PROTOCOL

STUDY TITLE:	**A Phase 1 Open Label Dose Ranging Study to Assess the Safety and Tolerability of N-Acetylcysteine (NAC) in Patients with Retinitis Pigmentosa (FIGHT-RP1 Study)**
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1. BACKGROUND

1.1 PATHOPHYSIOLOGY

Retinitis Pigmentosa (RP) is the term used for a genetically heterogeneous group of inherited retinal degenerations caused by a mutation in one of the many genes which cause rod photoreceptors to die. Photoreceptors are cells in the retina that are stimulated by light, initiating the translation of visual images into nerve impulses that travel to the brain. There are two types of photoreceptors, rods and cones. Rods enable vision in dim illumination; cones are responsible for more detailed central vision, vision in bright light, and perception of color. The vast majority (95%) of photoreceptors are rods.

Rods are the major consumers of oxygen in the retina and the loss of rods causes an increase in the tissue oxygen level in the outer retina.[1] This activates NADPH oxidase causing accumulation of superoxide radicals in the cytosol [2] and also increases their generation in mitochondria of cones. The excess superoxide radicals overwhelm superoxide dismutase 1 (SOD1) and SOD2 and cause a chain reaction by which other free radicals are generated including some that are even more damaging than superoxide radicals, such as hydroxyl radicals and peroxynitrite.[3] The free radicals attack proteins, lipids, and DNA causing specific modifications that indicate that oxidative damage has occurred. The most common modification to proteins from oxidative damage is the formation of carbonyl adducts.[4, 5] Measurements of these markers of oxidative damage provide a quantitative assessment of the amount of oxidative damage that has occurred in a tissue.[6] These modifications can impair the function of macromolecules and while there are endogenous repair processes, they are overwhelmed by severe oxidative stress resulting in reduced cellular function and eventually apoptosis. After rods are eliminated from the photoreceptor layer, oxidative stress in the outer retina is severe and leads to gradual cone cell death usually starting in the midperiphery where cone density is low and then spreading peripherally and posteriorly.[7-9] The posterior spread of cone death results in visual field constriction and eventually a central island of vision and its elimination causes blindness.

Clinical signs of RP include pigmentary changes in the retina, often around blood vessels and characterized as “bone spicule-like pigmentation”, constriction of retinal vessels, and optic disc pallor. Spectral domain optical coherence tomography can show thinning of the retina in areas of photoreceptor cell loss and with segmentation the loss is seen in the outer nuclear layer. Visual field testing shows constriction of the visual fields and electroretinography shows reduced a- and b-wave amplitudes. .

1.1 STUDY RATIONALE

Retinitis Pigmentosa (RP) is a devastating eye disease and at present there are no known treatment options that can alter the rate of vision loss and eventual blindness. In a series of studies in animal models, the effects of exposing cones in the periphery of the retina to a large excess of oxygen results in progressive oxidative damage to cone photoreceptors and cone cell death.[2, 3, 7-11] Cone cell death gradually spreads from the periphery of the retina toward its center, narrowing the visual field and eventually resulting in tunnel vision. Compared to control patients, those with RP showed significant reduction in the reduced to oxidized glutathione ratio (GSH/GSSG) in aqueous humor and a significant increase in protein carbonyl content.[12] This demonstration of oxidative stress and oxidative damage in the eyes of patients with RP, suggests that oxidative damage-induced cone cell death in animal models of RP may translate to humans with RP and support the hypotheses that (1) potent antioxidants will promote cone survival and function in patients with RP and (2) aqueous GSH/GSSG ratio and carbonyl content on proteins provide useful biomarkers of disease activity in this patient population. Orally administered N-Acetylcysteine (NAC) has been found to be a particularly effective antioxidant that promotes prolonged cone survival and maintenance of cone function in a mouse model of RP.[13] Since oral and/or topical administration of NAC is feasible for long-term treatment in humans, and NAC has a good safety profile, there is good rationale to test the effect of NAC in patients with RP. The first step is to do test different dosing regimens to identify the lowest dose that is able restore aqueous GSH/GSSG ratio and reduce carbonyl adducts on aqueous proteins.

