
    - Females of childbearing potential with a positive urine pregnancy test (UPT) result at Visit 1 or Visit 2 are excluded.
    - An adult woman is considered to be of childbearing potential unless she is one year post-menopausal or three months post-surgical sterilization.

4.3 Lifestyle Considerations

Substance abuse is not permitted during the study and subjects with a history of substance abuse 1 year prior to Visit 1 will be excluded from the study.

Subjects are required to refrain from wearing contact lenses for the duration of the study. Subjects not willing or able to refrain from wearing contact lenses will be excluded from the study.

Subjects should not have any planned ocular surgeries while participating in the study (Visit 1 – Visit 6). Investigators should discuss with the Medical Monitor should an ocular surgery become necessary while a subject is participating in the study.

4.4 Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but are not subsequently randomly assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event.

Individuals who do not meet the criteria for participation in this trial (screen failure) because of the following may be rescreened.:

- Prohibited medications from which the subject may discontinue for the purposes of eligibility and / or participating in the study (with approval from the Investigator based on clinical safety).
- Exclusionary medical history status at the time of screening that will change (e.g., ocular infection, surgical history duration).

Rescreened subjects should be assigned a new subject number.

4.5 Strategies for Recruitment and Retention

It is anticipated that approximately 90 subjects will need to be screened to achieve 60 qualified completed subjects (20 per arm) for the study. Subjects will be mostly recruited from the patient population at participating study centers in the US. Subject retention is not considered a major issue for this study due to the relatively short duration.

5 Study Treatment(s) or Intervention(s)

5.1 Investigational Product(s)

Three (3) investigational products (IPs) will be administered during this study:

- VVN539 ophthalmic solution 0.02% (3mL in 5 mL bottles)
- VVN539 ophthalmic solution 0.04% (3mL in 5 mL bottles)
- VVN539 ophthalmic solution vehicle (3mL in 5 mL bottles)

5.1.1 Description

VVN539 ophthalmic solution will be supplied to study sites in 5mL low-density polyethylene LPDE multidose droppers filled with at least 3 mL solution. Table 1 describes the composition and general information about VVN539 supplied for this study. The VVN539 vehicle control has the same composition as VVN539 ophthalmic solution except that it does not contain VVN539. Additional detail can be found in the Investigator's Brochure.

Table 1: VVN539 Ophthalmic Solution General Information

Dosage Form	Sterile solution
Active Ingredient	0.02% or 0.04% VVN539
Inactive Ingredients	Benzalkonium chloride, Polyvinylpyrrolidone (PVP) K90, Poloxamer 407, glycerin, boric acid, sodium borate, water
Route of Administration	Topical ocular administration
Physical Description	Isosmotic, clear solution
pH	Approximately 5.0
Pharmacokinetics	VVN539 metabolite and nitric oxide active moieties dosed q.d. or b.i.d
Stability	12 months expiry for samples stored at 5°C temperature

5.1.2 Dosage and Administration

Eligible subjects will be randomized in a 1:1:1 ratio to receive either 0.02% or 0.04% VVN539 or vehicle control. The IP will be administered as 1 drop either q.d. or b.i.d in the inferior conjunctival fornix in each eye.

The first dose of VVN539 will be administered in clinic after the 8:00AM IOP has been measured at Visit 3. The subjects will then receive the bottle of IP and will self-administer the IP in the morning (suggested timing between 7:00AM and 9:00AM) each day for 5 days. The study site should call the subject the day prior to Visit 4 to remind them to NOT dose in the morning at home on the day of the visit. The last day of q.d. morning dosing will be Visit 4 when subjects will be dosing in clinic after the 8:00AM IOP has been measured.

Starting the day after Visit 4, subjects will self-administer the IP in the evening (suggested timing between 7:00PM and 9:00PM) each day for 6 days. The last q.d. evening dose will be administered in the evening Visit 5 after the last IOP has been measured.

Starting the day after Visit 5, subjects will self-administer the IP b.i.d (suggested timing between 7:00AM and 9:00AM for the morning dose and between 7:00PM and 9:00PM for the evening dose) each day for 6 days. The study site should call the subject the day prior to Visit 6 to remind them to NOT dose in the morning at home on the day of the visit. The last morning dose will be administered in clinic after the 8:00AM IOP dose has been measured. Subjects will return the IP at Visit 6 and will resume their standard of care treatment.

Subjects should be instructed to administer missed doses as soon as possible, as long as it is not administered within 12 hours of the next scheduled dosing during the q.d. dosing periods or not within 4 hours of the next scheduled dosing during the b.i.d dosing period. Missed doses will be recorded as deviations in the electronic case report form (eCRF).

5.2 Preparation/Storage/Handling/Accountability

5.2.1 Acquisition and Accountability

VVN539 ophthalmic solution and vehicle will be provided to study sites in identical bottles so that masking is maintained. Shipment records will be verified by comparing the shipment inventory sheet to the actual quantity received at the site. Accurate records of receipt and disposition of the IP (e.g. dates, quantity, subject number, date dispensed, date returned) must be maintained by the study site. At the end of the study, all used and unused IP will be returned to VivaVision (or designee), following approval by VivaVision. The study monitor (or designee) will verify IP accountability prior to return of the IP. All accountability procedures must be completed before the study is complete or before the study site is closed, whichever comes first.

5.2.2 Product Packaging and Labeling

Each randomization kit contains one dropper bottle of IP sufficient for 14-18 days of q.d. and 7-9 days of b.i.d. dosing. Double masked labels will minimally contain the following information:

- Protocol number
- Sponsor’s name
- Kit number
- Instructions for use
- Storage temperature
- Required statement(s) per regulatory agency

5.2.3 Product Storage and Stability

IP and active comparator should be stored at controlled refrigerated temperatures from 2°C to 8°C and protected from light. Once dispensed to the subject, IP bottles may be stored by the subject in the refrigerator (approximately 2-8°C/35-46°F) or at ambient room temperature until use.

5.3 Measures to Minimize Bias: Randomization and Blinding

Subjects will be randomized to a treatment assignment. A randomization schedule will be generated by qualified personnel independent of the project team and study conduct and maintained in a secure and limited-access location separate from the Investigators and members of the project team. Treatment will not be blocked by study site which essentially stratifies the treatment by study site. Treatment assignments will be masked to the Sponsor, subjects, Investigators, and investigative staff, until completion of the study and the final database is locked. Appropriate precautions will be taken to prevent unauthorized access to the randomization scheme.

Both doses of VVN ophthalmic solution as well as vehicle will be provided in identical dropper bottles to prevent inadvertent unmasking. If unmasking is required, the integrity of the study assessments and objectives will be maintained by limiting access to the unmasked data. Unless the subject's safety requires otherwise and if time permits, the decision to unmask an individual subject's treatment assignment is to be made jointly by the Investigator and medical monitor after consultation with the Sponsor, thus leaving the masking of the remaining subjects intact.

