Protocol Title: Pilot Phase I/II Study of the Evaluation of Interferon Gamma-1b Administered Topically for Macular Edema/Intraretinal Schisis Cysts in Rod-Cone Dystrophy (RCD) and Enhanced S-Cone Syndrome (ESCS)

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Human Research Protections Program Investigator and Staff Training:

For this protocol, the following "Just in time" human subjects protection training courses are required for investigators and staff:

Biomedical-Vulnerable Subjects-Research with Children

Coordinating Center: The Emmes Corporation (Emmes)
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Total Requested Accrual: 12 Participants:

- Up to 5 Participants with Rod-cone dystrophy and macular edema
- Up to 5 Participants with Enhanced S-cone syndrome and macular edema
- Up to an additional two participants may be enrolled to account for participants who withdraw from the study prior to receiving five days of treatment

0 Healthy Volunteers

Project Uses Ionizing Radiation: ☒ No ☐ Yes

IND/IDE: ☐ No ☒ Yes

Drug #: 105,631

Sponsor: Wadih Zein, MD, National Eye Institute

Durable Power of Attorney: ☒ No ☐ Yes

Multi-institutional Project: ☒No ☐ Yes

Data and Safety Monitoring Board: ☒ No ☐Yes

Technology Transfer Agreement: ☐ No ☒ Yes

Agreement type and number: CTA 00935-13 with Horizon Pharma [Vidara Therapeutics Research Limited (Vidara)]

Expiration Date: September 30, 2016

Samples Being Stored for Future Research: ☐No ☒ Yes

Flesch-Kincaid Reading Level of Consent Form: 8.6

Flesch-Kincaid Reading Level of Assent Form: 5.7

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Protocol Abbreviation List

Abbreviation	Term
°C	Degrees Celsius
°F	Degrees Fahrenheit
µg	Microgram
µL	Microliter
AE	Adverse Event
BCVA	Best-corrected visual acuity
BP	Blood pressure
CAI	Carbonic Anhydrase Inhibitor
CBC	Complete Blood Count
CC	Clinical Center
CD	Clinical Director
CFP	Color Fundus Photography
CFR	Code of Federal Regulations
CNS IRB	Combined Neuroscience Institutional Review Board
CNV	Choroidal Neovascularization
CRIS	Clinical Research Information System
CTA	Clinical Trial Agreement
DSMC	Data and Safety Monitoring Committee
Emmes	The Emmes Corporation
EMR	Electronic Medical Record
EOG	Electrooculography
ERG	Electroretinogram/Electroretinography
ESCS	Enhanced S-Cone Syndrome
ETDRS	Early Treatment Diabetic Retinopathy Study
EVA	Electronic Visual Acuity
FA	Fluorescein Angiography
FAF	Fundus Autoflourescence Imaging
FDA	Food and Drug Administration
FSH	Follicle-stimulating Hormone
HRPP	Human Research Protection Program
HSPU	Human Subjects Protection Unit
IFN	Interferon
IND	Investigational New Drug
IOP	Intraocular Pressure
IP	Investigational Product
iPSC	Induced pluripotent stem cell

Abbreviation	Term
IRB	Institutional Review Board
IU	International Unit
kg	Kilogram
logMAR	Logarithm of the Minimum Angle of Resolution
MD	Doctor of Medicine
mg	Milligram
mIU	Milli International Unit
mL	Milliliter
mm	Millimeter
mmol	Millimole
MS	Multiple Sclerosis
MSHSc	Master of Science in Health Sciences
NEI	National Eye Institute
NEI QA	National Eye Institute Quality Assurance
NEIS	National Eye Institute Support
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
NR2E3	Nuclear Receptor Subfamily 2, Group E, Member 3
OCT	Optical Coherence Tomography
OHSRP	Office of Human Subjects Research Protections
OMIM	Online Mendelian Inheritance in Man
PhD	Doctor of Philosophy
PI	Principal Investigator
RCD	Rod-cone dystrophy
RN	Registered Nurse
RP	Retinitis Pigmentosa
RPE	Retinal Pigment Epithelium
SAE	Serious Adverse Event
ScM	Master of Science
SOP	Standard Operating Procedures
VA	Visual Acuity
VFQ	Visual Field Questionnaire

PRÉCIS

Objective: Rod-cone dystrophy (RCD) is a term applied to a number of genetically heterogenous diseases presenting with night vision abnormalities, visual field defects and reduced rod electroretinography responses. Enhanced S-Cone syndrome (ESCS) is a rare autosomal recessive retinal disease with a developmental and a degenerative aspect. Macular cystic changes, often florid and usually resulting in a reduction of central acuity, are frequently associated with both diseases. The reason for this association is not well understood. Acetazolamide (Diamox) and Dorzolamide (Trusopt) have been reported to have variable success in reducing these cystic changes but the effect is frequently inadequate. The objective of this study is to evaluate the safety and potential efficacy of Interferon (IFN) gamma-1b administered topically for macular edema/retinal schisis cysts in RCD and ESCS. Possible disease-related pathophysiologic mechanisms will be explored using induced pluripotent stem cell (iPSC) protocols leading to iPSC-derived retinal pigment epithelium (RPE) and photoreceptor generation.

Study Population: Up to five participants with RCD with significant macular cystic changes and up to five participants with ESCS with significant macular cystic changes will be enrolled to receive IFN gamma-1b administered topically in one eye. However, up to an additional two participants may be enrolled in order to obtain the five participants in each disease group to be included in the primary analysis if any participants withdraw from the study prior to receiving five days of treatment.

Design: This is a single-center, prospective, uncontrolled, unmasked pilot Phase I/II study of the safety, tolerability and possible efficacy of IFN gamma-1b in participants with RCD and ESCS and macular cystic changes. One eye of up to five participants with RCD with significant macular cystic changes and up to five participants with ESCS with significant macular cystic changes [evidenced by optical coherence tomography (OCT) >275 microns central macular thickness and/or disruption of foveal contour] will receive topical IFN gamma-1b instilled as drops on the cornea. The initial stage of the study will include two participants from each disease category. Once all four participants have completed the 8-week visit, enrollment will be halted. Safety Adverse Event Review Committee members unaffiliated with the study will review the

data as a preliminary assessment of safety and efficacy and to determine whether enrollment should continue. If the committee determines enrollment will continue, three additional participants with RCD and three participants with ESCS will be enrolled. The study will be completed once the final participant has received one year of follow-up.

Outcome Measures: The primary outcome measure related to the safety and tolerability of IFN gamma-1b administered topically at the prescribed dosage for macular cystic changes in participants with RCD and ESCS will be assessed by the number and severity of adverse events related to the IP and the number of withdrawals at 52 weeks (one year) post-administration. Additional safety of IFN gamma-1b administered topically in participants with RCD and ESCS will be determined from the assessment of retinal function, ocular structure and occurrence of adverse events at all time points. Secondary outcomes include changes in visual function including visual acuity and microperimetry, and retinal imaging with OCT and fluorescein angiography.

1.0 INTRODUCTION/SCIENTIFIC RATIONALE

RCD is a broad category of genetically heterogeneous diseases that includes many different forms of primary photoreceptor abnormalities − typically affecting rods first and cones later. Retinitis pigmentosa (RP) is the most common form of RCD. The prevalence is in the range of 1:3000–1:5000.[1] The natural history of the disease involves progressive visual field loss and difficulties with night vision. Reduction in central vision can develop secondary to macular atrophy in advanced stages of the disease or due to cystic macular changes at any stage. Early studies on macular changes in RP gave macular edema a prevalence of around 13% based on clinical examination and fluorescein angiography.[2, 3] Cystic macular changes were noted in up to one third of patients on OCT with visual loss that is correlated with the degree of macular thickening.[4,5] The cystic changes are frequently bilateral and may respond to treatment with carbonic anhydrase inhibitors (CAIs).[6-8] Some patients do not respond well to CAI therapy and a rebound effect has been noted with extended use.[9]

ESCS (OMIM 268100; http://www.ncbi.nlm.nih.gov/omim/ Online Mendelian Inheritance in Man; NCBI, Bethesda, MD) is a rare autosomal recessive disease primarily associated with mutations in the gene *NR2E3* (OMIM 604485). The disorder was first recognized as a separate entity by Jacobson and Marmor in 1990[10,11] and association with the *NR2E3* gene was described by Haidar et al. in 2000.[12] The phenotype is variable but early hemeralopia, visual field constriction, nummular pigmentary clumping in the retinal midperiphery and pathognomonic electroretinographic changes aid in suspecting the diagnosis.[10,11,13-15] The disease is commonly associated with significant central retinal cystic changes showing variable response to treatment with topical or systemic CAIs.[13,14,16-19] No leakage has been noted on fluorescein angiography, leading Audo et al. to ascribe these changes to schisis rather than edema.[13] The exact mechanism behind these often dramatic cystoid changes, with associated reduction in acuity, is still elusive.

Milam et al. reported on the histopathology in a 77-year-old patient with ESCS (confirmed molecularly) indicating that the retina was disorganized with densely packed cones in the center (most of which were labeled with S-cone opsins). They note that the peripheral retina was degenerative with no rods identified. The RPE was variably depigmented.[20]

Multiple investigators have studied electroretinographic responses in ESCS. The main findings include almost absent rod responses, delayed scotopic maximal response and photopic flash responses (thus the two waveforms look similar in shape) and a more robust S-cone response compared to the reduced L- and M-cone responses.[10,11,13,14,21-24] Pattern electroretinography showed variable reduction in amplitude and delay of the P50 component.[13,23] Variable degrees of dyschromatopsia have been reported on color vision testing with many patients demonstrating relative tritan axis sparing and moderately elevated protan and deutan thresholds.[13]

The *NR2E3* gene was identified in 1999 as a photoreceptor cell-specific nuclear receptor.[25] The association of mutations in this nuclear receptor gene with ESCS was made by Haider et al.[12] The molecular biology of this gene is also of interest because of the role it plays in photoreceptor development, differentiation, and maintenance.[20,26-34] It has also been noted that mutations in other genes that interact with *NR2E3* can, less commonly, cause a similar phenotype.[35] Recently, Mustafi et al. reported on defective photoreceptor phagocytosis in a mouse model of ESCS as a possible contributor to the progressive degeneration.[36] They do not indicate defective RPE but rather an abnormal interaction between the photoreceptors and the RPE. Personal communication with Kapil Bharti, PhD indicates that *NR2E3* is part of the adult RPE transcriptome (based on work by Strunnikova et al.).[37] All previous reports had indicated that the gene is photoreceptor specific. Given the above body of work, it would be very helpful if iPSC-derived photoreceptor and RPE cells were available to further study the disease mechanisms.

The fluid transport role of the RPE is important in the control of subretinal fluid.[38] The active "pump" role of the RPE is believed to be important in the maintenance of retinal attachment and fluid absorption. This role can be modified with the use of medications.[39] *In vivo* rodent studies have shown that IFN gamma-1b applied to the anterior surface of the eye results in significant, rapid decrease in retinal detachment volume.[40] A number of National Eye Institute (NEI) protocols address the question of the safety (and possible efficacy) of topical IFN gamma-1b for the treatment of macular edema in different disease models (Central Serous Chorioretinopathy (CSC) in NIH protocol 12-EI-0013; Uveitis in NIH protocol 09-EI-0191 and NIH protocol 11-EI-0167). Topical IFN gamma-1b was first evaluated in uveitic macular edema at the NEI (NIH protocol 09-EI-0191). This pilot, phase I, dose-escalation study found topical IFN

gamma-1b to be safe and well tolerated with no systemic adverse events or abnormal laboratory values (unpublished data). Two participants received 10 micrograms (μg) of topical IFN gamma-1b (one instillation), two participants received 20 μg (two instillations) and one participant received 30 μg (three instillations). Variable changes in retinal thickness were noted using OCT testing; for that study, topical IFN gamma-1b was administered as a one-time instillation or series of instillations and OCT testing was performed over a three-hour time period. We propose to study the safety and potential efficacy of topical IFN gamma-1b for macular cystic changes in the setting of RCD and ESCS. In the proposed protocol, ESCS would serve as a model of hereditary retinal diseases associated with significant macular cystic/schisis changes and RCD would serve as a model for the more common presentation of macular changes in a hereditary retinal degeneration.