Oxidative damage has been implicated in several diseases including cystic fibrosis, chronic obstructive pulmonary disease (COPD), and Idiopathic Pulmonary Fibrosis. NAC also has a mucolytic effect which makes it particularly useful for treatment of these pulmonary diseases. The effect of oral NAC has been tested in these indications in several clinical trials providing extensive safety data. In COPD, NAC 600mg bid improves airway function and reduces the frequency of acute exacerbations.[14, 15] Doses of up to 1800mg/day have been well-tolerated in the treatment of Idiopathic Pulmonary Fibrosis.[16] Paracetamol (acetaminophen) toxicity is treated with a loading dose of 140 mg/kg NAC followed by 70 mg/kg every 4 hours for 17 doses.[17] Normal volunteers tolerated a dose of 11.2 grams NAC/day for three months without any serious undesirable effects [18] and in another study a dose of 500mg/kg/day was tolerated.[19] The most frequent adverse events associated with the oral administration of NAC are

gastrointestinal in nature and include vomiting, diarrhea, stomatitis, abdominal pain and nausea (incidence rate ≥1/1000 to <1/100). Hypersensitivity reactions including anaphylactic shock and anaphylactic/anaphylactoid reaction (incidence rate <1/10,000), dyspnea, bronchospasm (incidence rate ≥1/10,000 to <1/1000), angioedema, tachycardia, urticaria, rash and pruritus (incidence rate ≥1/1000 to <1/100) have been reported less frequently. Finally, reports of headache, tinnitus, pyrexia, blood pressure decreased (incidence rate ≥1/1000 to <1/100), face edema and hemorrhage have also been collected with oral NAC. In this study, we will use NAC 600 mg effervescent tablets which are generally more pleasant and more tolerable than large capsules; however, because the formulation contains sodium, we will exclude patients with uncontrolled arterial hypertension defined as diastolic blood pressure > 95 mm Hg or systolic blood pressure > 160 mm Hg.

In patients with Idiopathic Pulmonary Fibrosis, polymorphisms within the *TOLLIP* gene were found to influence outcomes of NAC-treated patients.[20] The product of the *TOLLIP* gene, toll-interacting protein, is an inhibitory adaptor protein acting downstream of toll-like receptors, mediators of innate and adaptive immunity. The identification of the influence of *TOLLIP* polymorphisms on the effect of NAC in Idiopathic Pulmonary Fibrosis provides rationale for collecting DNA and genotyping the same SNPs in the current trial. In addition to this candidate gene genetic analysis, we plan to collect and bank patient RNA for future transcriptome analysis. The rationale for this is to identify gene expression changes that modify disease progression in RP. There is substantial variability in rate of progression among patients with RP. A patient who loses all vision early in life can have a sibling with the same mutation who maintains vision into advanced age. This suggests that modifier genes can have a major impact on cone survival. We hypothesize that level of expression of gene products that contribute to the antioxidant defense system may influence cone cell death and hence the rate of loss of visual field. It is also possible that gene expression differences may contribute to differences in response to NAC. For these reasons we plan to collect RNA from patients which will allow us to do next generation sequencing in the future to understand the transcriptome background on which our intervention has been performed. This is an ambitious undertaking that will require additional funding, but it is critical to plan for it and collect the samples as part of this protocol. Also, while it is not critical to know the pathogenic mutation in each patient for our intervention that is directed at cone survival, the rate of rod cell death influences the rate of cone cell death and it may be useful to know the underlying mutation in each of our patients. The underlying mutation may be known in some patients who enter the trial, but it is not a

requirement. In those patients for whom the pathogenic mutation is not known, we will collect DNA to enable future genotyping studies including next generation sequencing. These studies are not part of the current study and will require additional funding, but it is important to obtain and store the samples for future use.

2. OBJECTIVES

2.1 Primary Objectives

- To assess the safety and tolerability of N-acetylcysteine (NAC) in patients with RP

2.2 Secondary Objectives

- To measure the change from baseline aqueous GSH/GSSG ratio and protein carbonyl content.
- To measure change from baseline central retinal sensitivity by microperimetry.
- To measure change from baseline spectral domain optical coherence tomography (SD-OCT).
- To genotype *TOLLIP* SNPs
- To collect samples for future genotyping and transcriptome analysis.

3. STUDY DESIGN

3. DESCRIPTION OF THE STUDY

This study is a phase 1 dose escalation study with 3 cohorts, designed to assess the safety and tolerability of NAC in subjects with RP. Thirty subjects with RP will be enrolled from the clinic population of the Wilmer Eye Institute, Johns Hopkins. Informed consent will be obtained from all study subjects prior to enrollment. Each cohort will have an experimental and an exploratory arm. Subjects with RP will be enrolled in the experimental arm if they have a high carbonyl content ($\geq$0.6) and a reduced GSH/GSSG ratio ($\leq$3.0) in the aqueous. Subjects with RP who don't have a high carbonyl content ($\geq$0.6) and a reduced GSH/GSSG ratio ($\leq$3.0) but otherwise are good candidates for the study will be enrolled in the exploratory arm. The reason for this stratification is that the primary outcome is the change from baseline carbonyl content and GSH/GSSG caused by each dose of NAC and patients who have reduced chance to show improvement could skew the data and reduce the ability to identify the minimally effect dose. In a previous study, 17 of 20 patients with RP fit these criteria, so we anticipate that 8-9 subjects in each cohort will be in the experimental group. We do not want to

completely exclude RP patients who do not fit these criteria, because they may experience substantial decrease in carbonyl content and substantial increase in GSH/GSSG and their inclusion in the exploratory arm will help to determine if patients who do not fit the criteria should be excluded from the next trial, a longer trial with a functional endpoint.