5.4 Treatment Adherence

Adherence to the treatment schedule will be assessed by site staff by querying subjects at Visits 4-6 whether they missed any doses in the dosing period preceding the visit and if so, whether 2 or more doses were missed. Subjects should be counseled on the importance of adhering to the dosing scheduled. Missed doses will be recorded as deviations in the eCRF.

5.5 Concomitant Therapy

All medications that the subject has taken 2 months prior to Visit 1 and / or is taking through Visit 6 (or discontinuation from the study) will be recorded in the subject chart and the eCRF. The generic name of the drug, dose, route of administration, duration of treatment (including start and stop dates), frequency, indication and whether or not the medication was taken due to an AE will be recorded for each medication.

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the case report form are concomitant prescription medications, over-the-counter medications and supplements.

The medications and therapies listed in Table 2 are exclusionary for the study and / or are prohibited during study participation. Investigators should discuss with the Medical Monitor should any of these medications or procedures become medically necessary for a subject during study participation.

Table 2: Medications and Procedures Not Permitted

Medication / Procedure	Eye	Limitation
Intraocular implant for IOP treatment, glaucoma filtering surgery, placement, or removal of minimally invasive glaucoma implant (MIGS)	Study Eye	Exclusionary any time prior to Visit 1
Laser IOP lowering surgery in the study eye	Study Eye	Exclusionary if performed within 6 months of Visit 1
Laser peripheral iridotomy for narrow angle in study eye	Study Eye	Exclusionary if performed within 3 months of Visit 1
Ocular trauma in the study eye	Study Eye	Exclusionary if occurred within 6 months of Visit 1
Surgery involving the ocular surface (including cataract surgery) or non-IOP-lowering laser in the study eye	Study Eye	Exclusionary if performed within 3 months of Visit 1
Refractive surgery (including but not limited to radial keratotomy, PRK, LASIK)	Study Eye	Exclusionary any time prior to Visit 1 and prohibited during study participation
Intravitreal corticosteroid injection	Either Eye	Exclusionary if performed within 6 months of Visit 1
Subtenon/subconjunctival steroid injection	Either Eye	Exclusionary if performed within 3 months of Visit 1
Ocular infection, inflammation, clinically significant blepharitis, conjunctivitis, or of herpes simplex keratitis	Either Eye	Exclusionary if occurred within 2 months of Visit 1
Contact lens use	Either Eye	Prohibited during study participation
Ocular medication of any kind other than: • Ocular hypotensive medications which must be washed out according to the provided schedule • Ocular Medication as part of an eye exam • Lubricating drops for dry eye	Either Eye	Exclusionary within 28 days of Visit 1 and prohibited during study participation
Ocular hypotensive medication	Either Eye	Subjects on ocular hypotensive medication must wash out prior to Visit 2 See section 5.5.1 for use of ocular hypotensive medication other than IP during study participation
Changes of systemic beta blockers that in the opinion of the investigator could have a substantial effect on IOP	N/A	Exclusionary within 30 days of Visit 1 and prohibited during study participation
Systemic corticosteroids (oral, injectable, inhaled) within 30 days of Visit 1 or planned use during the study. • Topical dermatologic steroids are allowed if used for less than 3 consecutive days on no more than 3 separate areas of the body and the dosage is not expected to increase during the study. • Intranasal steroids are allowed if used less than or 4 times per week and the dosage is not expected to increase during the study.	N/A	Exclusionary within 30 days of Visit 1 and prohibited during study participation

Medication / Procedure	Eye	Limitation
Substance abuse	N/A	Exclusionary within one year of Visit 1 and prohibited during study participation

5.5.1 Management of Elevated IOP During Treatment

Any subject who has an elevated IOP that the PI considers unsafe for the subject may be rescued and placed on appropriate alternate therapy. The choice of rescue medication is at the Investigator's discretion. Rescue medication should be entered into the eCRF with annotation indicating that the medication was used for rescue.

Rescued subjects will be considered as treatment failures, and the need for rescue therapy will not be considered an adverse event (AE). Rescued subjects will stop the investigational product and should not be withdrawn from the study.

The following guidance is provided to Investigators for rescue and the decision to rescue is at the discretion of the investigator’s judgement:

- Ocular hypotensive medication (other than IP) is prohibited during study participation unless the subject is rescued.
- If the subject’s IOP is > 36 mm HG, the Investigator may recheck the IOP within 3 day of the visit and rescue medication may be considered if the IOP remains elevated
- Rescued subjects should discontinue the investigational product.
- Rescued subjects should continue in the study for safety follow-up after receiving the rescue medication.

6 Study Discontinuation/Subject Withdrawal

6.1 Discontinuation of Study Treatment or Intervention

IP may be discontinued by a subject at any time. In addition, the Investigator or Sponsor can discontinue a subject from further IP administration for other reasons related to the best interest of the subject.

6.2 Subjects Discontinuation/Withdrawal from the Study

Subjects are free to withdraw from participation in the study at any time upon request. An Investigator may discontinue or withdraw a subject from the study for the following reasons:

- IOP measurement of 36 mmHg or higher in either eye
- Severe ocular irritation, burning, pain, or hyperemia
- Study terminated by the Sponsor, Food and Drug Administration (FDA), or other regulatory authorities
- Any woman who becomes pregnant while participating in the study. Information on the pregnancy and outcome will be requested.
- Significant study treatment/intervention non-compliance
- If any clinical adverse event, laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject
- Disease progression which requires discontinuation of the study intervention
- If the subject meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

The reason for subject discontinuation or withdrawal from the study will be recorded on the case report form. Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Those who sign the informed consent form, and are randomized and receive the study treatment, and subsequently withdraw, or are withdrawn or discontinued from the study, will not be replaced.

Additionally, the trial or parts of the trial may be discontinued by the Sponsor or at the recommendation of the Investigator after consultation with VivaVision Biotech, Inc. This may be based on a significant number of AEs of a similar nature that warrant such action.

In the event study discontinuation of a randomized subject is necessary, the Investigator should make every attempt to have the subject complete all Visit 6 assessments. If a non-serious AE is unresolved at the time of the subject's final study visit, an effort will be made to follow up until

the AE is resolved or stabilized, the subject is lost to follow-up, or there is some other resolution of the event. The Investigator should make every attempt to follow all SAEs to resolution.

6.3 Lost to Follow up

A subject will be considered lost to follow-up if he or she fails to return for scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a subject fails to return to the clinic for a required study visit:

- The site will attempt to contact the subject and reschedule the missed visit within 1-2 days and counsel the subject on the importance of maintaining the assigned visit schedule and ascertain if the subject wishes to and/or should continue in the study. Subjects should continue the current dosing regimen until the rescheduled visit.
- Before a subject is deemed lost to follow-up, the Investigator or designee will make every effort to regain contact with the subject (where possible, 3 telephone calls and, if necessary, a certified letter to the subject's last known mailing address or local equivalent methods). These contact attempts should be documented in the subject's medical record or study file.
- Should the subject continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

7 Study Procedures

Written informed consent and Health Insurance Portability and Accountability Act (HIPAA) authorization will be obtained from all subjects prior to any study-related procedures being performed. The Schedule of Procedures and Assessments (Appendix 1) lists the procedures that should occur at each study visit. Details for the study assessments can be found in Appendices 3-12.