2.0 STUDY OBJECTIVES

The primary objective of this study is to explore the safety, tolerability, and possible efficacy of the topical application of IFN gamma-1b used as a therapeutic option in patients with macular cystic changes associated with RCD and ESCS. Participants will be assessed for systemic and ocular side effects. The study is a safety trial but will also provide information about the possible therapeutic effect of IFN gamma-1b in a model of florid cystic macular edema and in hereditary retinal degenerations. Insight into the pathophysiology of the diseases will be possible by obtaining skin biopsy and blood samples for stem cell derived studies.

3.0 PARTICIPANTS

Up to five participants with RCD with significant macular cystic changes and up to five participants with ESCS with significant macular cystic changes will be enrolled to receive topical IFN gamma-1b in one eye. However, up to an additional two participants may be enrolled in order to obtain the five participants in each disease group to be included in the primary analysis if any participants withdraw from the study prior to receiving five days of treatment.

The inclusion and exclusion criteria are outlined in the following sections.

3.1 Participant Eligibility Criteria

3.1.1 Inclusion Criteria

To be eligible, the following inclusion criteria must be met, where applicable.

1. Participant must be 12 years of age or older.
2. Participant (or legal guardian or legal representative) must understand and sign the protocol informed consent.
3. Participant is willing to comply with the study procedures and is expected to be able to return for all study visits.
4. Participant must carry a clinical diagnosis of RCD or ESCS.
5. ESCS participant must have molecular confirmation with two alleles for *NR2E3* gene mutations
6. Female participant of childbearing potential (see Appendix 5 for definition) must not be pregnant or breast-feeding, must have a negative pregnancy test at screening and must be willing to undergo pregnancy tests at scheduled study visits.
7. Female participant of childbearing potential (see Appendix 5), and any male participant able to father children must have (or have a partner who has) had a hysterectomy or vasectomy, be completely abstinent from intercourse or must agree to practice two reliable methods of contraception while taking the IP and six weeks after completion. Acceptable methods of contraception include:
    - Hormonal contraception (i.e., birth control pills, injected hormones, dermal patch or vaginal ring);
    - Intrauterine device;
    - Barrier methods (diaphragm, condom) with spermicide; or
    - Surgical sterilization (tubal ligation).

3.1.2 Exclusion Criteria

A participant is not eligible if any of the following exclusion criteria are present.

1. Participant has a history of other ocular disease likely to contribute significantly to visual disruption (e.g., optic neuropathy, glaucoma, uveitis, or other retinal disease).
2. Participant has had diagnosis or treatment of a malignancy (excluding non-melanoma skin cancer) within the previous five years.

3. Participant has received investigational treatment in another clinical study related to an ocular condition in the last six months.
4. Participant is pregnant, lactating, planning to become pregnant (or father a child) during the study follow-up period.
5. Participant is allergic to fluorescein dye.
6. Participant has a systemic condition that, in the opinion of the investigator, would preclude participation in the study (e.g., multiple sclerosis (MS), as IFN gamma may cause MS exacerbations).

3.2 Study Eye Eligibility Criteria

A participant must have at least one eye meeting all inclusion criteria and none of the exclusion criteria listed below.

3.2.1 Study Eye Inclusion Criteria

1. The study eye must retain adequate fixation to allow for completion of protocol assessments.
2. The study eye must have macular cystic changes (>275 microns and/or disruption of foveal contour on OCT).

3.2.2 Study Eye Exclusion Criteria

1. The study eye has lens, cornea, or other media opacities that preclude adequate visualization and testing of the retina.
2. The study eye has undergone intraocular surgery within 6 months prior to enrollment.
3. The study eye has a disease that may confound the outcome of the study [e.g., choroidal neovascularization (CNV) in the fovea or parafoveal area].
4. Participant is unwilling to discontinue wearing a contact lens in the study eye during IP administration.

3.2.3 Study Eye Selection Criteria in Cases of Bilateral Disease

RCD and ESCS usually affect both eyes to a similar degree. In case both eyes of a participant meet the study eye eligibility criteria, the following criteria will be used to select the study eye:

- The eye with more intraretinal fluid will be selected as the study eye;

- If both eyes have similar levels of intraretinal fluid, the eye with worse visual acuity will be selected as the study eye;
- If both eyes have the similar levels of intraretinal fluid and visual acuities, the right eye will be selected as the study eye.

4.0 STUDY DESIGN AND METHODS

4.1 Study Overview

This is a single-center, prospective, uncontrolled, unmasked pilot Phase I/II study of the safety, tolerability and possible efficacy of IFN gamma-1b in participants with RCD and ESCS and macular cystic changes. Initially, one eye of two RCD participants with macular cystic changes and one eye of two ESCS participants with macular cystic changes will receive topical IFN gamma-1b. Enrollment will be halted and the Serious Adverse Event Review Committee will conduct a preliminary assessment of safety and efficacy after two participants in each disease category have completed the 8-week visit. If the committee approves study continuation, three additional participants with RCD and three participants with ESCS will be enrolled. All participants receiving IP will be followed for 52 weeks (one year). During the first study visit, participants receiving IP will be provided with on-campus lodging to evaluate and monitor the participants (baseline, Days 1 and 2). The visits at Weeks 2, 5, 8 and 52 will be outpatient visits. The study will be completed once the final participant has received 52 weeks of follow-up.

4.2 Recruitment

Participants may enroll in this study after referral by a medical practitioner in the private sector, or by another clinic, hospital or medical institution. Participants may enroll in this study concurrently with another NEI or NIH protocol that does not offer ocular treatment with an investigational product, or following completion of another study. Self-referral is permitted. Participants enrolled or previously enrolled in other active NEI protocols may be contacted. If any recruitment materials are used, IRB-approval will be obtained prior to distribution. It is expected that recruitment will be complete within a 12-month period.

4.3 Screening

Screening may be performed using the *NEI Screening Study for the Evaluation and Diagnosis of Potential Research Participants* (08-EI-0102) or the *NEI Evaluation and Treatment Protocol for Potential Research Participants with Ocular Diseases* (08-EI-0169). Participants may also enter this protocol while enrolled in another study or after exiting another. Participants will undergo a standardized medical ophthalmic history, family history and a comprehensive standard-of-care ophthalmologic examination for their disease. Female participants of childbearing potential (see Appendix 5) will undergo a pregnancy test. Positive pregnancy test results from female minors will be shared with both the legal guardian(s) and the minor. Participants who are sexually active will be referred to the NIH gynecologist consult service to discuss appropriate contraception methods prior to completing any study procedures or dispensing investigational product. Consent will be obtained before any study procedures are performed. Screening procedures may include assessment of visual acuity, visual field, color vision, biomicroscopy and fundus examination, electroretinography (ERG), fundus imaging, and OCT (see Appendix 1). A blood sample for genetic testing of the *NR2E3* gene or of RCD-associated genes may be obtained under other NEI protocols.

4.4 Study Visits

Participants receiving IP will be provided with on-campus lodging for the first three days to complete the baseline assessments and for frequent monitoring to ensure good compliance and correct eye drop instillation technique (Days 1, 2 and 3). Scheduled clinic visits will occur at baseline, Days 1, 2 and 3, and Weeks 2, 5 and 8. A 52-week (one year) safety visit may be conducted at the NIH (on site) or with a local ophthalmologist (if the participant is unable/unwilling to come to NIH). Although there may be differences in testing among the various private physicians, at a minimum, all participants receiving IP must provide documentation of the assessments noted in Week 52 on the study flowsheet. After the participant signs a release of information with his/her ophthalmologist, the examination results will be sent to NEI. NEI will pay for the costs associated with obtaining medical records.

In addition, one of the investigators will conduct a phone interview with participants receiving IP at Weeks 1 and 6 to inquire about ocular complications. One or more unscheduled visits for safety assessment can be added based on the discretion of the investigators.

The regimen of IFN gamma-1b administration will consist of two weeks of therapy, dosing the study eye four times daily, with each dose consisting of a four-drop series, each drop spaced by 5 minutes, for a total of 16 drops delivered per day. The daily dose of IFN gamma-1b will be 112 μg topically (7 μg per drop, applied in 16 drops). Punctal occlusion will be employed to reduce systemic absorption. Investigators will train participants receiving IP on proper drop administration and monitor participants during the initial visits.

If at any time during the study participants experience ocular redness or other ocular concerns, they will be evaluated as soon as possible and the investigator may choose to terminate or reduce the dosage of the IFN gamma-1b eye drops. The dosage may be reduced to a three-drop series four times daily if symptomatic ocular surface disease and/or red eye develops.

Participants receiving IP may be restarted on the drops if the ocular condition resolves, at the investigator's and participant's discretion. Follow-up visits to manage ocular complications will be arranged, independent of and in addition to the prescribed visit schedule of the protocol.

If the participants agree, a skin biopsy and/or blood samples will be obtained, to develop fibroblast and lymphoblast cell lines that will be used to develop stem cells. Participants will be required to submit a blood sample but they will have the option to provide a skin biopsy. Participants who do not agree to a skin biopsy will *not* be excluded from the protocol.

It is anticipated that participants in this study will occasionally miss or fail to complete an assessment, procedure or study visit. These omissions will be considered expected events and not protocol deviations provided they are infrequent and do not include data needed to assess safety or the primary study outcome. Cumulative proportions of these missed events in the study population will be presented to the IRB annually. In addition, the rate of omissions will be monitored by the Investigators and biannually by the NEI SAE Review Committee. If an individual misses more than 15% of the required investigational product doses, assessments/procedures or study visits (or completes them outside the visit window), or if more

than 15% of the participants miss completion of the same assessment or procedure, it will be considered a deviation and a deviation report will be sent to the IRB within two weeks.

4.4.1 Study Evaluations

The following examinations will be performed at the study visits as indicated in the study flowsheet (Appendix 1). Examinations will be performed both as clinical care and for research purposes.

1. Medical/Ophthalmic History
2. Brief Physical Examination
3. Concomitant Medication Assessment
4. Adverse Event Assessment
5. Vital Signs (weight, blood pressure (BP), respiration, temperature)
6. Best-corrected Visual Acuity (BCVA) using EVA (Appendix 4)
7. Manifest Refraction
8. Slit Lamp Examination
9. Dilated Fundus Examination
10. Intraocular Pressure (IOP)
11. Color Vision Assessment (Cambridge Color Test)
12. Color Fundus Photography (CFP) / Fundus Autofluorescence Imaging (FAF)
13. Lens photography
14. Optical Coherence Tomography (OCT)
15. Fluorescein Angiography (FA)
16. Microperimetry
17. Electroretinography (ERG)
18. Electrooculography (EOG)
19. National Eye Institute Visual Functioning Questionnaire - 25 (VFQ – 25) (Appendix 3)
20. Serum Chemistry Panels (Acute Care and Hepatic)
21. Serum Chemistry to obtain IFN gamma levels and antibody titers
22. Complete Blood Count (CBC) with differential

23. Pregnancy Testing
24. Skin Biopsy and blood sample to derive iPSCs*
    a. A 3 mm skin biopsy may be collected. The same procedure will be used for adults and children. **Participants who do not agree to a skin biopsy will **not** be excluded.*
    b. Thirty (30) mL of blood may be collected by phlebotomy to derive iPSCs. For children, no more than the lesser of 5 mL/kg or 30 mL of blood will be collected.

4.5 Study and Concomitant Therapies

4.5.1 Product Overview

IFN gamma-1b, (Actimmune®, Horizon Pharma), a biologic response modifier, is a single chain polypeptide containing 140 amino acids. Actimmune® is a highly purified sterile solution consisting of non-covalent dimmers of two identical 16,465 dalton monomers.

Actimmune® is a sterile, clear, colorless solution. Each 50 microliters (µL) of solution contains 10 µg (200,000 IU) of IFN gamma-1b with 2 mg mannitol, 14 µg succinic acid, 37 µg of disodium succinate hexahydrate and 5 µg of polysorbate 20 in sterile water for injection. This solution has a pH of approximately 5.2 and an osmolality of 221 mmol/kg.

4.5.2 Investigational New Drug (IND) Requirement

The study will be conducted under IND 105,631.