Baseline testing will include: (1) detailed medical and ophthalmological history, (2) measurement of vital signs (3) slit lamp examination, (4) funduscopic examination, (5) best-corrected visual acuity (BCVA), (6) MP1 and MAIA microperimetry, (7) SD-OCT, and (8) ultrawide angle fundus photographs. One eye will be selected as the study eye and an anterior chamber tap will done for measurement of GSH/GSSG and protein carbonyl content. Serum GSH/GSSG, protein carbonyl content and bilirubin levels will also be measured. Blood will be obtained for RNA and DNA samples. The following is the dosing of NAC 600 mg effervescent tablets in each cohort.

	Baseline	Week 4	Week 8	Week 12	Week 20	Week 24
Cohort 1	600mg bid	600mg bid	600mg bid	600mg tid	600mg tid	600mg tid
Cohort 2	1200mg bid	1200mg bid	1200mg bid	1200mg tid	1200mg tid	1200mg tid
Cohort 3	1800mg bid	1800mg bid	1800mg bid	1800mg tid	1800mg tid	1800mg tid

There will be 8 study visits (screening, baseline and month 1-6), all study visits after the baseline visit will be 28±7 days apart, followed by a 12 week observation period. At each visit, patients will have medical history, measurement of vital signs (blood pressure, heart rate, respiratory rate and temperature), review of concomitant medications, review of study drug diary, recording of adverse events, BCVA, eye examination, and anterior chamber tap. MP1 microperimetry will be done at screening and baseline visit. Optical Coherence tomography will be done at the screening and baseline visit and repeated every 3 months. MAIA microperimetry will be done at screening and baseline visit and repeated every month. Fundus autofluorescence and Ultrawide field fundus photographs will be obtained at screening and month 6 visit. At all visits where needed, microperimetry will be performed first followed by OCT, FAF and UWFP. Serum levels of NAC pre- and post-dose will be measured at baseline, week 12 and 24. When all 10 subjects in cohort 1 have had a week 4 visit, the PI will review safety and tolerability with study team members and if there has been good safety and tolerability, enrollment will begin for cohort 2. When all 10 subjects in cohort 2 have had a week 4 visit, the PI will

review safety and tolerability with study team members and if there has been good safety and tolerability, enrollment will begin for cohort 3.

3.2 OUTCOME MEASURES

3.2.1 Primary Outcome Measures

- Assessment of safety and tolerability of NAC including incidence and severity of systemic and ocular adverse events (AEs) and changes from baseline vital signs and physical examination.

3.2.2 Secondary Outcome Measures

- Change from baseline aqueous and serum carbonyl content and GSH/GSSG ratio at 1, 2, 3, 4, 5, 6, 7, 8 and 9 months after initiation of NAC.
- Change from baseline BCVA 6 months after initiation of NAC.
- Change from baseline central retinal sensitivity by microperimetry 3, 6, and 9 months after initiation of NAC.
- Change from baseline ellipsoid zone (EZ) width by SD-OCT 6 month after initiation of NAC .

3.3 SAFETY PLAN

The safety assessments to be conducted for this study are listed in Section 5

Potential safety issues are associated with anterior chamber taps and peripheral blood draw. Subjects will be instructed to contact their physician at any time should they have health-related concerns. All adverse events (serious and non-serious) will be recorded for up to 14 days after the last study visit.

Anterior chamber tap in the clinic in patients with RP

The patient will be seated at a slit lamp and a drop of topical anesthetic will be placed in the study eye. A 30 gauge needle will be passed through the limbus into the anterior chamber and 0.15 ml of aqueous humor will be removed. There are no nerves at the limbus below the epithelium and therefore the topical anesthetic provides complete anesthesia and there is no pain associated with the procedure. Anterior chamber tap is a routine procedure that is extremely safe. It is done to deal with severe

increased intraocular pressure after intravitreous injections, or injection of gas during pneumatic retinopexy. Anterior chamber taps have been done as part of several prior studies and there have been no related complications or adverse events.

Peripheral blood draw

The peripheral blood will be drawn to collect RNA and DNA samples and serum bilirubin levels at the screening visit (BL-28 to 56 days). Blood draw for measurement of serum carbonyl content and GSH/GSSG ratio will be performed at each study visit. At the baseline (Day 1 +7days), week 12 and week 24 visit, peripheral blood will be drawn before as well as 1 and 2 hours after administration of oral NAC in the clinic for measurement of plasma NAC levels. The 2 hour blood draw will be optional, based on the convenience of the subject and at the discretion of the investigator. The peripheral blood draw is commonplace procedure and there are no risks to exposure to transmissible diseases if ones blood is being drawn using aseptic conditions. A patient may feel light headed or faint, or experience pain or discomfort at the site of puncture during the procedure. There may be possible bruising or swelling at the puncture site, rarely there may be an infection.