7.1 Visit Descriptions

7.1.1 Screening (Visit 1, Day –35 to -1)

The following procedures will be performed on all subjects at the screening visit. Ophthalmic procedures should be performed in the order suggested below:

- Explain the purpose and conduct of the study to the subject, answer the subject's questions, and obtain written informed consent and HIPAA authorization.
- Obtain information including: demographics, concomitant medications, ocular and systemic medical and medication history and surgical history.
- Determine study eligibility based on Inclusion/Exclusion criteria.
- Best corrected visual acuity (BCVA)
- Perimetry
    - Visual field within 3 months of Visit 1 is acceptable, provided that it is reliable meets the requirement for automated threshold visual field (e.g., 30-2 or 24-2 Humphrey). Unreliable entry fields must be repeated prior to study drug dispensation.
- OCT
    - OCT within 3 months of Visit 1 is acceptable.
- Slit-Lamp Biomicroscopy
- IOP measurement
    - Measured using Goldmann applanation tonometer and a two-person reading method.
    - Measure twice in each eye; if results differ by >1 mm Hg, a third measurement will be taken. The mean will be taken if two measurements are used, for 3 measurements record the median.
- Pachymetry
- Gonioscopy
    - Gonioscopy performed within 3 months of Visit 1 is acceptable
- Dilated ophthalmoscopy

  - Determine cup-disc ratio
- Blood pressure & heart rate
- Blood chemistry, hematology
- Urine pregnancy test (UPT) for women of childbearing potential
  - An adult woman is considered to be of childbearing potential unless she is one year post-menopausal or three months post-surgical sterilization.
- Instruct subjects who were on ocular hypotensive medication prior to the visit to start washout based on the table below. Subjects may be changed to a another IOP lowering therapy with a shorter washout period as long as the appropriate washout is met prior to Visit 2.

Ocular Hypotensive Medication Class	Minimum washout period
Prostaglandins β-adrenoceptor antagonists Kinase inhibitors	4 weeks
Adrenergic agonists	2 weeks
Muscarinic agonists Carbonic anhydrase inhibitors	5 days

7.1.2 Randomization (Visit 2, Day 0)

The following procedures will be performed on Day 0 (randomization). Ophthalmic procedures should be performed in the order suggested below:

- Determine study eligibility based on Inclusion/Exclusion criteria.
- BCVA
- Biomicroscopy
- Diurnal IOP
  - Measured using Goldmann applanation tonometer and a two-person reading method.
  - Readings at:
    - 8:00AM (±30 mins)
    - 10:00AM (±30 mins)
    - 4:00PM (±30 mins)
  - Measure twice in each eye; if results differ by >1 mm Hg, a third measurement will be obtained. The mean will be taken if two measurements are used, for 3 measurements record the median.
- Blood pressure & heart rate
- Randomization
- Urine pregnancy test (UPT) for women of childbearing potential

  - An adult woman is considered to be of childbearing potential unless she is one year post-menopausal or three months post-surgical sterilization.
- Assess subject ability to instill drops in eye using artificial tears. Subjects should be retrained on the correct way to instill drops if they are not able to do so.
- Update medical and ocular history, as needed
- Update concomitant medications, as needed

7.1.3 Visit 3 (Day 1)

The following procedures will be performed at Visit 3 (Day 1). Ophthalmic procedures should be performed in the order suggested below:

- BCVA
- Biomicroscopy
- IOP
  - Measured using Goldmann applanation tonometer and a two-person reading method.
  - Reading at:
    - 8:00AM (±30 mins)
  - Measure twice in each eye; if results differ by >1 mm Hg, a third measurement will be obtained. The mean will be taken if two measurements are used, for 3 measurements record the median.
- Administer first dose of study drug in clinic after IOP is taken.
- Dispense study drug
- Instruct subject to dose daily in the morning (suggested timing between 7:00AM and 9:00AM).
- Blood pressure & heart rate
- Update concomitant medications, as needed
- Adverse event assessment

7.1.4 Visit 4 (Day 7 + 2 days)

Visit 4 needs should be scheduled 5 days after Visit 3 in order to ensure at least 7 days of q.d. dosing in the morning.

The following procedures will be performed at Visit 4 (Day 7). Ophthalmic procedures should be performed in the order suggested below:

- BCVA
- Biomicroscopy
- Diurnal IOP

  - Measured using Goldmann applanation tonometer and a two-person reading method.
  - Readings at:
    - 8:00AM (±30 mins)
    - 10:00AM (±30 mins)
    - 4:00PM (±30 mins)
  - Measure twice in each eye; if results differ by >1 mm Hg, a third measurement will be obtained. The mean will be taken if two measurements are used, for 3 measurements record the median.
- Administer last q.d. morning dose in clinic after 8:00AM IOP is taken.
- Collect used study drug and dispense a new bottle.
- Instruct subject to start dosing daily the day after the visit in the evening (suggested timing between 7:00PM and 9:00PM).
- Blood pressure & heart rate
- Update concomitant medications, as needed
- Adverse event assessment

7.1.5 Visit 5 (Day 14 + 2 days)

Visit 5 needs should be scheduled 6days after Visit 4 in order to ensure at least 7 days of q.d. dosing in the PM. The following procedures will be performed at Visit 5 (Day 14). Ophthalmic procedures should be performed in the order suggested below:

- BCVA
- Biomicroscopy
- Diurnal IOP
  - Measured using Goldmann applanation tonometer and a two-person reading method.
  - Readings at:
    - 8:00AM (±30 mins)
    - 10:00AM (±30 mins)
    - 4:00PM (±30 mins)
  - Measure twice in each eye; if results differ by >1 mm Hg, a third measurement will be obtained. The mean will be taken if two measurements are used, for 3 measurements record the median.
- Collect used study drug and dispense a new bottle.
- Instruct subject to instill the last daily evening dose in the evening after the visit (suggested timing between 7:00PM and 9:00PM).

- Instruct subject to start dosing twice daily the day after the visit in the morning (suggested timing between 7:00AM and 9:00AM for the morning dose and between 7:00PM and 9:00PM for the evening dose).
- Blood pressure & heart rate
- Update concomitant medications, as needed
- Adverse event assessment

7.1.6 End of Study (Visit 6, Day 21 + 2 days)

Visit 6 needs should be scheduled 6 days after Visit 5 in order to ensure at least 6 days of b.i.d. dosing.