4.5.3 Preparation and Administration

Commercially-available Actimmune®, manufactured by Horizon Pharma, will be used in this study and provided to the NIH CC Pharmacy. Vials of Actimmune® will be placed in a 2-8° C (36-46° F) refrigerator immediately upon receipt to ensure optimal retention of physical and biochemical integrity. The vials will not be frozen. Excessive or vigorous agitation will be avoided and the vials will not be shaken. The vials will be shipped from the NIH CC Pharmacy under controlled conditions to Pine Pharmaceuticals (100 Colvin Woods Parkway, Tonawanda, New York) to be re-packaged into single daily use 1 mL dropper bottles. IFN gamma-1b will be supplied to participants in the re-packaged single daily-use dropperettes to be stored in a 2-8° C (36-46° F) refrigerator. A single dropperette contains 1 mL of IFN gamma-1b (Actimmune®) at a

concentration of 200 µg/mL, which is equivalent to 28 drops. Each drop provides a dose of 7 µg of investigational product. Between daily uses, participants will store the open dropperette in the refrigerator or in a cooler with a cold pack at 2-8° C. Participants will be given cooler bags for transporting dropperettes. Once opened, each dropperette should be discarded within 24 hours. Participants will be given containers for disposal of the used dropperettes.

Participants will use a new dropperette each day as instructed from the Day 1 visit through the day prior to the Week 2 visit (see Appendix 2). Administration of topical IFN gamma-1b drops should be spaced throughout the day (approximately at breakfast, lunch, dinner and bedtime depending upon the dosage received) as instructed. Participants will be instructed to immediately discard dropperettes after each day's use in the provided disposal container which will be returned to the clinic at their next visit. Participants will be instructed on the proper administration techniques during their initial visit.

4.5.4 Drug Accountability

The NIH CC Pharmacy is responsible for the accountability of all unused investigational product. Adequate drug accountability records include documentation of all investigational products and supplies shipped, received, prepared and dispensed by the NIH CC Pharmacy. Participants will be asked to complete an eye drop diary documenting the number of drops as well as the days and times the investigational product was administered. At each applicable visit, participants will return the eye drop diary, unused investigational product and used investigational product dropperettes in the disposal container. The investigator/study staff will review the eye drop diary with the participant for completeness and accuracy and document the number of returned dropperettes (both used and unused). Missed doses of investigational product and incorrect doses below the prescribed amount will be documented in the eye drop diary but will not be reported to the IRB as protocol deviations. The study staff will dispose of returned investigational product dropperettes in the hazardous waste disposal bins.

4.5.5 Concomitant Medications and Treatments

Attempts at treating the macular cystic changes in retinal degenerations with CAIs [e.g., Acetazolamide (Diamox) or topical Dorzolamide (Trusopt)] have had variable success. The half-life of the systemic Acetazolamide is 3-9 hours and a rebound effect has been

described. If the potential participant receiving IP is using a CAI at the time of screening, s/he will be asked to continue using the medication at the same dosage during the study. The investigator should only initiate changes to the participant's CAI regimen for safety reasons. The effect of the addition of IFN gamma-1b on recalcitrant macular cystic changes will be assessed.

4.6 Follow-up/Termination Procedures

At the conclusion of the study, the participants will no longer be able to be seen under this protocol. Follow-up care will be arranged with an outside ophthalmologist or the participant will continue to be seen at the NIH under another protocol, if available and the participant is eligible. The participants and their physicians, with written consent, will be informed of the participant's disease status during this study. Clinical data obtained during participation will be shared with the participants and their private physicians (with written permission from the participant). Results from the overall study will be shared once the study team has analyzed the data from all participants.

4.7 Storage of Samples and Data

Skin biopsy and blood samples to develop fibroblast and lymphoblast cell lines that will be used to develop stem cells may be stored. Personal identifiers will be removed from samples prior to processing and all samples will be coded. After processing, specimens will be stored in freezers in secured buildings on the NIH campus. The manipulation of somatic cells may include the propagation, creation, and study of iPSCs, as well as the establishment of *in vitro* cultures. Cells may be genetically altered in the laboratory for experimental purposes and may be used for *in vivo* research. No cells, tissue or other sample components will be used for reproduction, cloning, or growth of new organisms.

Research samples may be stored for future use at the NEI as specified in the informed consent document. The participant has a choice to limit sample usage by selecting one of the following consent options:

Your data and samples may be used for other research projects, including those not related to retinal diseases, if you agree. Please initial on the line below that reflects your choice:

_____ *YES. You may use my data and samples for other research projects, including those not related to retinal diseases.*

_____ *NO. I do not want my data and samples used for other research projects. Please destroy my samples once this project is complete.*

The clinical data will be stored in the NEI's research database, the Clinical Research Information System (CRIS) and The Emmes Corporation's database. All individual data will remain confidential.

5.0 RISKS AND DISCOMFORTS

5.1 Risks and Discomforts Associated with the Clinical Assessments

The clinical visits will involve assessments that are commonly performed in the regular care of patients with RCD and ESCS.

1. Dilating drops may sting, cause an allergic reaction, precipitate an attack of angle closure glaucoma, worsen the participant's light sensitivity and distort near vision making tasks such as reading and computer work difficult. Vision typically returns to normal after two to four hours but, may be blurry up to 24 hours. Serious side effects (e.g., high blood pressure, confusion, hallucinations and seizures) are very rare and pupillary dilation is accepted as part of the standard of care for individuals with RCD and ESCS.
2. In rare instances, the cornea may be abraded during measurement of intra-ocular pressure or use of a contact lens (used for examination purpose only and not a contact lens used to correct one's refractive error). A corneal abrasion of this sort may be painful, but it heals quickly with no lasting effects.
3. The 3 mm punch skin biopsy can cause some post procedural pain and lead to limited scarring at the site of the biopsy.
4. Blood draws can cause discomfort and bleeding/bruising at the site of venous puncture. There is a remote risk of fainting or local infection. If any of these conditions arise, they will be treated.
5. The fluorescein dye used in fluorescein angiography can make a participant's skin turn yellow for several hours; also, the participant's urine will turn orange for up to 24 hours after the exam. Some participants may be slightly nauseated during the exam, but their nausea usually lasts only a few seconds. If the dye extravasates during the injection, the skin around the injection site may feel mildly uncomfortable or become yellow. In rare

cases, participants may have an allergic reaction to the dye. Participants with known allergies to the procedure are excluded from this study. Treatment typically consists of an oral antihistamine medication but may require intravenous antihistamine administration if the symptoms are severe. Very rarely will a participant experience anaphylaxis. This would be treated immediately by trained personnel with drugs or, if necessary, intubation.

6. The medical/ophthalmic history, microperimetry and physical examination entail no medical risk. No additional risks or discomforts are expected for the other clinical procedures.

5.2 Risks and Discomforts Associated with the Investigational Product

Systemic use of IFN gamma-1b is approved by the Food and Drug Administration (FDA) for use in chronic granulomatous disease and malignant osteoporosis. Limited information is available regarding the risks of the topical administration of IFN gamma-1b; however, it has previously been found to be safe and well-tolerated in humans. No significant side effects were noted at the 30 μg dose previously tested at NEI (NIH protocol 09-EI-0191). NIH Protocol 12-EI-0013 (completed in December 2013) explored the use of IFN gamma-1b in patients with CSC. Two of four participants on that protocol experienced painless ocular redness during the second week of a two-week dosing regimen at the same dosing concentration and frequency proposed in this study. The ocular redness resolved within one day upon cessation of IFN gamma-1b therapy. One participant developed cataract in the study eye almost six months after stopping the investigational product. The participant denied trauma to the eye as a possible etiology (diabetes and steroid intake were also ruled out). The participant's age (58 years old) was consistent with the possibility of early senile cataract. In fact, this participant was noted to have an early nuclear cataract bilaterally prior to enrollment. Although cataract formation was observed six months after the last study point, its causal relationship to the investigational product cannot be absolutely ruled out. However, previous studies have shown that elevation of IFN gamma is completely dissipated after a period of four weeks. It is also noteworthy that the participant who developed the cataract had received a higher total cumulative dose of the investigational product than the one proposed for the present study. Also notable is the absence of lenticular opacities in all of the other 14 study participants who had received the investigational product (some at much higher doses) and who were examined specifically for a similar adverse event. Potential

theoretical discomforts of the investigational product include transient conjunctival edema or erythema. Other theoretical side effects may include intraocular inflammation, corneal toxicity and cataract. Possible discomforts associated with punctal pressure application include eye irritation and redness. Possible risks associated with eye drop administration include eye inflammation and infection if the dropperette tip becomes contaminated. This risk is reduced by using daily dropperettes for each set of instillations.

6.0 PARTICIPANT MONITORING

Participants will be monitored for adverse events over the course of the study by the study investigators. The principal investigator (PI) will be responsible for individual safety monitoring. Monitoring will be performed through interim systemic and ocular history, ophthalmic examination and assessment of the results of study examinations. In particular, the following adverse events will be closely monitored for safety:

1. Anterior surface injection/ocular erythema
2. Ocular inflammation
3. Increase or decrease in intraocular pressure
4. Formation of cataract or lenticular opacities
5. Development of CNV in the study eye, in which case it may be considered a safety event and treatment termination considered
6. Changes in visual function, different from those expected in the natural history of RCD or ESCS - (Adverse changes in visual function such as a decrease in visual acuity of > 0.3 logMAR from baseline).
7. Serious adverse reactions where there is a reasonable possibility that the IP caused the event

6.1 Participant Withdrawal Criteria

Participants may choose to withdraw from this study for any reason at any time without penalty or prohibition from enrolling in other clinical protocols. Investigators may withdraw a participant from study participation if a new health condition becomes evident that requires care proscribed by the protocol. Investigators may also withdraw a participant if s/he experiences a serious adverse reaction.

6.2 Reporting of Pregnancy

If the staff becomes aware that a female study participant has become pregnant during the study, investigational product will be discontinued. The investigator and participant will determine whether to continue any remaining study visits or to exit the study.

If the staff becomes aware that a male study participant impregnates his partner during the study, the investigator will remind the participant of the potential risks to the unborn fetus.

In either case of reported pregnancy, participant (or partner) will be referred to the NIH OB/GYN consultation service for evaluation and counseling. The investigator must follow the participant (or partner) until the pregnancy outcome.

7.0 OUTCOME MEASURES

7.1 Primary Outcome Measures

The primary outcome measure related to the safety and tolerability of IFN gamma-1b administered topically at the prescribed dosage for macular cystic changes in participants with RCD and ESCS will be assessed by the number and severity of adverse events related to the IP and the number of withdrawals.

7.2 Secondary Outcome Measures

Evidence of efficacy will be assessed from any improvement in visual function and reduction in macular cystic changes with respect to baseline measurements and with respect to the fellow untreated eye, herein referred to as the control eye.

The following secondary outcomes of visual function will be assessed on both the study (and control eyes where applicable):

- Change in BCVA from baseline at all time points;
- Change in the maximum subretinal fluid volume as measured on OCT at all applicable time points compared to baseline;
- Change in the central retinal thickness as measured on OCT at all applicable time points compared to baseline;

- Change in central visual field sensitivity as measured by microperimetry testing at Day 2 and Week 5 compared to baseline.

For those participants who enroll on CAIs, the effect of the addition of IFN gamma-1b on recalcitrant macular cystic changes will also be assessed.

8.0 STATISTICAL ANALYSIS

This study is not designed to detect or estimate accurately the true pre-post changes in secondary outcomes related to visual function and macular cysts. Therefore, no formal statistical hypothesis tests will be performed. Analyses will be primarily descriptive and by per participant.

8.1 Analysis

All reported adverse events will be tabulated and presented by system organ class, severity and relationship to study therapy.

Secondary outcomes will be presented for each participant and tabulated over time, as described in Section 7.2. Additionally, these outcomes will compare the changes within the study eye from baseline and the changes between the study eye and control eye over time.

8.2 Sample Size

No formal sample size calculation has been performed as this is a Phase I/II pilot study to evaluate safety. Up to five eyes in each disease group will be evaluated for safety and toxicity following IFN gamma-1b administered topically.