The Principal Investigator will review all adverse events on an ongoing basis. JHU IRB 3 will be notified immediately of any serious adverse events and will be notified in yearly renewals of all other adverse events. The IRB will be notified of any interruption in enrollment or change in the conduct of the study.

3.4 COMPLIANCE WITH LAWS AND REGULATIONS

This study will be conducted in accordance with current U.S. Food and Drug Administration (FDA) Good Clinical Practices (GCPs), and local ethical and legal requirements.

4. MATERIALS AND METHODS

4.1 SUBJECTS

4.1.1 Subject Selection

Thirty eligible subjects with RP will be enrolled. Subjects will be identified and recruited through the clinic population at the Wilmer Eye Institute and it is expected that enrollment will be completed over an 12 month period.

4.1.2 Inclusion Criteria

All subjects must meet the following criteria to be eligible for study entry:

- Signed informed consent and authorization of use and disclosure of protected health information
- Age ≥18 years
- Patients diagnosed with RP by the investigators, based on clinical phenotype and diagnostic tests

4.1.3 Exclusion Criteria

Subjects who meet any of the following criteria will be ineligible for study entry:

- Patients with concurrent retinal pathologies that result in vision loss, including but not limited to retinal vein occlusion, diabetic retinopathy and neovascular age-related macular degeneration. If one eye does not have any retinal pathology other than RP, it may be enrolled in the study.
- Patients with uncontrolled arterial hypertension defined as diastolic blood pressure > 95 mm Hg or systolic blood pressure > 160 mm Hg despite medical therapy.

4.2 STUDY ASSESSMENTS

For a complete overview of the study assessments, see Flow Charts in Appendix section.

Screening Visit

- Obtain Informed Consent
- Record demographics, medical and ocular history; concomitant medications, smoking history, alcohol intake and supplement usage

- Vital sign measurements (blood pressure, pulse rate, temperature and respiratory rate)
- Slit lamp examination
- Indirect Ophthalmoscopy
- BCVA by Early Treatment Diabetic Retinopathy Study (ETDRS) Protocol
- SD-OCT
- Fundus autofluorescence
- Ultrawide field fundus photography
- MP1 and MAIA microperimetry
- Anterior chamber tap (for measurement of carbonyl content and GSH/GSSG)
- Blood draw for RNA and DNA samples, serum carbonyl content, GSH/GSSG ratio and bilirubin levels.

Baseline Visit (Day 1)

- Record any change in medical and ocular history; concomitant medications, smoking history, alcohol intake, supplement usage and adverse events
- Vital sign measurements (blood pressure, pulse rate, temperature and respiratory rate)
- Slit lamp examination
- Indirect Ophthalmoscopy
- BCVA by Early Treatment Diabetic Retinopathy Study (ETDRS) Protocol
- SD-OCT
- MP1 and MAIA microperimetry
- Anterior chamber tap (for measurement of carbonyl content and GSH/GSSG)
- Obtain blood for serum carbonyl content, GSH/GSSG ratio and blood for plasma NAC

Levels (before and after drug administration).

- Initiation of NAC treatment, first dose of NAC to be administered in the clinic

Four Week Visit (Week 4/ 28±7days)

- Record any change in medical and ocular history; concomitant medications, smoking history, alcohol intake, supplement usage and adverse events
- Vital sign measurements (blood pressure, pulse rate, temperature and respiratory rate)

- Slit lamp examination
- Indirect Ophthalmoscopy
- BCVA
- MAIA microperimetry
- Anterior chamber tap (for measurement of carbonyl content and GSH/GSSG)
- Blood Draw for serum carbonyl content and GSH/GSSG ratio

Eight Week Visit (Week 8/ 56±7days)

- Record any change in medical and ocular history; concomitant medications, smoking history, alcohol intake, supplement usage and adverse events
- Vital sign measurements (blood pressure, pulse rate, temperature and respiratory rate)
- Slit lamp examination
- Indirect Ophthalmoscopy
- BCVA
- MAIA microperimetry
- Anterior chamber tap (for measurement of carbonyl content and GSH/GSSG)
- Blood Draw for serum carbonyl content and GSH/GSSG ratio

Twelve Week Visit (Week 12/ 84±7days)

- Record any change in medical and ocular history; concomitant medications, smoking history, alcohol intake, supplement usage and adverse events
- Vital sign measurements (blood pressure, pulse rate, temperature and respiratory rate)
- Slit lamp examination
- Indirect Ophthalmoscopy
- BCVA
- SD-OCT
- MAIA microperimetry
- Anterior chamber tap (for measurement of carbonyl content and GSH/GSSG)
- Obtain blood for serum carbonyl content, GSH/GSSG ratio and blood for plasma NAC levels (before and after drug administration).