The following procedures will be performed at the End of Study visit (Visit 6) on Day 21. Ophthalmic procedures should be performed in the order suggested below:

- BCVA
- Biomicroscopy
- Diurnal IOP
    - Measured using Goldmann applanation tonometer and a two-person reading method.
    - Readings at:
        - 8:00AM (±30 mins)
        - 10:00AM (±30 mins)
        - 4:00PM (±30 mins)
    - Measure twice in each eye; if results differ by >1 mm Hg, a third measurement will be obtained. The mean will be taken if two measurements are used, for 3 measurements record the median.
- Administer last dose of b.i.d. study drug in clinic after 8:00AM IOP is taken.
- Pachymetry
- Dilated ophthalmoscopy
    - After last IOP measurement is taken.
- Blood pressure & heart rate
- Blood chemistry, hematology
- UPT for women of childbearing potential
    - An adult woman is considered to be of childbearing potential unless she is one year post-menopausal or three months post-surgical sterilization.
- Collect used study drug.
- Update concomitant medications, as needed
- Adverse event assessment

- If a non-serious AE is unresolved at the time of the subject's final study visit, an effort will be made to follow up until the AE is resolved or stabilized, the subject is lost to follow-up, or there is some other resolution of the event.
- The Investigator should make every attempt to follow all SAEs to resolution.

7.1.7 Unscheduled Visits

Any visits or procedures performed beyond those specified within the protocol must be documented in the Unscheduled Visit pages of the eCRF. Subjects IOP should be checked at all unscheduled visits. Unscheduled visits may include but are not limited to reporting AEs, changes in concomitant medications, or ophthalmic assessments as deemed appropriate by an appropriately qualified physician.

7.1.8 Early Termination

In the event that a subject exits or is terminated from the study prior to the End of Study visit (Visit 6), every attempt will be made to ensure that the subject completes all Visit 6 assessments. If a non-serious AE is unresolved at the time of the subject's final study visit, an effort will be made to follow up until the AE is resolved or stabilized, the subject is lost to follow-up, or there is some other resolution of the event. The Investigator should make every attempt to follow all SAEs to resolution.

8 Study Assessments

The Schedule of Procedures and Assessments (Appendix 1) provides a list of study assessments and evaluations to be performed and the timing of each.

8.1 Efficacy Evaluations

Efficacy assessments will be conducted at the time points indicated on the Schedule of Procedures and Assessments (Appendix 1) and as detailed in the sections below.

8.1.1 IOP

IOP will be measured per the instructions in Appendix 5.

8.2 Safety Evaluations

Safety assessments will be performed at the time points indicated on the Schedule of Procedures and Assessments (Appendix 1) and as detailed in the sections below.

- AE monitoring
- BCVA (Appendix 3)
- Slit-Lamp Biomicroscopy (Appendix 4)
- Central corneal thickness (Appendix 7)
- Dilated ophthalmoscopy (Appendix 6)
- Vital signs (heart rate and blood pressure) (Appendix 12)
- Clinical laboratory tests (blood chemistry, hematology) (Appendix 11)

8.2.1 Adverse Events (AEs) and Serious Adverse Events (SAEs)

AEs will be monitored throughout the study. Subjects will be encouraged to report any adverse findings during the study whether or not they are related to IP. These can be collected either in an unsolicited fashion without any prompting or in response to a general question such as: “Have you noticed anything different since you started the study; began the IP, etc?”

All AEs will be captured on the appropriate eCRF. Information to be collected at minimum includes event description, onset, assessment of severity, relationship to IP, and outcome.

The Investigator will record all AEs with start dates occurring any time after informed consent is obtained until 7 (for nonserious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the Investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the study subject's condition deteriorates at any time during the study, it will be recorded as an AE.

8.2.1.1 Definitions

An AE is any untoward medical occurrence in a patient or clinical study subject, temporally associated with the use of study intervention, whether or not considered related to the study intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.

An AE or suspected adverse reaction is considered "serious" if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death,
- A life-threatening AE,
- Inpatient hospitalization or prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly or birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.2.1.2 Classification of Adverse Events

Severity of Adverse Events

The severity of all AEs will be assessed by the Investigator and graded as follows:

- **Mild:** requires minimal or no treatment and do not interfere with the subject's daily activities;
- **Moderate:** results in a low level of inconvenience or concern and may cause some interference with functioning;
- **Severe**: interrupts a subject's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. The term "severe" does not necessarily equate to "serious".

Relationship of Adverse Events

All AEs must have their relationship to study intervention assessed by the Investigator who examines and evaluates the subject based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical study, the IP must always be suspect.

- **Unrelated:** no reasonable possibility that the administration of the IP caused the event, no temporal relationship between the IP and event onset, or an alternate etiology has been established.
- **Related:** is known to occur with the IP, is a reasonable possibility that the IP caused the AE, or there is a temporal relationship between the IP and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the IP and the AE.

Expectedness

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure (IB), package insert, or device labeling or is not listed at the specificity or severity that has been observed; or, if an IB is not required or available, is not consistent with the risk information described in the protocol, as amended. "Unexpected," as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the IB, package insert, or device labeling as occurring with a *class of drugs* (or other medical products) or as anticipated from the pharmacological properties or other characteristics of the IP, but are not specifically mentioned as occurring with the particular IP under investigation.

The Investigator will be responsible for determining whether an AE is unexpected, i.e., if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the IP.

8.2.1.3 Adverse Event Reporting Requirements

According to federal regulations, an Investigator must immediately (within 24 hours) report to the Sponsor or a designee any SAE, whether or not considered drug related, including those listed in the protocol or IB and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

According to federal regulations, the Sponsor must notify the FDA and all participating Investigators as soon as possible, but in no case later than 15 calendar days after the Sponsor determines that a potential serious risk arising from a clinical study qualifies for reporting. Sponsor must report any suspected adverse reaction that is both serious and unexpected. The Sponsor must report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event (See 21 CFR 312.32(c)(1)).

Furthermore, the Sponsor must also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the Sponsor's initial receipt of the information (See 21 CFR 312.32(c)(2)).

8.2.1.4 Pregnancy

All pregnancies are to be reported from the time informed consent is signed until 14 days after the last study visit.

Any report of pregnancy for any female study subject must be reported within 24 hours to the Sponsor or its delegate using the Pregnancy Report Form. The pregnant female study subject must be withdrawn from the study.

Every effort should be made to gather information regarding the pregnancy outcome and condition of the infant. It is the responsibility of the Investigator to obtain this information within 30 calendar days after the initial notification and approximately 30 calendar days after delivery.

Pregnancy complications such as spontaneous abortion/miscarriage or congenital abnormality are considered SAEs and must be reported to the Sponsor or its delegate using the Serious Adverse Event Form. **Note**: An elective abortion is not considered an SAE.

In addition to the above, if the Investigator determines that the pregnancy meets serious criteria, it must be reported as an SAE to the Sponsor or its delegate.

9 Statistical Considerations

Continuous measures will be summarized descriptively by the mean, standard deviation, median, minimum, and maximum values. Categorical measures will be summarized by the frequency and percentage of subjects.

A separate Statistical Analysis Plan (SAP) will be prepared prior to unmasking of study data.

Baseline for computing change from baseline for IOP at each time point will be time-matched.