9.0 HUMAN SUBJECTS PROTECTION

9.1 Equitability

Accrual for this study will be equitable among participants meeting the enrollment criteria. Participants of any ethnic background, gender, sexual orientation, or health status will be included according to the inclusion and exclusion criteria previously listed.

9.1.1 Justification for Exclusion of Children Below 12 Years of Age

Children below 12 years of age will be excluded from the study due to limited experience with the treatment in that group of pediatric age patients.

9.1.2 Justification for Inclusion of Children Ages 12 and Above

Children ages 12 and above are eligible for this study as the age of symptom onset for RCD and ESCS is frequently late childhood to early teenage years and there are many affected teenagers. Individuals who are affected earlier tend to have the most severe disease presentation and are the most likely to enroll in future clinical trials. It is anticipated that many children ages 12 and above will be able to comply with the extensive examinations required for this study; however, those who are unable to do so will be excluded.

9.1.3 Safeguards for Vulnerable Populations

The standard in the community is for retinal specialists and general ophthalmologists to provide care and perform procedures for children with RCD and ESCS. Dr. Brooks and Dr. Zein are fellowship-trained pediatric ophthalmologists and the clinic is designed to accommodate children. All of the investigators on this protocol have experience working with children. A pediatrician will be available for consultation if necessary.

9.1.4 Consideration of Persons Who Cannot Consent

Adults unable to understand and sign an informed consent are not excluded from this study unless they are unable to cooperate with evaluation and testing that will be done under this protocol. Experience at the NEI clinic suggests that few, if any, such participants who cannot consent will be able to comply with the extensive examinations required for this study, given that sedation will not be used. In the event that such persons are considered for enrollment, consultation to assess whether an individual has the capacity to provide consent or is able to assign a surrogate, as well as the appropriateness of a surrogate, will be obtained by contacting the NIH Ability to Consent Assessment Team (ACAT) (301-496-9675 or 301-496-2429), which is a group trained to conduct these assessments and includes members from Psychiatry, Bioethics and other disciplines.

9.1.5 Justification for Exclusion of Pregnant or Lactating Women

Pregnant women are excluded because the teratogenicity of IFN gamma-1b on a fetus is unknown. Lactating women will also be excluded as it is not known whether or not IFN gamma-1b is excreted in breast milk.

9.2 Qualifications of Investigators

The investigators who will be performing study procedures are experienced in caring for patients with RCD and ESCS. In addition, they are experienced in conducting studies similar to this protocol. Credentialed NEI staff physicians/fellows may also conduct procedures under the direction of the Investigator physicians.

The Principal Investigator has verified that all individuals working on this protocol required to take HRPP training under OHSRP SOP 25 (Training requirements for the NIH Human Research Protections Program) have completed all required training. The investigators and roles are as follows:

Wadih Zein, MD is the Principal Investigator and is responsible for the conduct and oversight of the study. He is responsible for obtaining informed consent, obtaining participant history, performing ophthalmic and physical examinations, performing skin biopsies, dispensing medication, completing regulatory documentation and IRB submissions, generating clinical source documents and overseeing data entry and data query resolution. Dr. Zein is a fellowship-trained pediatric ophthalmologist, has clinical experience and has conducted research studies as a Principal and Associate Investigator on related ocular diseases.

Catherine Cukras, MD, PhD, Emily Chew MD, Laryssa Huryn, MD, Henry Wiley, MD, Wai T. Wong, MD, PhD, Brian Brooks, MD, PhD, Robert Hufnagel, MD, PhD and H. Nida Sen, MD, MHSc are Associate Investigators with the ability to obtain informed consent. They are responsible for obtaining participant history, performing ophthalmic and physical examinations, performing skin biopsies, dispensing medication and generating clinical source documents. They have clinical experience and conducted research studies as Principal and Associate Investigators on related ocular diseases. Dr. Brooks is a fellowship-trained pediatric ophthalmologist. Dr. Sen

was the Principal Investigator on NIH protocol 09-EI-0191 and NIH protocol 11-EI-0167. Dr. Chew is the Principal Investigator on NIH protocol 12-EI-0013.

Amy Turriff, ScM is an Associate Investigator participating as an integral part in the consenting process but without the ability to obtain informed consent. She is a certified genetic counselor with NEI and is responsible for generating clinical source documents. Ms. Turiff currently serves as an Associate Investigator on related ocular research studies.

Arvydas Maminshkis, PhD is an Associate Investigator without the ability to obtain informed consent. He is responsible for providing input on the scientific question and study design, as well as study analysis. He works on the basic research used to develop the scientific question. Dr. Maminshkis was an Associate Investigator on the NIH protocol 09-EI-0191.

Sheldon Miller, PhD is an Associate Investigator without the ability to obtain informed consent. He is responsible for providing input on the scientific question and study design as well as study analysis. He is currently the Scientific Director of the NEI and oversees the basic research used to develop the scientific question. Dr. Miller was the Lead Associate Investigator on the NIH protocol 09-EI-0191.

Brett Jeffrey, PhD is an Associate Investigator without the ability to obtain informed consent. He is an ocular electrophysiologist by training with experience in clinical electrophysiology, psychophysics and implementation of clinical studies and will assist in the interpretation of eye examinations.

Kapil Bharti, PhD is an Associate Investigator without the ability to obtain informed consent. He will be responsible for the processing and storage of samples collected from participants. Dr. Bharti has expertise in experimental models of eye disease and has published widely on the biophysical, molecular, and developmental characteristics of the RPE. He also has expertise in creating iPSCs from somatic cells.

Anand Swaroop, PhD is an Associate Investigator without the ability to obtain informed consent. He will be involved in the processing and storage of certain samples collected from the participants. Dr. Swaroop has expertise in experimental models of eye disease and has published widely on the biophysical and molecular biological characteristics of the retina.

Angel Garced, BSN, MPH is an Associate Investigator and the primary research contact participating as an integral part in the consenting process but without the ability to obtain informed consent. Mr. Garced has previous experience in clinical trials as a study coordinator. He is responsible for obtaining participant history, ensuring that the study visits are scheduled per protocol, generating clinical source documents, entering data and resolving data queries.

Allison Bamji, RN is an Associate Investigator and will be the back-up study coordinator. Ms. Bamji has led several NEI protocols as the clinical trials coordinator on related ocular diseases. As a back-up study coordinator, she will be involved in the consenting process but without the ability to obtain informed consent. She is responsible for obtaining participant history, ensuring that the study visits are scheduled per protocol, generating clinical source documents, entering data and resolving data queries if Mr. Garced is unavailable.

10.0 ANTICIPATED BENEFIT

Participants receiving IP may benefit directly from this study if topical IFN gamma-1b improves visual function. This may include an improvement in visual acuity, visual field, or color vision. Any improvements are expected to persist through the study duration. It is unknown whether participants will maintain any improvements beyond the duration of the trial. The study will also lead to generalizable knowledge that will enhance the investigators' understanding of the use of IFN gamma-1b in macular cystic disease in RCD or ESCS.

11.0 CLASSIFICATION OF RISK

Risk is classified as more than minimal risk for participants receiving IP. For minor children receiving IP, risk is classified as research involving greater than minimal risk, but presenting the prospect of direct benefit to the individual subjects (45CFR 46.405). The risks of this study are reasonable given that the participants suffer significant visual disruption due to macular cystic changes associated with RCD or ESCS. For adults unable to consent, risk is classified as research involving greater than minimal risk but presenting the prospect of direct benefit to the individual subjects.

12.0 CONSENT DOCUMENTS AND PROCESS

Study investigators with consenting privileges will obtain informed consent. All study investigators obtaining informed consent have completed the NIMH HSPU "Elements of Successful Informed Consent" training. Assent will be obtained from minor participants. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study. Participants must have the ability to understand and sign an informed consent form, which must be signed prior to enrollment. Participants will have the opportunity to carefully review the consent and ask questions regarding this study prior to signing and they will be informed that they may withdraw from the study at any time without prejudice to themselves.

If a participant requires the consent to be in larger font in order to read it well, this will be provided. If a participant is visually impaired to the point of being unable to read the consent, s/he can take the consent back with them to read it over with a family member or with the use of magnifying devices. If a participant chooses, the investigator can also read the consent verbatim to the participant and answer any questions that may arise.

The investigator obtaining consent will document the consent process in the participant's medical record. A signed copy of the informed consent form will be provided to the participant to take home.

12.1 Non-English Speaking Participants

If a non-English speaking participant is unexpectedly eligible for enrollment, the participant will be provided with the CC Short Written Consent Form for Non-English Speaking Research Subjects in the participant's native language and a verbal explanation of the purpose, procedures and risks of the study. The IRB-approved English consent form will serve as basis for the verbal explanation of the study. The investigator will obtain an interpreter unless the investigator is fluent in the prospective participant's language. Preferably, the interpreter will be someone who is independent of the participant (i.e., not a family member). Attempts to locate independent interpreters will be made through the Clinical Center. The interpreters will translate the IRB-approved English consent form verbatim and facilitate discussion between the participant and investigator.

The IRB-approved English consent form will be signed by the investigator obtaining consent and a witness to the oral presentation. The CC Short Written Consent Form will be signed by the participant and a witness who observed the presentation of information. The interpreter may sign the consent document as the witness and, in this case, will note "Interpreter" under the signature line. A copy of both signed forms will be provided to the participant to take home.

The investigator obtaining consent will document the consent process in the participant's medical record, including the name of the interpreter. Further, all instances of use of the CC Short Written Consent Form will be reported to the IRB at the time of annual review. If the CC Short Written Consent Form is used three times or more for the same language within an IRB approval period, this will be reported to the IRB immediately.

Interpreters will also be present for other protocol procedures as necessary.

13.0 DATA AND SAFETY MONITORING

The NEI Serious Adverse Event (SAE) Review Committee is responsible for monitoring data and safety and will exercise oversight of the clinical investigation independently from the study investigators.

13.1 Coordinating Center

The Emmes Corporation (Emmes) has been assigned as the Coordinating Center for this trial to conduct data collection, protocol monitoring, data analysis and reporting. The Coordinating Center provides routine monitoring of study participants' data. Monitoring visits will occur on a schedule depending on the status of the study. More frequent monitoring visits will be performed at the beginning of the study when enrollment is open. Monitoring will decrease as enrollment closes and as participant follow-up continues.

Although Emmes advises the NEI Clinical Director (CD) and Principal Investigator on data and statistical activities, the Coordinating Center staff does not have direct access to or interaction with participants.

13.2 NEI Serious Adverse Event Review Committee

The NEI Serious Adverse Event Review Committee, which consists of the NEI Clinical Director and three other NEI physicians, will be responsible for reviewing any reported serious safety events, if they occur under this protocol. The Committee will review accumulating data on a semiannual basis to determine whether the study should continue. If changes to the protocol are indicated, recommendations will be made to the NEI Director and IRB who will consider and act on such recommendations in a timely manner. Should any serious suspected adverse reactions occur, the NEI Clinical Director may, at his discretion, assemble the Review Committee before the scheduled date to consider whether the study should move forward. The Review Committee or Principal Investigator can stop the study at any time if it appears there are any unexpected serious suspected adverse reactions that would outweigh any potential benefits of treatment. In addition, if three or more participants experience non-serious suspected adverse reactions that require temporary or permanent cessation of the investigational product, the Principal Investigator shall report this to the NEI Clinical Director. The NEI Clinical Director may convene the Review Committee before the scheduled time to consider the cessation of the study as a whole. The NEI Serious Adverse Event Review Committee or IRB may recommend temporarily suspending or closing enrollment, or stopping the study at any time due to safety concerns, demonstration of efficacy or lack of efficacy or slow recruitment.

13.3 Criteria for Stopping the Study

The NEI SAE Review Committee may recommend temporarily suspending or closing enrollment, or stopping the study at any time due to safety concerns. Enrollment will be temporarily suspended if a participant experiences a suspected serious adverse reaction. The NEI SAE Review Committee will review the event and determine whether enrollment may be restarted. Enrollment will be permanently suspended if two or more participants experience serious adverse reactions assessed as related to the investigational product itself. Irrespective of study status, participants who are already enrolled will be followed up for the previously delineated time period.