- NAC to be administered in the clinic

Sixteen Week Visit (Week 16/ 112±7days)

- Record any change in medical and ocular history; concomitant medications, smoking history, alcohol intake, supplement usage and adverse events
- Vital sign measurements (blood pressure, pulse rate, temperature and respiratory rate)
- Slit lamp examination
- Indirect Ophthalmoscopy
- BCVA
- MAIA microperimetry
- Anterior chamber tap (for measurement of carbonyl content and GSH/GSSG)
- Blood Draw for serum carbonyl content and GSH/GSSG ratio

Twenty Week Visit (Week 20/ 140±7days)

- Record any change in medical and ocular history; concomitant medications, smoking history, alcohol intake, supplement usage and adverse events
- Vital sign measurements (blood pressure, pulse rate, temperature and respiratory rate)
- Slit lamp examination
- Indirect Ophthalmoscopy
- BCVA
- MAIA microperimetry
- Anterior chamber tap (for measurement of carbonyl content and GSH/GSSG)
- Blood Draw for serum carbonyl content and GSH/GSSG ratio

Twenty-Four Week Visit (Week 24/ 168±7days)

- Record any change in medical and ocular history; concomitant medications, smoking history, alcohol intake, supplement usage and adverse events
- Vital sign measurements (blood pressure, pulse rate, temperature and respiratory rate)
- Slit lamp examination
- Indirect Ophthalmoscopy

- BCVA
- SD-OCT
- Fundus auto-fluorescence
- Ultra-wide field fundus photography
- MAIA microperimetry
- Anterior chamber tap (for measurement of carbonyl content and GSH/GSSG)
- Blood Draw for serum carbonyl content, GSH/GSSG ratio and blood for plasma NAC levels (before and after drug administration)
- NAC to be administered in the clinic

Twenty-Eight Week Visit (Week 28/ 196±7days)

- Record any change in medical and ocular history; concomitant medications, smoking history, alcohol intake, supplement usage and adverse events
- Vital sign measurements (blood pressure, pulse rate, temperature and respiratory rate)
- Slit lamp examination
- Indirect Ophthalmoscopy
- BCVA
- MAIA microperimetry
- Anterior chamber tap (for measurement of carbonyl content and GSH/GSSG)
- Blood Draw for serum carbonyl content and GSH/GSSG ratio

Thirty-Two Week Visit (Week 32/ 224±7days)

- Record any change in medical and ocular history; concomitant medications, smoking history, alcohol intake, supplement usage and adverse events
- Vital sign measurements (blood pressure, pulse rate, temperature and respiratory rate)
- Slit lamp examination
- Indirect Ophthalmoscopy
- BCVA
- MAIA microperimetry
- Anterior chamber tap (for measurement of carbonyl content and GSH/GSSG)

- Blood Draw for serum carbonyl content and GSH/GSSG ratio

Thirty-Six Week Visit (Week 36/ 252±7days)

- Record any change in medical and ocular history; concomitant medications, smoking history, alcohol intake, supplement usage and adverse events
- Vital sign measurements (blood pressure, pulse rate, temperature and respiratory rate)
- Slit lamp examination
- Indirect Ophthalmoscopy
- BCVA
- SD-OCT
- MAIA microperimetry
- Anterior chamber tap (for measurement of carbonyl content and GSH/GSSG)
- Blood Draw for serum carbonyl content and GSH/GSSG ratio

4.3 DOSING AND DOSE ESCALATION

This is a dose-escalation study in which 6 doses of NAC effervescent tablets are being tested: 600mg bid, 600mg tid, 1200mg bid, 1200mg tid, 1800mg bid and 1800mg tid. Patients will be given the dose pre-determined for the cohort they enter upon enrollment. The most common side effect of NAC is nausea and vomiting. If a patient cannot tolerate the assigned dose of NAC due to nausea and/or vomiting, the dose will be reduced to the dose immediately below the current dose. If that dose cannot be tolerated, the dose will be reduced to the next lowest level. This will be done until a tolerated dose is identified and the patient will be maintained on that dose throughout the trial. If a patient cannot tolerate the starting dose of 600mg bid, they will be reduced to 600mg qd. If a patient cannot tolerate a dose of 600mg qd, they will be exited from the trial. If 3 of the first 5 subjects in a cohort or a total of 5 subjects are unable to tolerate a particular dose of NAC, no additional subjects will be treated with that dose and the dose immediately below it will be designated the maximum tolerated dose. All subsequent subjects enrolled in the study will be treated with the maximum tolerated dose.