9.1 Statistical Hypothesis

The null hypothesis is that VVN539 has an equivalent effect compared to vehicle for mean IOP at each diurnal time point (8AM, 10AM, and 4PM) at Visit 4 (Day 7), Visit 5 (Day 14), and Visit 6 (Day 21). The alternative hypothesis is that VVN539 is superior to vehicle for mean IOP at these time points.

9.2 Sample Size Determination

With a sample size in each group of 20, the study will have 80% power to detect a difference of 3.0 mm Hg between an VVN539 dose compared to vehicle at each diurnal time point assuming a common standard deviation of 3.3 mm Hg, $\alpha = 0.05$ (two-tailed).

There is no correction for multiplicity for multiple time points or comparisons for high or low VVN539 doses.

9.3 Analysis Populations

The Full Analysis Set (FAS) consists of all subjects who were randomized. Subjects will be analyzed in the group to which they were randomized. This set will be used for the analysis of all efficacy endpoints as the primary analysis.

The Per Protocol (PP) Analysis Set will include all subjects who completed study-required treatment and who followed the protocol without significant deviations. The determination of significant protocol violations will be made prior to locking the final database and unmasking. Subjects will be analyzed in the group to which they were randomized.

The Safety Analysis Set will include all subjects who took at least one dose of investigational product as indicated on the dosing record. Subjects will be analyzed in the group according to the treatment received. All safety variables will be analyzed using the Safety Analysis Set and only observed data will be included (i.e., missing data will remain missing for the safety analysis).

9.4 Statistical Analyses

The statistical analysis of the study will be performed on the data through Day 21 and will be performed after all subjects have either completed the Day 21 visit or discontinued early from the study and after the study database has been cleaned, verified, and locked. It is planned that the data from all clinical sites that participate in this study will be combined so that the target sample size will be available for analysis.

9.4.1 Baseline Descriptive Analyses

Demographic characteristics including age (years), sex, race, ethnicity, and baseline disease characteristics will be summarized by cohort and overall. Medical history (coded using the most recent version of MedDRA), and prior and concomitant medications (coded using World Health Organization Dictionary) will be summarized by cohort and overall.

The numbers of subjects who were enrolled and completed each visit of the study will be provided, as well as the reasons for all enrollment discontinuations, grouped by major reason (e.g., lost to follow-up, adverse event, poor compliance, rescue due to lack of efficacy). A list of discontinued subjects, protocol deviations, and subjects excluded from the analysis sets will be provided as well.

Exposure and compliance to study treatment will be summarized by treatment and overall.

9.4.2 Efficacy Analyses

Efficacy analyses will be based on the study eye using the FAS.

9.4.2.1 Primary Efficacy Analyses

Primary Estimands: The primary estimands are the treatment differences between VVN539 and vehicle at 8:00 AM, 10:00 AM, and 4:00 PM at each of Days 7, 14, and 21 in the study eye using the FAS. The primary estimands treat subjects as if they do not receive rescue/prohibited therapy.

Target Population: Subjects with open-angle glaucoma or ocular hypertension that meet the study entry criteria.

Endpoint: Observed IOP measurements at each time point in the study eye.

Treatment Condition(s): Treatment condition is based on the randomized treatment group.

Population-level Summaries: The difference in the mean IOP at the nine time points of interest and their corresponding p-values and 95% confidence intervals.

Intercurrent Events and Strategies to Address Intercurrent Events

- Discontinuation of study therapy with continued participation in the study without receipt of prohibited/rescue therapy
    - Treatment Policy – no imputation; use observed data

- Receipt of prohibited/rescue therapy in the study eye
    - Hypothetical Approach – data for visits after the receipt of rescue therapy will be set to missing and will be analyzed assuming missing at random (MAR) using a longitudinal model (mixed model repeated measures, MMRM)

- Missing data without withdrawal or with withdrawal regardless of reason
    - Hypothetical Approach: visits that are missing data will be analyzed assuming MAR using MMRM

Time-matched longitudinal models adjusting for baseline and including a random effect for center will be used to test time-specific comparisons.

Data from Days 7, 14, and 21 will be analyzed using MMRM with an unstructured covariance assumed for each treatment with treatment, visit, and visit by treatment interaction as a fixed effects, baseline measurement as a covariate, and a random effect for site.

Sample code for the model run separately for 8AM, 10 AM, and 4 PM:

```
PROC MIXED DATA=indata;
       CLASS site visit treatment;
       MODEL IOP = baseline visit treatment visit*treatment / solution DDFM=KR;
       REPEATED visit / SUBJECT=subject TYPE=UN group = treatment;
       RANDOM site;
       LSMEANS treatment*visit / slice=visit CL;
RUN;
```

Sensitivity analyses include a time-matched last observation carried forward (LOCF) of the last available IOP measurement as well as analysis of covariance (ANCOVA) analyses at each visit. Additional sensitivity analyses will be performed on the PP Analysis Set as necessary.

9.4.2.2 Secondary Efficacy Analyses

Analyses of change from baseline and observed and change from baseline for diurnal IOP will be performed in a manner similar to the primary efficacy analysis.

9.4.3 Safety Analyses

Safety analyses will be performed on all subjects in the Safety Analysis Set. The assessment of safety will be based on the summary of ocular and non-ocular treatment emergent adverse events (TEAEs), BCVA, central corneal thickness, vital signs, clinical laboratory tests, and ophthalmic exams using slit-

lamp biomicroscopy and dilated ophthalmoscopy. Summaries will be provided by treatment group, and for ocular assessments separately by study-eye and non-study-eye.

9.4.3.1 Adverse Events

AEs will be coded using MedDRA (most current version) and categorized by system organ class using preferred terms. Separate summaries of AEs related to treatment (as reported by the Investigator) and by severity will be presented. The number of deaths and SAEs will also be presented, and events leading to discontinuation from the study will be listed and tabulated.

9.4.3.2 Clinical Laboratory Tests

Summary statistics for observed and change from baseline for serum chemistry and hematology laboratory tests will be presented.

9.4.3.3 Other Safety Evaluations

Summary statistics for observed and change from baseline for BCVA, central corneal thickness, and vital signs will be presented. Abnormalities in slit-lamp biomicroscopy and dilated ophthalmoscopy will be summarized by frequency and percentage.

9.5 Interim Analysis

Not applicable.

9.6 Subgroup Analyses

Subgroup analyses will be described in the SAP.

9.7 Exploratory Analyses

Not applicable.

9.8 Missing or Unused Data

Missing data for IOP will be imputed using LOCF methodology as a sensitivity analysis for the primary endpoint and diurnal IOP. Otherwise, observed data will be analyzed at each visit and analyzed using MMRM which assumes MAR.

9.9 Tabulation of Individual Subject Data

All data collected in this study will be presented in individual subject data listings for all subjects.

10 Supporting Documentation and Operational Considerations

10.1 Regulatory Issues, Ethical Concerns, and Study Oversight

10.1.1 Informed Consent Process

10.1.1.1 Consent/Assent Documents

Consent forms describing in detail the study intervention, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to starting intervention/administering study intervention.