14.0 QUALITY ASSURANCE

The NEI and Emmes maintain quality control by adhering to standard operating procedures (NEI QA program and NEIS standard operating procedures). These procedures cover the full protocol cycle beginning with staff credentialing and training, and protocol development and approval, through database development, data collection, monitoring and analysis, and finally manuscript preparation at the conclusion of the study. Data quality assurance is of the utmost importance to the NEI and Emmes. The two groups use a quality assurance system that relies on real-time data checks and reports throughout the course of a study to ensure the accuracy of information. This system is a secure and confidential data management system that stores data and provides quality assurance and reporting. Emmes has developed a number of routine reports specifically designed for monitors (e.g., listings of serious adverse events, etc.).

Additionally, Emmes has developed summary reports of discrepancies, as well as reports of the exceptions databases, which include requests and reasons for exceptions. The results of the reports are communicated back to site staff and along with protocol compliance issues, to the DSMC (if applicable).

Following the monitoring plan for this study, Emmes will perform monitoring activities, including on-site audits, review of database entries and the resolution of study issues. In addition to monitoring, Emmes performs various detailed automated and manual data quality checks. The results from these checks and any protocol compliance issues are communicated back to site staff and to the NEI Project Officer, NEI Clinical Director and applicable regulatory bodies.

15.0 REPORTING OF UNANTICIPATED PROBLEMS, PROTOCOL DEVIATIONS AND ADVERSE EVENTS

The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems, adverse events (AEs), including serious adverse events (SAEs), and deviations in accordance with NIH policy, IRB requirements, and federal regulations. Relatedness to the research of all serious adverse events will be determined by the PI in consultation with the Clinical Director (CD).

Serious unanticipated problems, serious adverse events (including deaths) that are not unanticipated problems and serious protocol deviations will be reported to the IRB and CD as soon as possible and in writing not more than 7 days after the PI first learns of the event, unless immediate reporting is waived for specific serious adverse events as noted below. Not serious unanticipated problems and not serious deviations will be reported to the IRB and CD as soon as possible and in writing not more than 14 days after the PI first learns of the event. Written reports will be submitted in PTMS.

All adverse events, deviations, and unanticipated problems will be summarized and reported at the time of Continuing Review.

The PI will immediately report SAEs to the Sponsor according to the requirements of 21 CFR 312.64(b). The PI will record non-serious AEs and report them to the Sponsor as soon as possible.

16.0 ALTERNATIVES TO PARTICIPATION

Current therapy for patients with RCD or ESCS and macular cystic changes is treatment with a topical or systemic CAI. This study calls for recruitment of patients that have not been exposed to CAI, have been unable to use CAI due to side effects, or have not had an adequate response to CAI.

17.0 PRIVACY

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

18.0 CONFIDENTIALITY

All medical records will be kept confidential and will only be reviewed by the participating investigators. Data will be kept in password-protected computers held at the NEI and The Emmes Corporation. Only study investigators and Emmes staff will have access to the study data. The participants' names will not appear on any of the data forms reported to the coordinating center. A unique identifier, a study registration number, will identify the participant to the coordinating center. Participants' personal information will be kept as private as possible. However, records can be inspected by organizations for quality assurance and data analysis.

These include the members of the IRB and the NEI SAE Review Committee. Clinical and demographic information will be kept in the Eye Clinic Electronic Medical Record (EMR). Access will be password-protected and restricted to authorized users. In most cases, the NIH will not release any information about research involvement without written permission. However, if the participant signs a release of information form, the NIH will give the requestor information from the participant's medical record. We will try to keep personal information as private as possible. However, medical records may be inspected by various governmental and regulatory entities for auditing and other purposes. De-identified results from this clinical trial will be posted on http://www.clinicaltrials.gov.

19.0 CONFLICT OF INTEREST

The NIH guidelines on conflict of interest were distributed to all the investigators and none of the NIH investigators had any conflicts of interest.

20.0 TECHNOLOGY TRANSFER

Horizon Pharma (formerly Vidara) will provide the investigational product (IFN gamma-1b injection solution, ACTIMMUNE®) as outlined in a signed clinical trial agreement with NEI (CTA #00935-13). NEI may provide summary information and de-identified coded data to Horizon Pharma, if requested. NEI will ship collected, coded samples to Horizon Pharma or their designee for IFN gamma level testing.

21.0 RESEARCH AND TRAVEL COMPENSATION

For this study, there is no compensation for participation. This protocol will include reimbursement for travel and subsistence.

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APPENDIX 1: STUDY FLOWSHEET

Visit Schedule	Baseline	Day 1	Day 2	Day 3	Week 1	Week 2	Week 5	Week 6	Week 8	Week 52
Visit Number	**000**	**000A**	**000B**	**000C**	**001**	**002**	**005**	**006**	**008**	**SFTY[8]**
Target Day from Baseline	0	1	2	3	7	14	35	42	56	365
Visit Window					±1 day	±5 days	±7 days	±7 days	±7 days	±30 days
Investigational Product										
Interferon Gamma-1b Droperrettes Dispensed		X[1]	X[1]	X[1]						
Drug Accountability Review						X				
General Assessments										
Medical/Ophthalmic History	X									
Brief Physical Examination	X									
Concomitant Medications Assessment	X	X	X	X		X	X		X	X
Adverse Event Assessment	X	X	X	X		X	X		X	X
Vital Signs	X	X	X			X			X	
Telephone Assessment					X			X		
Ophthalmic Assessments										
BCVA (EVA)	X	X	X	X		X	X		X	X
Manifest Refraction[2]	X								X	
Slit Lamp Examination	X	X	X	X		X	X		X	X
Dilated Fundus Examination	X	X	X	X		X	X		X	X
Intraocular Pressure (IOP)	X	X	X	X		X	X		X	X
Color Vision Assessment (Cambridge Color Test)	X[3]						X			
Color Fundus Photography (CFP)	X[3]					X			X	
Fundus Autofluorescence (FAF)	X[3]					X			X	
Lens Photography	X[3]					X			X	
Optical Coherence Tomography (OCT)	X[4]	X[4]	X[4]	X[4]		X	X		X	
Fluorescein Angiogram (FA)	X[3]					X				
Microperimetry	X[3]		X				X		X	
Electroretinography (ERG)	X						X			
Electrooculography (EOG)	X		X			X	X		X	
NEI VFQ-25	X[3]					X			X	
Research Sample Collection										
Skin Biopsy for iPSCs[5]		X								
Blood Sample for iPSCs[5]		X								

Visit Schedule	Baseline	Day 1	Day 2	Day 3	Week 1	Week 2	Week 5	Week 6	Week 8	Week 52
Visit Number	**000**	**000A**	**000B**	**000C**	**001**	**002**	**005**	**006**	**008**	**SFTY[8]**
Laboratory Testing										
CBC with differential	X[3]					X			X	
Chemistry Panels (Acute Care and Hepatic)	X[3]					X			X	
Serum IFN gamma levels and antibody titers[6]	X		X			X				
Pregnancy Testing[7]	X						X		X	

1 Investigational product may be instilled by clinic staff during the first three days of IP administration and will be prescribed as necessary to maintain the dosing regimen.

2 BCVA with manifest refraction must be performed when scheduled and when there is a change in EVA of >10 E-ETDRS letters (> 0.20 logMAR) as compared with relevant baseline.

3 These procedures can be completed under NEI protocols 08-EI-0102 or 08-EI-0169 if performed within 9 days prior to Visit 000.

4 This test will be performed at least once but can be performed up to three times daily to assess for acute/subacute changes in macular cysts.

5 These procedures (skin biopsy for iPSCs and blood sample for iPSCs) may be performed at any study visit. The skin biopsy is only for participants who consent to providing skin biopsy samples for iPSC generation as this procedure is optional.

6 Collected samples will be shipped to Horizon Pharma (or their designee) for analysis.

7 This test is for women of childbearing potential only and they must have a negative test prior to the investigational product administration on enrollment day.

8 Visit records from a local ophthalmologist can substitute for the Week 52 safety visit if participant is unwilling or unable to come to NIH.

APPENDIX 2: STUDY AND CONCOMITANT MEDICATION INSTRUCTION SHEET

Participant Reminders

1. **At your Day 1 visit, we will give you your first dose of your interferon gamma-1b eye drops in your study eye in the clinic. For the first three days of treatment, we will show you how to use the drops.**
2. **Before you leave NIH, you will be given a supply of study eye drops and instructed to take the rest of your daily doses in your study eye. You will be called after you have been home one week to see how you are doing. You will continue taking your daily dose of interferon gamma-1b eye drops in your study eye until the day before your Week 2 visit.**
3. **The study eye drops will be in daily-use dropperettes. The dropperettes should be stored in your refrigerator or in a cooler with a cold pack when you are not using them. You will be given a small cooler to transport the dropperettes. Do not shake the dropperettes.**
4. **To take your study eye drops after opening your dropperette, you will use your fingers to press on the area between your study eye and your nose. This is known as "punctal pressure." Punctal pressure helps the study eye drops stay on your eye and should be used for each eye drop. After applying punctal pressure to your study eye, you will take the opened dropperette and apply one drop of interferon gamma-1b directly into your study eye without touching the surface of your eye, wait 5 minutes and then apply the next drop until the dose is complete (for a total of 4 drops). You will repeat this procedure four times a day as prescribed (around breakfast, lunch, dinner and bedtime).**
5. **To avoid an eye infection, it is very important that the dropperette tip not touch anything, including the surface of your eye. However, it is not necessary to dispose of the dropper if this happens.**
6. **Each dropperette must be thrown out within 24 hours after it is opened. We will give you a container to dispose of your empty dropperettes.**
7. **You must use a new dropperette each day. Take the study eye drops at approximately the same time every day to help you to remember to take them. Remember to place each study drop in your study eye 5 minutes apart.**
8. **Do not forget to make notes on your eye drop diary about when you took the study eye drops and ANYTHING that you feel is a side effect.**
9. **Please inform any physician who is prescribing new medication that you are using interferon gamma-1b eye drops. If a new medication is prescribed to you, please inform a study team member at your next visit.**
10. **You may use other eye drops during the study. If you need to use other eye drops, you should wait at least 5 minutes before using the interferon gamma-1b eye drops.**
11. **If you forget to take your study eye drops, you should take them when you remember and take the next dose at your regularly scheduled time. If you forget to take your study eye drops and it is more than 6 hours after the missed dose, you should skip that dose and take the next dose at your regularly scheduled time.**

12. **Be sure to always bring your eye drop diary, your disposal container with your used, empty dropperettes and any unused dropperettes with you to your next appointment.**
13. **If for any reason you miss a study visit, contact a member of the study team so that your appointment can be rescheduled.**
14. **Do not wear a contact lens in your study eye during the two weeks of using interferon gamma-1b eye drops.**

Contact your study team with any questions:

NEI Study Coordinator: ***Angel Garced, BSN, MPH***

301-594-3141- Eye Clinic - OP10

Principal Investigator: ***Wadih Zein, MD***

301-496-8118

Please note: If any questions or issues arise after normal business hours or on the weekend, you may call the page operator at 301-496-1211 and ask to speak to the Ophthalmology Fellow on call. Be sure to identify the study you are on.

YOUR STUDY IS: 15-EI-0052

"Pilot Phase I/II Study of the Evaluation of Interferon Gamma-1b Administered Topically for Macular Edema/Intraretinal Schisis Cysts in Rod-Cone Dystrophy (RCD) and Enhanced S-Cone Syndrome (ESCS)"

APPENDIX 3: NATIONAL EYE INSTITUTE VISUAL FUNCTIONING QUESTIONNAIRE-25

Instructions:

I'm going to read you some statements about problems which involve your vision or feelings that you have about your vision condition. After each question I will read you a list of possible answers. Please choose the response that best describes your situation.

Please answer all the questions as if you were wearing your glasses or contact lenses (if any).

Please take as much time as you need to answer each question. All your answers are confidential. In order for this survey to improve our knowledge about vision problems and how they affect your quality of life, your answers must be as accurate as possible. Remember, if you wear glasses or contact lenses for a particular activity, please answer all of the following questions as though you were wearing them.