4.4 STUDY DISCONTINUATION

The Wilmer Eye Institute may terminate this study at any time. Reasons for terminating the study may include the following:

- The incidence or severity of adverse events in this or other studies indicates a potential health hazard to subjects
- Subject enrollment is unsatisfactory

4.5 STATISTICAL METHODS

4.5.1 Safety Analysis

The primary outcome measure is the safety and tolerability of N-acetyl cysteine (NAC) in patients with RP. Adverse events and results of ocular examinations from all 30 patients will be utilized to summarize safety data. The number and percentage of patients with treatment emergent adverse events (i.e. started or increased in severity after the patient received study treatment and includes abnormal lab results etc) will be summarised. The adverse events recorded prior to NAC administration will not be summarised and will be separated from the adverse events that are recorded post NAC administration in the final listings.

4.5.2 Secondary Analysis

To measure the change from baseline aqueous and serum carbonyl content and GSH/GSSG ratio at 1, 2, 3, 4, 5, 6, 7, 8 and 9 months after initiation of NAC treatment and correlate it with changes in BCVA, retinal sensitivity, and ellipsoid zone (EZ) width.

4.5.3 Power Analysis

Power analysis was conducted and a sample size of 30 was found to achieve 89% power to detect a mean of paired difference of 50% in aqueous carbonyl content with an estimated standard deviation of 0.8 and with significance level of 0.05 (95% confidence interval) using a 2 sided paired t-test.

4.5.4 Missing Data

Analyses of efficacy and safety will be based on available cases without imputation for missing values.

4.5.5 Interim Analysis

No formal schedule of interim analyses is planned. Reports of adverse events from this study may be reviewed and summarized periodically while the study is ongoing to ensure the safety of subjects.

4.5.6 Early Termination and Replacement of Subjects

Subjects withdrawn from the study prior to completion will be asked to return for an end of treatment assessment visit (all study procedures on the EOT assessment visit will be similar to those conducted on the month 9 visit) and the 3 months observational period. Subjects who are terminated prior to the week 24 visit will be replaced.

4.6 DATA QUALITY ASSURANCE

- Accurate, consistent, and reliable data will be ensured through the use of standard practices and procedures.

5. ASSESSMENT OF SAFETY

5.1 ADVERSE EVENTS

For this protocol, an AE is any "on study" untoward medical occurrence (e.g., sign, symptom, disease, syndrome, intercurrent illness) that occurs in a study subject, regardless of the suspected cause.

Unchanged, chronic conditions are NOT AEs and should not be recorded on the AE pages of the CRF. An exacerbation or worsening of a chronic condition should be recorded as an AE.

Both serious and non-serious AEs should be graded on a three-point scale (mild, moderate, severe) and reported in detail on the appropriate AE page of the CRF.

The suggested definitions are as follows:

Mild:	Discomfort noticed but no disruption of normal daily activity
Moderate:	Discomfort sufficient to reduce or affect normal daily activity
Severe:	Incapacitating with inability to work or perform normal daily activity

Using the following criteria, investigators also need to assess whether there is a reasonable possibility that study intervention caused or contributed to the AE.

- Yes (possibly or probably)

There is a clinically plausible time sequence between onset of the AE and study intervention; and/or

There is a biologically plausible mechanism for study intervention causing or contributing to the AE; and

The AE may or may not be attributed to concurrent/underlying illness, other drugs, or procedures.

- No

A clinically plausible temporal sequence is inconsistent with the onset of the AE and study intervention; and/or

A causal relationship is considered biologically implausible.

5.2 BASELINE MEDICAL CONDITIONS

It is not necessary to complete an AE CRF page for chronic medical conditions present at enrollment that do not worsen in intensity or frequency during the trial. These medical conditions should be adequately documented on the appropriate page of the CRF (medical history and/or physical examination). However, medical conditions present at enrollment that worsen in intensity or frequency during the treatment or post-treatment periods and ongoing AEs that started during the previous study should be reported and recorded as AEs.

5.3 PROCEDURES FOR RECORDING AND REPORTING ADVERSE EVENTS

5.3.1 Recording Adverse Events

To improve the quality and precision of acquired AE data, investigators should observe the following guidelines:

- Whenever possible, use recognized medical terms when recording AEs on the AE pages of the CRF. Do not use colloquialisms and/or abbreviations.

- If known, record the diagnosis (i.e., disease or syndrome) rather than component signs and symptoms on AE pages of the CRF (e.g., record congestive heart failure rather than dyspnea, rales, and cyanosis). However, signs and symptoms that are considered unrelated to an encountered syndrome or

disease should be recorded as individual AEs on the CRF (e.g., if congestive heart failure and severe headache are observed at the same time, each events should be recorded as an individual AE).