10.1.1.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Investigational Review Board (IRB)-approved and the subject will be asked to read and review the document. The Investigator will explain the research study to the subject and answer any questions that may arise. A verbal explanation will be provided in terms suited to the subject's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research subjects. Subjects will have the opportunity to carefully review the written consent form and ask questions prior to signing. The subjects should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The subject will sign the informed consent document prior to any procedures being done specifically for the study. Subjects must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the subjects for their records. The informed consent process will be conducted and documented in the source document (including the date), and the form signed, before the subject undergoes any study-specific procedures. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the Sponsor to the Investigators and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study subjects and the reviewing IRB. Study subjects will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension may include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the Sponsor, IRB and/or FDA.

10.1.3 Confidentiality and Privacy

Subject confidentiality and privacy will be strictly held in trust by the participating Investigators, their staff, and the Sponsor(s) and their interventions. This confidentiality will be extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to subjects. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

All research activities will be conducted in as private a setting as possible.

The Study Monitor, other authorized representatives of the Sponsor, representatives of the IRB, regulatory agencies or pharmaceutical company supplying study product will be able to inspect all documents and records required to be maintained by the Investigator, including but not limited to, medical records (i.e., office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

The study subject's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or Sponsor requirements.

Study subject research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at Lexitas Pharma Services, Inc. This will not include the subject's contact or identifying information. Rather, individual subjects and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by Lexitas research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at Lexitas.

10.1.4 Key Roles and Study Governance

The Manual of Procedures (MOP) will include a list of study team roles and responsibilities of those involved in the conduct, management, or oversight of the trial.

10.1.5 Clinical Monitoring

Lexitas Pharma Services, Inc. will conduct the clinical monitoring for this study. A clinical Monitoring Plan is to be used, which will describe in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed and the distribution of monitoring reports.

10.1.6 Quality Assurance and Quality Control

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council for Harmonisation (ICH) Good Clinical Practice (GCP), and applicable regulatory requirements (e.g., Good Laboratory Practice [GLP], Good Manufacturing Practices [GMP]).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by local and regulatory authorities.

10.1.7 Data Handling and Record Keeping

10.1.7.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site's Investigator. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to insure accurate interpretation of data. Data recorded in the eCRF derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including AEs, concomitant medications, and expected adverse reactions data) will be entered into IBM Clinical Development, a 21 CFR Part 11-compliant data capture system provided by Lexitas Pharma Services, Inc. The data system includes password protection and

internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.1.7.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the Sponsor, if applicable. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained.

10.1.8 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the subject, the Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site's Investigator to use continuous vigilance to identify and report deviations. All deviations must be addressed in study source documents, reported to the reviewing IRB (as applicable per the IRB policies), the Contract Research Organization and/or Sponsor. The site Investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

10.1.9 Publication and Data Sharing Policy

This study will comply with the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. The institution and Investigators participating in this trial shall have no right to publish or present the results of this study without the prior written consent of the Sponsor.

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12 Appendices

12.1 Appendix 1: Schedule of Procedures and Assessments

Study Period	Screening	Washout[1]	Baseline	Treatment Period			End of Treatment
Visit No.	**1**		**2**	**3**	**4**	**5**	**6**
Visit Window	**Day-35 to -1**		**Day 0**	**Day 1**	**Day 7 (+2)**	**Day 14 (+2)**	**Day 21 (+2)**
Informed consent[2]	**X**						
Ocular and medical history	**X**		**X**				
Demographics	**X**						
Inclusion/Exclusion criteria	**X**		**X**				
BCVA	**X**		**X**	**X**	**X**	**X**	**X**
Biomicroscopy[3]	**X**		**X**	**X**	**X**	**X**	**X**
IOP	**X**			**X**[4,]			
Diurnal IOP[5]			**X**		**X**	**X**	**X**
Cup-to-disc ratio	**X**						**X**
Dilated ophthalmoscopy	**X**						**X**[6]
Pachymetry[7]	**X**						**X**
Gonioscopy[8]	**X**						

[1] Washout period: Prostaglandins, β-adrenoceptor antagonists, kinase inhibitors (4 weeks), adrenergic agonists (2 weeks), muscarinic agonists and carbonic anhydrase inhibitors (5 days)
[2] Informed consent will be obtained prior to the conduct of any study specified or associated procedures or evaluations.
[3] Biomicroscopy of anterior segment includes corneal epithelium, bulbar and lower conjunctiva, and lens.
[4] Measured at 8:00AM
[5] Measured at 8:00AM, 10:00AM and 4:00PM
[6] After the last IOP measurement
[7] Central corneal thickness will be measured at entry for IOP interpretation. Pachymetry performed within 3 months of screening is acceptable. Ultrasound and optical pachymetry is permissible provided that the same methodology is used at Visits 1 and 6.
[8] Gonioscopy performed within 3 months of Visit 1 is acceptable.

Study Period	Screening	Washout[1]	Baseline	Treatment Period			End of Treatment
Visit No.	**1**		**2**	**3**	**4**	**5**	**6**
Visit Window	**Day-35 to -1**		**Day 0**	**Day 1**	**Day 7 (+2)**	**Day 14 (+2)**	**Day 21 (+2)**
Visual field[9]	**X**						
Optical Coherence Tomography	**X**[10]						
Blood chemistry, hematology	**X**						**X**
BP, HR	**X**		**X**	**X**	**X**	**X**	**X**
Pregnancy test[11]	**X**		**X**				**X**
Randomization			**X**				
Study drug administration in clinic				**X**[12]	**X**[13]		**X**[13]
Dispense investigational product				**X**	**X**	**X**	
Collect study drug					**X**	**X**	**X**
Prior/Concomitant medications	**X**		**X**	**X**	**X**	**X**	**X**
Adverse event assessment				**X**	**X**	**X**	**X**
Abbreviation key: BCVA=best corrected visual acuity; BP=blood pressure; HR=heart rate; IOP=intraocular pressure							

[9] Visual field within 3 months of Visit 1 is acceptable, provided that it is reliable and meets the requirement for automated threshold visual field (e.g., 30-2 or 24-2 Humphrey). Unreliable entry fields must be repeated prior to study drug dispensation.

[10] Optical Coherence Tomography within 3 months of Visit 1 is acceptable.