PART 1 - GENERAL HEALTH AND VISION

1. In general, would you say your overall health is*:

   READ CATEGORIES: *(Circle One)*

   Excellent 1
   Very Good 2
   Good 3
   Fair 4
   Poor 5

2. At the present time, would you say your eyesight using both eyes (with glasses or contact lenses, if you wear them) is excellent, good, fair, poor, or very poor or are you completely blind?

   READ CATEGORIES: *(Circle One)*

   Excellent 1
   Good 2
   Fair 3
   Poor 4
   Very Poor 5
   Completely Blind 6

3. How much of the time do you worry about your eyesight?

READ CATEGORIES: *(Circle One)*

None of the time 1
A little of the time 2
Some of the time 3
Most of the time 4
All of the time 5

4. How much pain or discomfort have you had in and around your eyes (for example, burning, itching, or aching)? Would you say it is:

READ CATEGORIES: *(Circle One)*

None 1
Mild 2
Moderate 3
Severe 4
Very severe 5

PART 2 - DIFFICULTY WITH ACTIVITIES

The next questions are about how much difficulty, if any, you have doing certain activities wearing your glasses or contact lenses if you use them for that activity.

5. How much difficulty do you have reading ordinary print in newspapers? Would you say you have:

(READ CATEGORIES AS NEEDED) *(Circle One)*

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

6. How much difficulty do you have doing work or hobbies that require you to see well up close, such as cooking, sewing, fixing things around the house, or using hand tools? Would you say:

(READ CATEGORIES AS NEEDED) *(Circle One)*

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

7. Because of your eyesight, how much difficulty do you have finding something on a crowded shelf?

(READ CATEGORIES AS NEEDED) *(Circle One)*

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

8. How much difficulty do you have reading street signs or the names of stores?

(READ CATEGORIES AS NEEDED) *(Circle One)*

No difficulty at all 1
A little difficulty 2
Moderate difficult 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

9. Because of your eyesight, how much difficulty do you have going down steps, stairs, or curbs in dim light or at night?

(READ CATEGORIES AS NEEDED) *(Circle One)*

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

10. Because of your eyesight, how much difficulty do you have noticing objects off to the side while you are walking along?

(READ CATEGORIES AS NEEDED) *(Circle One)*

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

11. Because of your eyesight, how much difficulty do you have seeing how people react to things you say?

(READ CATEGORIES AS NEEDED) *(Circle One)*

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

12. Because of your eyesight, how much difficulty do you have picking out and matching your own clothes?

(READ CATEGORIES AS NEEDED) *(Circle One)*

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

13. Because of your eyesight, how much difficulty do you have visiting with people in their homes, at parties, or in restaurants ?

(READ CATEGORIES AS NEEDED) *(Circle One)*

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

14. Because of your eyesight, how much difficulty do you have going out to see movies, plays, or sports events?

(READ CATEGORIES AS NEEDED) *(Circle One)*

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

15. Now, I'd like to ask about driving a car. Are you currently driving, at least once in a while?

(Circle One)

Yes 1

Skip To Q 15c

No 2

15a. IF NO, ASK: Have you never driven a car or have you given up driving?

(Circle One)

Never drove 1

Skip To Part 3, Q 17

Gave up 2

15b. IF GAVE UP DRIVING: Was that mainly because of your eyesight, mainly for some other reason, or because of both your eyesight and other reasons?

(Circle One)

Mainly eyesight 1

Skip To Part 3, Q 17

Mainly other reasons 2

Skip To Part 3, Q 17

Both eyesight and other reasons 3

Skip To Part 3, Q 17

15c. IF CURRENTLY DRIVING: How much difficulty do you have driving during the daytime in familiar places? Would you say you have:

(Circle One)

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4

16. How much difficulty do you have driving at night? Would you say you have:

(READ CATEGORIES AS NEEDED) *(Circle One)*

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

16a. How much difficulty do you have driving in difficult conditions, such as in bad weather, during rush hour, on the freeway, or in city traffic? Would you say you have:

(READ CATEGORIES AS NEEDED) *(Circle One)*

No difficulty at all 1
A little difficulty 2
Moderate difficulty 3
Extreme difficulty 4
Stopped doing this because of your eyesight 5
Stopped doing this for other reasons or not interested in doing this 6

PART 3: RESPONSES TO VISION PROBLEMS

The next questions are about how things you do may be affected by your vision. For each one, I'd like you to tell me if this is true for you all, most, some, a little, or none of the time.

(Circle One On Each Line)

READ CATEGORIES:	All of the time	Most of the time	Some of the time	A little of the time	None of the time
17. Do you accomplish less than you would like because of your vision?	1	2	3	4	5
18. Are you limited in how long you can work or do other activities because of your vision?	1	2	3	4	5
19. How much does pain or discomfort in or around your eyes, for example, burning, itching or aching, keep you from doing what you'd like to be doing? Would you say:	1	2	3	4	5

For each of the following statements, please tell me if it is definitely true, mostly true, mostly false or definitely false for you or you are not sure.

(Circle One On Each Line)

READ CATEGORIES:	Definitely true	Mostly true	Not sure	Mostly false	Definitely false
20. I stay home most of the time because of my eyesight.	1	2	3	4	5
21. I feel frustrated a lot of the time because of my eyesight.	1	2	3	4	5
22. I have much less control over what I do, because of my eyesight.	1	2	3	4	5
23. Because of my eyesight, I rely too much on what other people tell me.	1	2	3	4	5
24. I need a lot of help from others because of my eyesight.	1	2	3	4	5
25. I worry about doing things that will embarrass myself or others, because of my eyesight.	1	2	3	4	5

That's the end of the interview. Thank you very much for your time and your help.

APPENDIX 4: ELECTRONIC VISUAL ACUITY (EVA) AND MANIFEST REFRACTION

1.0 REFRACTION PROTOCOL TECHNIQUE

A. Introduction

ALL refractionists should be proficient in the following optical fundamentals:

- Spherical equivalency
- Plus/minus spheres and cylinders
- Hyperopia, myopia and astigmatism
- "Push plus" refraction principles.

1.1 Refraction Chart

- Use of the refraction chart on the Electronic Visual Acuity Tester (EVA) at a distance of 3 meters is the method of choice for performing refraction (see RVACC Visual Acuity Testing Procedures Manual for EVA details). If the EVA is not working, either ETDRS chart R at 4 meters/1 meter can be used.

- For the EVA, the refraction chart on the EVA is displayed by tapping on the [Chart] icon on the Main Menu of the Palm Handheld or tapping the dropdown in upper right corner of screen and selecting 'EVA Applications'; select [Chart] icon.

- If the EVA is not functioning and the ETDRS chart R is used for refraction, the refraction protocol described beginning in Section B "Steps in Refraction" should be performed, starting at 4 meters. If the subject is unable to read at least 3 letters on both the 20/200 and 20/160 lines, the subject should be moved to 1 meter, a +0.75 diopter (D) sphere added to the spherical power in the trial frame, and the refraction performed using the appropriate lenses according to the vision level. The refraction obtained at 1 meter must be reported as a 4-meter equivalent by subtracting +0.75 (D) from the spherical power.

- Under no circumstances should the ETDRS charts be used interchangeably with the EVA during the same refraction session.

Check the room lighting level before beginning the refraction. For the EVA, dim incandescent lighting is required; fluorescent lighting should not be used. There should be no glare on the EVA screen and no spotlights. After warming up the EVA for at least 10 minutes, it should be calibrated for size and brightness (see RVACC Visual Acuity Testing Procedures Manual for EVA details).

1.2 Trial Frames/Phoropter

- Trial frames are preferred for use in refraction. If trial frames/lenses are not used, a phoropter may be used. If a phoropter is used, the final refraction MUST be put in trial frames and the final spherical refinement performed at 3 meters with the EVA, if the EVA is used, or at 4 meters with the ETDRS chart, if the EVA is not functioning.

- If a phoropter is used for a subject whose acuity is worse than 20/80, the +/-0.25 D or +/-0.50 D strength of the phoropter's mounted cross cylinder may not allow the subject to notice any change when checking for cylindrical axis and power. In this case, a separate +/-1.00 D handheld cross cylinder (as in the Protocol Summary at the end of this chapter) held in front of the phoropter instead of the mounted cross cylinder is recommended.

The protocol for the subjective refraction is described in terms of a trial frame, but a similar method can be followed with a phoropter.

The trial frame is placed and adjusted on the subject's face so that the lens cells are parallel to the anterior plane of the orbits and centered in front of the pupils. The left eye is occluded and the starting refraction is placed in the right lens cells, with the cylindrical correction anterior. The steps for the procedure are detailed below.

1.3 Contact Lens Use

If the subject wears contact lenses and has spectacle glasses as well, he/she should be instructed to refrain from wearing the contact lenses on the day of each examination. In the event that the subject either has no glasses or has forgotten the instructions and has reported for the examination wearing contact lenses, these should be removed and at least one-half hour should elapse before the refraction is performed. In this latter event, careful attention should be given to the cornea during the slit-lamp examination; any abnormalities should be noted in the subject's clinic record.

B. Steps in Refraction

1. Determine initial starting refraction
2. Refine sphere for the right eye
3. Refine cylinder axis for the right eye
4. Refine cylinder power for the right eye
5. Recheck sphere for the right eye
6. Repeat the process for the left eye

1.4 Determine Initial Starting Refraction

If subject has had study refraction at a prior visit, use the refraction results from the most recent visit.

If this is the first study refraction for the subject, use one of the following for the starting refraction:

- Retinoscopy
- Autorefractor
- Current spectacles
- Previous refraction (available in subject's chart)

In the exceptional case that none of the above is available, then start the refraction with 'plano.'

The refraction steps below are for visual acuities of 20/20 to 20/80 with the initial starting refraction. For acuities worse than 20/80, refer to the charts for appropriate sphere and cylinder powers to use. Whenever the acuity improves to a better range by improved correction (e.g., from 20/80 – 20/160 range to 20/20 – 20/80 range) smaller sphere and cylinder powers for the better acuity range according to the charts should be used.

1.5 Refine Sphere

a. Increase Plus

	Sphere for Checking	Sphere Incremental Change
20/20 - 20/80	+0.50	+0.50
<20/80 – 20/160	+1.00	+1.00
20/200 – 20/320	+2.00	+1.00
<20/320	+2.00	+1.00

The right eye is tested first and then the left eye. The starting refraction is placed in the trial frame; the left eye is occluded with an occluder lens and tissue or eye patch and the refractionist determines the lowest line that the subject can read.

With the subject focused on the smallest letters that he/she can read, a +0.50 D sphere is held in front of the trial frame over the right eye, and the subject is asked if the lens makes the vision clearer, blurrier, or keeps the vision exactly the same.

NOTE: "Clearer, Blurrier, or No change" preferred but "Better, Worse, or No change" can be used.

- If vision is clearer or there is no change, the sphere in the trial frame is replaced with a sphere that is 0.50 D more plus or less minus.

- The +0.50 D sphere is again held in front of the trial frame over the right eye and the subject is asked again if the lens makes the vision clearer, blurrier, or keeps the vision exactly the same.
- If vision is again clearer or there is no change, the sphere in the trial frame is replaced with a sphere that is 0.50 D more plus or less minus.
- This process of increasing the plus sphere or decreasing the minus sphere in the right eye is repeated until the +0.50 D sphere makes the vision blurrier.
- When the +0.50 D sphere makes the vision blurrier, no additional change in the sphere is made at this time.
- By this process the highest plus or least minus sphere for best vision is determined.

b. Increase Minus

	Sphere for Checking	Sphere Incremental Change
20/20 - 20/80	-0.50 (or -0.37)	-0.25
<20/80 – 20/160	-1.00	-0.50
20/200 – 20/320	-2.00	-1.00
<20/320	-2.00	-1.00

After determining the highest plus or least minus sphere, the subject is asked to read the smallest line possible (the reading should be at least as good as the initial reading).

The -0.50 (or –0.37) D sphere is held in front of the trial frame before the right eye and the subject is asked if the vision is improved so he can actually read more letters.