- AEs occurring secondary to other events (e.g., sequelae) should be identified by the primary cause.

A "primary" AE, if clearly identifiable, generally represents the most accurate clinical term to record on AE pages of the CRF. If a primary serious AE (SAE) is recorded on an SAE CRF, events occurring secondary to the primary event should be described in the narrative description of the case.

5.3.2 Serious Adverse Events

An AE should be classified as **SERIOUS** if:

- It resulted in death (i.e., the AE caused or led to death).
- It was life threatening (i.e., the AE placed the subject at immediate risk of death; it does not apply to an AE that hypothetically might have caused death if it were more severe).
- It required or prolonged inpatient hospitalization (i.e., the AE required at least a 24-hour inpatient hospitalization or prolonged a hospitalization beyond the expected length of stay; hospitalizations for elective medical/surgical procedures, scheduled treatments, or routine check-ups are not SAEs by this criterion).
- It was disabling (i.e., the AE resulted in a substantial disruption of the subject's ability to carry out normal life functions).
- It resulted in a congenital anomaly/birth defect (i.e., an adverse outcome in a child or fetus of a subject exposed to the molecule or study drug prior to conception or during pregnancy).
- It does not meet any of the above serious criteria but may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed above.

Sight-threatening adverse events

An adverse event is considered to be sight-threatening if it meets one or more of the following criteria and will be reported as a serious adverse event (SAE):

- The event causes a decrease in visual acuity of greater ≥30 letters (compared with the last assessment of visual acuity prior to the most recent treatment) lasting >1 hour.

- The event causes a decrease in visual acuity to the level of light perception or worse lasting >1 hour.
- The event requires surgical intervention (e.g, conventional surgery, vitreous tap or biopsy with intravitreal injection of antibiotics, or laser or retinal cryopexy with gas) to prevent permanent loss of sight.
- The event is associated with severe intraocular inflammation (i.e., 4+ anterior chamber cell/flare or 4+ vitritis).
- In the opinion of the investigator, the event may require medical intervention to prevent permanent loss of sight.

5.3.3 SAE Reporting

Sub-Investigators should report all SAEs to the PI within 48 hours of observing or learning of the event. For initial SAE reports, investigators should record all case details that can be gathered within 48 hours on the SAE page of the CRF. The PI will inform the IRB within 1 week of being informed of the SAE.

5.3.4 Type and Duration Of Follow-Up After Adverse Events

All reported AEs should be followed until resolution or until 1 month after the procedure. Subjects who have an ongoing study related SAE will be followed by the investigator or his or her designee until the event is resolved or determined to be irreversible, chronic, or stable by the investigator.

5.3.5 Plans to Decrease Potential Risks and Increase Safety

Detailed ocular examinations (e.g., VA, slit lamp examination and dilated fundus examination) will be performed every month. The Principal Investigator will review all adverse events. The appropriate IRB will be notified immediately of any serious adverse events and will be notified in yearly renewals of all other adverse events. The IRB and FDA will be notified of any interruption in enrollment or change in the conduct of the study. Study drug administration will be held for subjects who experience certain systemic or ocular adverse events related to the drug. In the event any subject develops an adverse event in the study eye that is considered by the designated evaluating physician to be severe in intensity, serious consideration should be given to withdrawing the subject from the study.

6. INVESTIGATOR REQUIREMENTS

6.1 STUDY INITIATION

Before the start of this study, the following documents must be on file at Johns Hopkins University or its appointed representative:

- Current curricula vitae of the Principal Investigator
- Written documentation of IRB approval of protocol (identified by Johns Hopkins University, protocol number or title and date of approval) and informed consent document (identified by Johns Hopkins University protocol number or title and date of approval)
- A copy of the IRB-approved informed consent document
- Written documentation of IRB review and approval of any advertising materials to be used for study recruitment, if applicable
- The informed consent document and any advertising materials must also be reviewed and approved by the Johns Hopkins University Legal Department.
- Certified translations of IRB approval letters, pertinent correspondence, and approved informed consent document (when applicable)

6.2 STUDY COMPLETION

The following data and materials are required by Johns Hopkins University before a study can be considered complete or terminated:

- Laboratory findings, clinical data, and all special test results from screening through the end of the study follow-up period (if applicable)
- Case Report Forms properly completed by appropriate study personnel and signed and dated by the investigator (if applicable)
- Copies of protocol amendments and IRB approval/notification (if applicable)

- A summary of the study prepared by the Principal Investigator (will accept IRB summary close letter) (if applicable)
- All regulatory documents (e.g., curricula vitae for each Principal Investigator)

6.3 INFORMED CONSENT

Informed consent documents will be provided to each subject.