[11] Women of childbearing potential only

[12] After 8:00AM IOP measurement

[13] After 8:00AM and prior to 10:00AM IOP measurement

12.2 Appendix 2: Abbreviations and Definition of Terms

AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ANCOVA	Analysis of Covariance
BCVA	Best Corrected Visual Acuity
b.i.d	Twice a Day (Bis in Die)
BP	Blood Pressure
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
CS	Clinically Significant
eCRF	Electronic Case Report Form
FAS	Full Analysis Set
FDA	Food and Drug Administration
ETDRS	Early Treatment Diabetic Retinopathy Study
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
hCG	Human Chorionic Gonadotropin
Hct	Hematocrit
HDL	High-Density Lipoprotein
Hgb	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HR	Heart Rate
IB	Investigator's Brochure
ICH	International Council for Harmonisation
IND	Investigational New Drug

IOP	Intraocular Pressure
IP	Investigational Product
IRB	Institutional Review Board
LASIK	Laser-Assisted *in situ* Keratomileusis
LDH	Lactate Dehydrogenase
LDL	Low-Density Lipoprotein
LOCF	Last Observation Carried Forward
logMAR	Logarithm of the Minimum Angle of Resolution
LPDE	Low-Density Polyethylene
MAR	Missing at Random
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MIGS	Minimally Invasive Glaucoma Surgery
MMRM	Mixed Model Repeated Measures
MOP	Manual of Procedures
NCE	New Chemical Entity
NCS	Not Clinically Significant
NO	Nitric Oxide
OAG	Open Angle Glaucoma
OCT	Optical Coherence Tomography
OHT	Ocular Hypertension
OU	Both Eyes (Oculus Uterque)
PI	Principal Investigator
PK	Pharmacokinetic
POAG	Primary Open Angle Glaucoma
PP	Per Protocol
PRK	Photorefractive Keratectomy

PVP	Polyvinylpyrrolidone
QC	Quality Control
q.d	Once a Day (Quaque Die)
RBC	Red Blood Cells
ROCK	Rho Kinase
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure
TEAE	Treatment Emergent Adverse Events
UPT	Urine Pregnancy Test
US	United States
VA	Visual Acuity
WBC	White Blood Cells

12.3 Appendix 3: Visual Acuity

Distance visual acuity (VA) will be assessed as outlined in Appendix 1 using ETDRS visual acuity charts either retro-illuminated or frontally illuminated (60-watt bulb or well-lit room, respectively). It is recommended that the ETDRS Chart 1 be used for testing the right eye and the Chart 2 be used for testing the left eye.

BCVA testing should precede slit lamp examination, intraocular pressure measurement, the administration of topical anesthetic agents or any examination requiring contact with the eye.

12.3.1 Manifest Refraction

Visual acuity can be tested using the subject's prescription eyeglasses for distance correction, provided the prescription is less than one year old. Sites should measure the eyeglasses and record the readings at Visit 1 in case that subjects forget to bring their eyeglasses to later visits.

Manifest refraction should be performed per the sites standard procedures if the subject's eyeglass prescription is more than one year old or if the subject forgets to bring their eyeglasses to Visit 1. This refraction should then be used for all following visits.

12.3.2 Best Corrected Visual Acuity

Visual Acuity will be assessed at each visit in monocularly in both eyes, testing the right eye first and then the left eye.

The subject should be positioned according to the elevation of the chart (either seated or standing) so that the chart is at a comfortable viewing angle. The subject should attempt to read each letter, line-by-line, left to right, beginning with line 1 at the top of the chart. The subject should be told that the chart has letters only, no numbers. If the subject reads a number, they should be reminded that the chart contains no numbers, and the examiner should then request a letter instead of the number. The subject should be asked to read slowly, about 1 letter per second, to achieve the best identification of each letter. The subject is not to proceed to the next letter until they have given a definite response. If the subject changes a response before he has read aloud the next letter, then the change must be accepted.

Maximum effort should be made to identify each letter on the chart; the subject should be encouraged to guess. When it becomes evident that no further meaningful readings can be made, the examiner should stop the test. The number of letters missed or read incorrectly should be noted.

In order to provide standardized and well-controlled assessments of visual acuity during the study, the same lighting conditions must be used consistently throughout the study

Calculations: LogMAR VA = Baseline value + (n x 0.02)

- where: the baseline value is the LogMAR number of the last line read (at least 1 letter read correctly in this line), and "n" is the total number of letters missed up to and including the last line read, and "0.02" is the value for each letter.

12.4 Appendix 4: Biomicroscopy

The slit-lamp biomicroscopy exam will be performed to examine eye structures at each study visit for each eye. Areas assessed will include the following: anterior chamber, eyelids, conjunctiva, cornea, iris, pupils, lashes and lens.

Note: the same Investigator should assess and score anterior chamber cells and flare across all study visits for an individual subject.

It should be performed with a slit lamp (halogen illumination system is required) using a beam width and intensity that provide optimal evaluation of the anterior segment.

This procedure will be performed in the same manner for all subjects observed at the Investigator's site. The site will record all ABNORMAL findings in the source document and the Investigator will evaluate the ABNORMAL findings as Not Clinically Significant (NCS) or Clinically Significant (CS). CS and NCS ABNORMAL findings will be recorded in the source documentation. However, only ABNORMAL CS descriptions from Visits 3-6 will be captured in the eCRF as AE.

The scoring scales below will be used:

Anterior Chamber Cells	0 = No cells seen 1 = 1 - 5 cells 2 = 6 - 15 cells 3 = 16 - 30 cells 4 = greater than 30 cells
Anterior Chamber Flare	0 = None 1 = Mild (trace to clearly noticeable, visible) 2 = Moderate (without plastic aqueous humor) 3 = Marked (with plastic aqueous humor) 4 = Severe (with fibrin deposits and/or clots)
Eyelid • Eyelid Hyperemia • Eyelid Edema	0 = None 1 = Mild 2 = Moderate 3 = Severe
Conjunctiva • Hyperemia • Edema (Chemosis) • Conjunctival Discharge/Exudate	0 = None 1 = Mild 2 = Moderate 3 = Severe

Cornea • Corneal Edema	0 = None 1 = Mild 2 = Moderate 3 = Severe
Iris	Brown Blue Hazel Grey Green Black Other
Pupil	0 = Normal 1 = Abnormal
Lashes	0 = Normal 1 = Abnormal
Lens	0 = Normal 1 = Abnormal

12.5 Appendix 5: Intraocular Pressure

IOP measurement should be conducted after the biomicroscopy exam is completed and prior to pupil dilation, as applicable.

IOP measurements will be performed utilizing a Goldmann applanation tonometer affixed to a slit lamp according to the Investigator's standard procedure (two-person reading method). Measurements will be taken with the subject seated. The subject and slit lamp should be adjusted so that the subject's head is firmly positioned on the chin rest and against the forehead rest without leaning forward or straining. All pressure will be recorded in mmHg. Two consecutive measurements are taken in each eye and recorded as the mean of the two measurements. If the two measurements differ by >1.0 mmHg, a third measurement is performed, and the median is recorded.

At Visit 1, IOP will be measured once during screening visit.

At Visit 3, IOP will be measured once at 8:00AM (±30 minutes) prior to the first q.d. AM dosing.

At Visits 2, 4, 5, and 6 diurnal IOP will be measured:

- At 8:00AM (±30 minutes), 10:00AM (±30 minutes) and 4:00PM (±30 minutes)
- At Visits 4 and 6, the 8:00AM measurement will be taken prior to the AM dosing.