- If vision is not improved, the +0.50 D sphere is held in front of the trial frame before the right eye once again to see if the subject will accept more plus.
- If the subject reports that the –0.50 (or -0.37) D lens improves vision, the subject is requested to read the smallest line possible while the –0.50 (or –0.37) D lens is held in front of the trial frame.
- If there is an actual improvement in acuity and the examiner is convinced that the subject is able to read at least one additional letter, then the sphere in the trial frame is replaced by a sphere that is 0.25 D less plus or more minus.

Minus spherical power is added in –0.25 D increments in this fashion as long as the subject continues to read at least one additional letter.

- If the subject is unable to read any more letters, the sphere is not changed, even if the subject reports that the vision with the extra minus is better (or sharper and darker or more distinct).

The final check in the initial sphere evaluation should be the presentation of a +0.50 D sphere to determine if any more plus sphere will be accepted initially.

Example: Assume that following the check with plus sphere, the sphere in the trial frame is -0.50. The subject is asked to read the lowest line possible with this correction and reads the 20/20 line perfectly and no letters on the 20/16 line. Then -0.50 (or -0.37) D is held in front of the trial frame and the subject is asked if the lens makes the vision clearer or blurrier. If the subject reports that the vision is clearer, he is again asked to read the chart. If more letters are read (e.g., 20/20+2), then the sphere in the trial frame is changed to -0.75.

The process is repeated with a -0.50 (or -0.37) D added over –0.75. If again the subject reports that vision is improved, but he cannot read any additional letters, the sphere should remain at –0.75 and a final sphere check with a +0.50 D lens done.

1.6 Refine Cylinder Axis

For purposes of this discussion, only plus cylinder techniques are presented. Minus cylinders may be used instead of plus cylinders to determine the axis and power of the cylinder. If minus cylinders are used, the procedure described must be revised to reflect this change in sign.

If the starting refraction contains a cylinder correction, changes in cylindrical axis are tested by holding a 0.50 D cross cylinder in front of the trial frame (or appropriate cross cylinder based on level of acuity), first with the positive axis 45 degrees to one side of the cylinder axis, and then with the positive axis 45 degrees to the opposite side of the cylinder axis (in most cases, the handle of the Jackson cross cylinder lens should be aligned directly over the axis of the cylinder lens in the trial frame).

Instruct the subject to focus on an “O” or “C” one-two lines above the smallest line of letters that he can read.

Explain to the subject: *I am going to show you two views of this “C” and neither view may be clearer than the view you have right now. I would like to know which of the two views is the clearer of the two, or are both views pretty much about the same or equally blurry.* Ask: *Is the “C” clearer on view 1 [flip the lens] or view 2, or are both views about the same or equally blurred?*

- Since neither position may produce a clear image, the subject is encouraged to select the position of least blur.

If the subject cannot choose between the two positions of the cross cylinder at the beginning of this test, the axis of the cylinder is moved 5-15 degrees, first in one direction and then in the other, with the cross cylinder being checked in each position to confirm that the original axis was indeed correct.

- If the subject does prefer one position of the cross cylinder to the other, the axis of the cylinder is moved 5-15 degrees toward the positive axis of the cross cylinder when in the position the subject said was better.

When the power of the cylinder is low and/or the subject's discrimination is poor, larger shifts will produce more clear-cut responses.

The cross cylinder is tried again with the positive axis 45 degrees to one side of the new cylinder axis and then with the positive axis 45 degrees to the opposite side of the new cylinder axis; the subject is asked which position he/she prefers.

- If the subject prefers one position to the other, the axis of the plus cylinder is moved toward the positive axis of the cross cylinder.

Testing for change of axis is repeated until the subject cannot decide that one position of the cross cylinder is clearer than the other by reporting that both views are about the same or equally blurry.

1.7 Refine Cylinder Power

Change in cylinder power is now tested by adding the 0.25 D cross cylinder (or appropriate cross cylinder based on level of acuity), first with the positive axis and then with the negative axis coincident with the cylinder axis.

Again, instruct the subject to focus on an "O" or "C" one-two lines above the smallest line of letters that he can read or on the smallest line of letters he can read. Explain to the subject: *Once again I am going to show you two views of this "C" and neither view may be clearer than the view you have right now. I would like to know which of the two views is the clearer of the two, or are both views pretty much about the same or equally blurry.* Ask: *Is the "C" clearer on view 1 [flip the lens] or view 2, or are both views about the same or equally blurred?*

- If the subject prefers the positive axis coincident with cylinder axis, the power of the correcting plus cylinder is increased by an additional plus 0.25 D.

- If the subject prefers the negative axis coincident with the cylinder, the power of the cylinder is reduced by 0.25 D.

The process is repeated until the subject cannot choose one of the cross cylinder positions as better than the other (i.e., until both positions are about the same or equally blurred).

Whenever the cylinder is changed by 0.50 D, 0.25 D of sphere of opposite sign is added as well (the changing of the sphere occurs during the procedure as soon as the cylinder has been changed by 0.50 D rather than making the adjustment following the completion of the refinement).

1.8 Checking Cylinder When Beginning Refraction is a Sphere

If the beginning refraction is a sphere and does not contain a cylinder, the presence of astigmatism can be tested by one of two methods:

1. Instruct the subject to focus on a letter "C" or "O" one-two lines above the smallest line of letters that he can read. Arbitrarily place a plus cylinder (for plus cylinder refraction) appropriate for the current acuity at 90 degrees, 180 degrees, 45 degrees, and 135 degrees in the trial frame and ask the subject if the lens makes the "C" or "O" clearer. If the subject reports that the cylinder makes the "C" or "O" clearer in any of these locations, continue the refraction by modifying the cylinder axis and power as described above.

2. Arbitrarily insert a +0.25 cylinder (or power appropriate for the current acuity) into the trial frame at axis 90 degrees, 180 degrees, 45 degrees, and 135 degrees. *Using a Jackson Cross Cylinder* appropriate for the current acuity, check for cylinder power at 90 degrees, 180 degrees, 45 degrees, and 135 degrees. If the subject accepts the cylinder in any of these locations, continue the refraction by modifying the cylinder axis and power as described above.

1.9 Refraction Recheck/Final Sphere Refinement

The power of the sphere is rechecked according to the sphere refinement protocol above by using +0.37 D and -0.37 D spheres and changing the spherical power by 0.25 D increments of the appropriate sign until the subject reports that the +0.37 lens blurs the vision and the -0.37 does not improve vision. If the sphere is changed at this point by 0.50 D or more, the cylinder axis and power should be rechecked. This process is repeated until no further significant lens changes are made. In refractions using the phoropter and the EVA, a final check of the sphere as described above **must** be repeated using the EVA (at a distance of 3 meters) and trial frames.

The entire process is then repeated for the left eye.

1.10 Refraction for Subjects with Poor Visual Acuity

For subjects with acuity worse than 20/100, the strong preference is to use the EVA at 3 meters since letters can be projected as large as 20/800. The EVA chart is a three-meter chart and should not be moved closer to the subject.

When the ETDRS chart R is used, if the subject is unable to read at least 3 letters on both the 20/200 and 20/160 lines, the subject should be moved to 1 meter, a +0.75 diopter (D) sphere added to the spherical power in the trial frame, and the refraction performed using the appropriate lenses according to the vision level. The refraction obtained at 1 meter must be reported as a 4-meter equivalent by subtracting +0.75 (D) from the spherical power.

If the subjective refraction cannot be performed because the subject's visual acuity is too poor, then the subject's most recent distance subjective refraction obtained at a previous visit should be considered as the refraction

Example 1: ETDRS chart R is used for refraction which could not be performed at 4 meters in the right eye because the subject could not see any letters on the refraction chart at that distance. When the subject was moved up to 1 meter, the following was obtained: + 2.00 + 1.00 x 180 degrees.

Example 2: ETDRS chart R is used for refraction which could not be performed at 4 meters in the right eye because the subject could not see any letters on the refraction chart at that distance. When the subject was moved up to 1 meter, the following was obtained: - 1.00 + 1.00 x 180 degrees.

In order to make this finding appropriate for visual acuity testing at 4 meters, a +0.75 D sphere must be subtracted from the above correction, resulting in -1.75 +1.00 x 180 degrees for the final refraction.

C. Refraction Protocol Summary

FLOW CHART OF REFRACTION PROTOCOL

1. SPHERE	**a. Plus** (lowest line)	Clearer	Increase plus.
		No Change	Increase plus.
		Blurrier	STOP
	b. Minus (lowest line)	Clearer	Increase minus if additional letter(s) read.
		No Change	STOP
		Blurrier	STOP
	c. Plus (lowest line)	Clearer	Increase plus.
		No Change	Increase plus.
		Blurrier	STOP
2. CYLINDER	**a. Axis** above smallest line)	Clearer at 1 or 2	Move axis toward preferred plus axis until (C or O position 1 and 2 are equal.
3. CYLINDER	**a. Power** (C or O on smallest line)	Clearer at 1 or 2	Increase or decrease plus power until neither position 1 or 2 is better. If power changes by >=0.50 adjust sphere.
(Not present)		JCC at 90/180 JCC at 45/135	Place 0.25 D cylinder at preferred axis. Then, check cylinder axis and power as above.

4. REFINE SPHERE Refine with +/- spheres as in step #1 until no improvement in vision.

NOTE: "Clearer, Blurrier, No Change" preferred; "Better, Worse, No change" may also be used.

Vision with Best Correction	Sphere		Cylinder			Sphere Refinement	
	Power (1)	Increment (1)	Axis (2)	Power (3)	Increment (3)	Power (4)	Increment (4)
20/20-20/80	+.50	+0.50	a. .50 JCC	a. .25 JCC	+.25 -.25	a. +.37	+0.25
	-50 - 0.37	-.25				b. -.37	-.25
	+.50	+.50				c. +.37	+.25
<20/80-20/160	+1.00	+1.00	a. 1.00 JCC	a. 1.00 JCC	+1.00 -1.00	a. +.50	+.50
	-1.00	-0.50				b. -.50	-.50
	+1.00	+1.00				c. +.50	+.50
20/200 – 20/320	a. +2.00	+1.00	a. 1.00 JCC	a. 1.00 JCC	+1.00 -1.00	a. +1.00	+1.00
	b. -2.00	-1.00				b. -1.00	-1.00
	c. +2.00	+1.00				c. +1.00	+1.00
<20/320	a. +2.00	+1.00	No cylinder test			No refinement	
	b. -2.00	-1.00					
	c. +2.00	-1.00					

2.0 VISUAL ACUITY TESTING USING THE EVA SYSTEM

It is essential to have standardized visual acuity measurements for each examination at each of the participating clinics to minimize the effects of acuity examiner and patient bias. Visual acuity testing is being performed with the Electronic Visual Acuity Tester (EVA) using a protocol called the Electronic ETDRS (E-ETDRS) Visual Acuity Testing Protocol. This protocol has been developed to provide a visual acuity score that is comparable to that using the manual testing protocol used in the Early Treatment of Diabetic Retinopathy Study (ETDRS). The ETDRS chart testing is used as a back-up in case the EVA is not functioning.

Visual acuity measurements for each eye are obtained by a certified visual acuity examiner before the patient's pupils have been dilated.

A. Electronic Visual Acuity Tester

2.1 EVA System Description

The Electronic Visual Acuity (EVA) Tester uses an Apple iPod Touch to communicate with a Windows-based computer.

Stimuli are high-contrast, black-and-white letters with luminance of 85 to 105 cd/m2 and contrast of 98%. The system can present single letters or lines of letters. Single letters are framed with crowding bars around the letter. For lines of letters, five (5) letters are displayed for sizes smaller than 20/250; a decreasing number of letters is displayed as letter size increases. With a 21.5-inch LCD/LED monitor at 1920 x 1080 pixels, the system is capable of displaying letters from 20/800 (1.6 logMAR) to 20/6 (-0.5 logMAR) at a test distance of 3 meters. Letter size corresponds to the logMAR progression of the ETDRS charts.

The iPod, communicating with the PC wirelessly via Wi-Fi, provides instructions to the technician and displays the letter that is being shown on the monitor. For the automated tests (ATS-HOTV, E-ETDRS) the size and sequence of letter presentations is determined from a computer algorithm based on the subject's responses.