The informed consent document must be signed and dated by the subject or the subject's legally authorized representative before his or her participation in the study. The case history for each subject shall document that informed consent was obtained prior to participation in the study. A copy of the informed consent document must be provided to the subject or the subject's legally authorized representative. If applicable, it will be provided in a certified translation of the local language.

Signed consent forms must remain in each subject's study file and must be available for verification at any time.

The following basic elements must be included:

A statement that the study involves research, an explanation of the purposes of the research and the expected duration of the patient's participation, a description of the procedures to be followed, and identification of any procedures or drug used for purposes which are experimental

- A description of any reasonably foreseeable risks or discomforts to the patients
- A description of any benefits to the patient or to others which may reasonably be expected from the research. A description that there may be no benefit from this research.
- A disclosure of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the patient
- A statement describing the extent, if any, to which confidentiality records identifying the patient will be maintained and that notes the possibility that the FDA and the Johns Hopkins University and the drug manufacturer may inspect the records

- For research involving more than minimal risk, an explanation as to whether any compensation and any medical treatments are available should injury occur and, if so, what they consist of or where further information may be obtained
- An explanation of whom to contact for answers to pertinent questions about the research and research patient's rights, and whom to contact in the event of a research-related injury to the patient
- A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the patient is otherwise entitled, and that the patient may discontinue participation at any time without penalty or loss of benefits to which the patient is otherwise entitled

6.4 INSTITUTIONAL REVIEW BOARD OR ETHICS COMMITTEE APPROVAL

This protocol, the informed consent document, and relevant supporting information must be submitted to the IRB/EC for review and must be approved before the study is initiated. The study will be conducted in accordance with U.S. FDA, applicable national and local health authorities, and IRB/EC requirements.

The Principal Investigator is responsible for keeping the IRB/EC apprised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case the IRB/EC must be updated at least once a year. The Principal Investigator must also keep the IRB/EC informed of any significant adverse events.

Investigators are required to promptly notify their respective IRB/EC of all adverse drug reactions that are both serious and unexpected. This generally refers to serious adverse events that are not already identified in the package insert and that are considered possibly or probably related to the study drug by the investigator. Some IRBs or ECs may have other specific adverse event requirements that investigators are expected to adhere to. Investigators must immediately forward to their IRB/EC any written safety report or update provided by Johns Hopkins University (e.g., IND safety report, Investigator Brochure, safety amendments and updates, etc.).

6.5 CASE REPORT FORMS

All CRFs should be filled out completely by appropriate personnel. The CRF should be reviewed, signed, and dated by the investigator.

All CRFs should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced CRF copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID ON THE ORIGINAL.

6.6 STUDY DRUG ACCOUNTABILITY

The Investigator is responsible for the control and distribution of study drug.

All partially used or empty containers should be disposed of at the study site according to institutional standard operating procedure.

6.7 DISCLOSURE OF DATA

Subject medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited.

Upon the subject's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare.

Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA, national and local health authorities, the drug manufacturer and the IRB/EC for each study site, if appropriate.

6.8 RETENTION OF RECORDS

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for 2 years after

the investigation is discontinued and the U.S. FDA and the applicable national and local health authorities are notified.

7. PAYMENTS, REMUNERATIONS AND COSTS TO PARTICIPANTS

7.1 PAYMENT AND REMUNERATION

There is no compensation for participants for this protocol.

7.2 COSTS

There are no costs for study procedures or study drug to participants.

8. REFERENCES

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Appendix A

Schedule of Activities for Subjects with RP											
Protocol Activities		BL	28± 7d	56± 7d	84±7 d	112± 7d	140± 7d	168± 7d	196± 7d	224± 7d	252± 7d
	Screening	Day1	W4	W8	W12	W16	W20	W24	W28	W32	W36
Informed Consent	X										
Eligibility Criteria	X										
Demographics	X										
Medical History/ Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X
BCVA	X	X	X	X	X	X	X	X	X	X	X
Slit Lamp Examination	X	X	X	X	X	X	X	X	X	X	X
Funduscopic Examination	X	X	X	X	X	X	X	X	X	X	X
Paracentesis (study eye)	X	X	X	X	X	X	X	X	X	X	X
Blood Draw for RNA & DNA samples	X										
Blood Draw for serum bilirubin levels	X										
Blood Draw for measurement of oxidative markers	X	X	X	X	X	X	X	X	X	X	X
Blood Draw for measurement of plasma NAC levels (before administration of oral NAC and 1 & 2 hours after administration – 2 hour draw optional)		X			X			X			
MP1 microperimetry	X	X									

MAIA microperimetry	X	X	X	X	X	X	X	X	X	X	X
SD-OCT	X	X			X			X			X
Fundus autofluorescence	X							X			
Ultrawide angle fundus photo	X							X			
Treatment with NAC		X	X	X	X	X	X	X			
Adverse Events		X	X	X	X	X	X	X	X	X	X