12.6 Appendix 6: Dilated Ophthalmoscopy

Dilated ophthalmoscopy will include assessment of the retina for any abnormal findings, optic nerve head for pallor and cupping (cup to disc ratio) for each eye. The status of the vitreous

(including haze), retina (including vessels), macula, optic nerve will be determined as Normal or Abnormal. The cup-to-disc ratio will be recorded with two decimal points (e.g., 0.80). After the Ophthalmoscopy procedure, the Investigator will determine if findings are within normal limits or are abnormal. For abnormal findings at Visit 1, the Investigator will determine whether or not the abnormality would exclude the subject from study participation.

12.7 Appendix 7: Pachymetry

Corneal thickness evaluation (in microns) will be performed using a calibrated ultrasonic or optical pachymeter, provided that the same method is used for all measurements. Measurements will be taken in the central cornea after instilling topical anesthetic. A total of three readings will be obtained for each eye. Care should be taken to place the probe perpendicular to the corneal surface and to not a compress the cornea.

Pachymetry is performed at Visit 1 and Visit 6.

- At Visit 1, pachymetry within 3 months of the visit is acceptable.
- At Visit 6, Pachymetry is to be performed after the last IOP measurement.

12.8 Appendix 8: Gonioscopy

Gonioscopy will be performed at Visit 1 using the Shaffer system (Shaffer, 1960) to grade the angle anatomy as follows:

Angle	Grade
closed	0
10 – 15º	I
15 – 25º	II
25 – 35º	III
>35 º	IV

- Gonioscopy performed within 3 months of Visit 1 is acceptable.

12.9 Appendix 9: Visual Field

Visual fields are performed at Visit using a 30-2 or 24.2 Humphrey perimeter. SITA Standard is preferred, SITA Fast is also allowed. Visual fields must be reliable, defined as:

- Fixation losses ≤ 33%
- False positives ≤ 33%
- False negatives ≤ 33%

The gaze track and blind spot should be turned on in order to calculate the fixation losses. Visual fields should be performed with a non-dilated pupil, unless, in the opinion of the Investigator, the pupil is so miotic that dilation is required.

- Visual fields performed within 3 months of Visit 1 are acceptable.

12.10 Appendix 10: Optical Coherence Tomography (OCT)

OCT will be used at the screening visit to determine the nerve fiber layer thickness at Visit 1.

12.11 Appendix 11: Blood Chemistry, Hematology

Laboratory requisition forms must be completed, and samples must be clearly labelled with the subject number, protocol number, site/center number and visit date. Refer to the Central Laboratory Manual for details for the collection, preparation and shipment of samples and reference ranges.

Laboratory reports must be reviewed and signed by the Investigator prior to Visit 2 and results cannot be indicative of any clinically significant or uncontrolled disease in the opinion of the Investigator. If any blood is not resulted, repeat laboratory collection must be performed and results reviewed by the Investigator prior to Visit 2.

Approximately 10 mL of blood will be obtained at Visit 1 and Visit 6. Fasting will not be required, therefore specific values may be out of the typical fasting range.

Abnormal laboratory values noted as clinically significant at Visit 6 that are changes from the Screening values are documented as AEs and is to be followed-up as appropriate.

The following serum chemistry and hematology parameters will be measured:

Chemistry	Hematology
Alanine aminotransferase (ALT)	Absolute/percent basophil count
Albumin	Absolute/percent eosinophil count
Alkaline phosphatase (ALP)	Absolute/percent lymphocyte count
Aspartate aminotransferase (AST)	Absolute/percent monocyte count
Bilirubin (total, direct, indirect)	Absolute/percent neutrophil count
Bun	Differential WBC
Calcium	Hematocrit (Hct)
Cholesterol (total, High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL))	Hemoglobin (Hgb)
Carbon dioxide	Mean corpuscular hemoglobin (MCH)
Chloride	Mean corpuscular hemoglobin conc. (MCHC)
Creatinine, serum	Mean corpuscular volume (MCV)

Glucose	Platelet count
Lactate dehydrogenase (LDH)	Red blood cells (RBC)
Potassium	White blood cells (WBC)
Sodium	
Total protein	
Triglycerides	

12.12 Appendix 12: Heart Rate & Blood Pressure

12.12.1 Heart Rate

Measure the heart rate after the subject has been seated in a quiet room for at least 5 minutes. Instruct the subject that they should refrain from talking during the procedure. Measure the pulse rate over a 30-second period by palpitation at the wrist, and multiply by 2. An electronic device may be used instead. The method of measurement (manual or electronic) must be documented in the subject records. Repeat measurements should be taken for out-of-range values. Confirmed heart rate readings outside the normal range must be evaluated by the Investigator for clinical significance.

12.12.2 Blood Pressure

Measure the blood pressure after heart rate (while the subject is in a resting state). Subject should be seated comfortably, with both feet resting on the floor; subject's arm should not be constrained by any clothing. Use a sphygmomanometer with appropriate size cuff with a stethoscope. A digital device may be used instead. All measurements will be obtained from the same arm using the same cuff size for all study visits. Repeat measurements should be taken for significantly high or low measurements. Confirmed BP readings outside the normal range must be evaluated by the Investigator for clinical significance.

12.13 Appendix 13: Pregnancy Test

A urine human chorionic gonadotropin (hCG) pregnancy test is performed at Visits 1, 2, and 6 for adult women of childbearing potential. An adult woman is considered to be of childbearing potential unless she is one year post-menopausal or three months post-surgical sterilization.

12.14 Appendix 14: Investigator Agreement

A phase 2, double-masked, randomized, vehicle-controlled, dose-response study assessing the safety and ocular hypotensive efficacy of VVN539 in subjects with primary open angle glaucoma or ocular hypertension.

Version No.: 2.0

Issue Date: 07/Jun/2022

I have read the clinical study protocol and understand it. I agree to conduct the study as outlined in this document and in accordance with Good Clinical Practice Guidelines, all local and federal requirements and regulations, and in compliance with those precepts set forth in the Declaration of Helsinki with respect to the use of human subjects in clinical studies and investigations.

Further, I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Signature of Investigator:

Name (printed)	Signature	Date

12.15 Appendix 15: Compliance Statement

The study will be conducted in accordance with International Council for Harmonisation Good Clinical Practice (ICH GCP), with the United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812), and as stipulated in the Declaration of Helsinki with respect to the use of human subjects in clinical studies and investigations.

The Principal Investigator (PI) will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) Sponsor, funding agency and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the study subjects. All personnel involved in the conduct of this study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from subjects who provided consent, using a previously approved consent form.

Sponsor's Representative(s):

Name (printed)	Signature	Date

Medical Monitor(s):

Name (printed)	Signature	Date

Principal Investigator(s):

Name (printed)	Signature	Date

12.16 Appendix 16: Protocol Amendments

Date	Description	Substantive/Non-Substantive
07 Jun 2022	Protocol Amendment 1 (Version 2.0)	This is a substantiative amendment; changes were made based on FDA comments on 25 May 2022 to minimize the risk of study subjects randomized to vehicle for potential glaucomatous loss. Detail can be found in the corresponding Summary of Changes document.
14 Apr 2022	Original Protocol (Version 1.0)	N/A

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