Figure 1: EVA - Model 10-WIN

Figure 2: Apple iPod Touch 4G 8GB

2.2 System Calibration

Two system calibrations are performed at regular intervals: (1) size calibration to confirm letters are accurately displayed and (2) luminance calibration to confirm the monitor screen is sufficiently bright for testing.

Size calibration must be performed at each study visit. For non-study use, size calibration is recommended at least quarterly. Luminance calibration must be performed at each study visit. Refer to the **EVA User's Manual - Model 10-WIN** for instructions specific to the EVA Model 10-WIN, monitor and light meter.

B. E-ETDRS Testing Protocol

The EVA runs a visual acuity testing program called E-ETDRS (which stands for Electronic Early Treatment of Diabetic Retinopathy). The program has been developed to provide a visual acuity letter score that is comparable to the ETDRS chart testing score.

As part of the development of the E-ETDRS protocol, a study was conducted in which high validity and test-retest reliability were demonstrated *(Moke PS, Turpin AH, Beck RW et al. A computerized method of visual acuity testing: adaptation of the amblyopia treatment study visual acuity testing protocol. Am J Ophthalmol 2001; 132:903-14).*

3.0 Overview of E-ETDRS Visual Acuity Testing Protocol

In brief, the E-ETDRS Visual Acuity Testing Protocol consists of an initial screening phase to obtain an approximation of the visual acuity threshold and then a testing phase to obtain the visual acuity score.

The protocol is summarized below. The complete algorithm is depicted in the figure that follows.

Electronic ETDRS (E-ETDRS) Visual Acuity Testing Protocol Overview

The E-ETDRS testing protocol:

- Screening phase: With single letter presentations, determines smallest logMAR level at which a letter is correctly identified.
- Testing phase: Starts testing letters by intermixing letter sizes of screening phase score and one level smaller.
- Test progress: If a letter is missed at a level, one level larger is added to the testing mix;if a letter is correct at a level, one level smaller is added to the testing mix.
- Acuity determination: Tests 5 letters at each level until smallest level with 5/5 correct and the smallest level with 0/5 correct are determined.

IN THE FOLLOWING EXAMPLE, C = CORRECT AND M = MISSED

Example:

Screening: 20/400c, 20/200c, 20/100c, 20/50c, 20/25m, 20/40m Score = 20/50

Test progress

1. Start by intermixing 20/50 and 20/40 letters: 20/50c, 20/40m, 20/50c, 20/40m, 20/50m.
2. Because a 20/50 letter was missed, add 20/63 to the letter mix (so now will have letters of 20/40, 20/50, and 20/63 intermixed): 20/63c, 20/50c, 20/40c.
3. Because 20/40 was correct, add 20/32 to the letter mix (mix is now 20/32, 20/40, 20/50, and 20/63): 20/32m, 20/63c, 20/63c, 20/50m.
4. Five letters at 20/50 have been tested, so it drops out of the mix (mix is now 20/32, 20/40, and 20/63): 20/63c, 20/40c, 20/32m, 20/40m.
5. Five letters at 20/40 have been tested, so it drops out of the mix (mix is now 20/32 and 20/63): 20/32m, 20/32m, 20/63c.
6. Five letters at 20/63 have been tested, so it drops out of the mix (mix is now 20/32 only): 20/32m.
7. Five letters at 20/32 have been tested; there are no letters left in the mix so test is over.

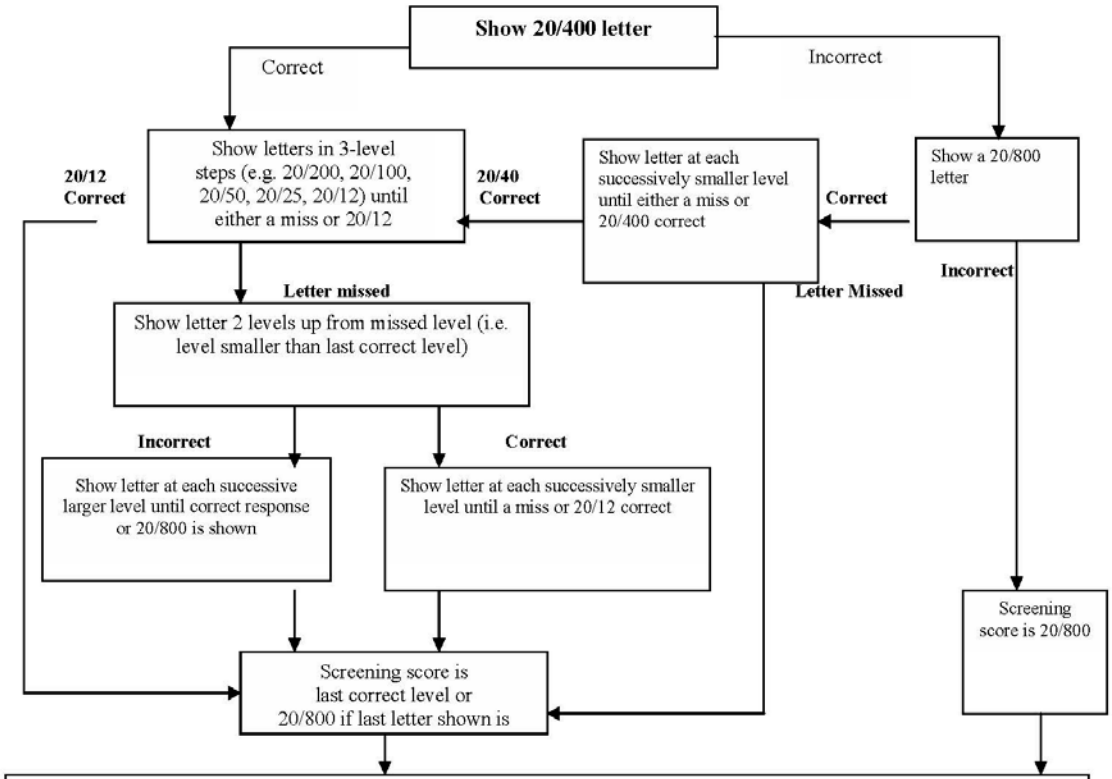
Test summary
20/63 5/5 correct
20/50 3/5 correct
20/40 2/5 correct
20/32 0/5 correct

Letter Score: 10 (number of letters correctly identified)
+ 55 (5 times the number of lines above (larger than) 20/63 and through 20/800)
= 65

Snellen Notation (smallest line with at least 3 of 5 letters correct): 20/50

Electronic ETDRS (E-ETDRS) Visual Acuity Testing Protocol Algorithm

In each step, one letter is shown at each logMAR level



Threshold Phase

1. To start, the letter pool consists of letters from 2 levels*: the level of the screening phase score and one level smaller.
2. Each letter presentation is randomly selected from the active pool of letters with the stipulation that every third letter must be from the largest level in the active letter pool.
3. A level remains in the active pool until 5 letters are tested at the level.
4. A new level is added to the active letter pool when:
   a. A letter from the largest level in the pool is missed: one level larger is added to the letter pool†
   b. A letter from the smallest level in the pool is correct: one level smaller is added to the letter pool‡
5. Testing continues until an upper logMAR level with 5 of 5 letters correct and a lower logMAR level with 0 of 5 letters correct are determined and 5 letters are tested on all levels in between the upper and lower logMAR levels.§
6. Visual acuity score is the number of letters correctly identified during threshold testing, plus 5 letters for each logMAR line above the upper logMAR level through 20/800.

ATS-HOTV© Visual Acuity Test Protocol

Single letters with surround bars ½ letter width from the letter are presented in four phases: screening, phase I (first threshold determination), reinforcement, and phase II (second threshold determination).

- In phase I and phase II, up to four single letters are sequentially presented at each logMAR level* that is tested.
- A level is considered to be 'passed' if 3 of 3 or 3 of 4 letters are correct and 'failed' if 2 letters at a level are missed.
- Testing of a level stops as soon as criteria are met for either 'pass' or 'fail'.

Screening Phase

Starting from either 20/100 or 20/400, single letters, in sequential descending logMAR sizes, are shown until one is missed.

1. Tester selects 20/100 or 20/400 size letter to present as starting point (depending on the expectation of visual acuity level based on previous testing or a pretest).
2. If response is correct, letter at next smallest logMAR level is presented and testing continues sequentially with one letter per logMAR level through 20/20 until there is an incorrect response.
3. If starting point was 20/100 and response is incorrect at either 20/100 or 20/80, screening is restarted at 20/400.
4. If starting point is 20/400 and response is incorrect at either 20/400 or 20/320, screening is restarted at 20/800.
    - If 20/800 is missed, 20/800 becomes the starting level for phase I.

Phase I

Starting 2 logMAR levels above the missed level in Screening, the smallest logMAR level at which 3 of 3 or 3 of 4 letters are correctly identified is determined.

1. Up to 4 single letters are sequentially presented 2 logMAR levels above the level missed in screening
   - Exception: if 20/20 was correct in Screening, phase I starts at 20/30.
   - Exception: if 20/800 or 20/640 was missed in Screening, phase I starts at 20/800
2. If first tested level is failed, testing continues at sequentially larger logMAR levels until a level is passed.
   - If 20/800 is failed, phase I ends, the Reinforcement phase is omitted, and 20/800 is retested in phase II
3. If first tested level is passed, testing continues at sequentially smaller logMAR levels until a level is failed.

Reinforcement Phase

In order to get the child whose attention is drifting back on track, 3 letters larger than the phase I threshold are sequentially presented.

1. Starting 3 levels larger than the level missed in phase I, 3 successively smaller single letters are presented.
   - Exception: if the level failed in phase I is 20/500 or 20/640, 3 20/800 letters are shown for reinforcement
   - Note: the reinforcement phase responses do not contribute to the visual acuity score and even if the responses are incorrect, the test proceeds to phase II.

Phase II

The last level missed in Phase I is retested and if 'passed', testing continues until a level is 'failed'

1. Up to 4 single letters are sequentially presented at the last level missed in phase I
2. If the level is failed, testing stops.
3. If the level is passed, testing continues at sequentially smaller logMAR levels until a level is failed.

Final Visual Acuity Score

The visual acuity score is the smallest logMAR level passed in Phase I or Phase II.
*logMAR levels (Snellen equivalents): 20/800, 20/640, 20/500, 20/400, 20/320, 20/250, 20/200, 20/160, 20/125, 20/100, 20/80, 20/63, 20/50, 20/40, 20/32, 20/25, 20/20, 20/16, 20/12

Testing Procedures Using the EVA

Before each patient study visit:

Calibrate monitor for letter size
Display the EVA splash screen. The outlined square in the middle of the screen is used for size calibration. Each side of the square should be 114 mm in length.

Luminance Calibration
For correct testing, the white area of the calibration square should be at least 95 cd/m2. The corresponding lux values are printed on the light meter label.

IMPORTANT!
1. Allow monitor to warm up.
a. For regular testing, the monitor should be on for at least 10 minutes.
b. For Low Contrast testing, the monitor should be on for at least 45 minutes

Before Every Test

Follow these instructions to perform visual acuity testing with the EVA Tester.

1. Insert the Model 10-WIN Flash Drive into a USB port. Power on the EVA PC.

2. Perform size and luminance calibration checks on the monitor.

3. Confirm test distance is 3 meters (118 inches) from the monitor screen to center of exam chair.

4. Confirm florescent lighting is not used in the exam room. Dim incandescent lighting is recommended.

5. Turn iPod on. The iPod screen should display test icons (E-ETDRS, ATS-HOTV, etc.). If the icons are not displayed, tap the EVA icon.

3.1 Visual Acuity Testing Procedures

a. Trial frames are to be used for refractive correction. In addition to the occluder in the trial frame, for testing the right eye, left eye is occluded with an eye patch or pad placed beneath the trial frames and vice versa.

b. If the protocol specifies that both eyes are to be tested, the right eye is always tested first.

c. Visual acuity testing is to be done without cycloplegia and without pupil dilation.

3.2 Safeguards to Avoid Bias

Masking of visual acuity testing will be achieved when feasible for a study. However, due to the automated nature of the computerized EVA testing, the potential for induction of bias on the part of the technician is minimized.

Technician instructions to the patient are to be minimal:

a. The patient should be told that there are only letters and no numbers and that each letter is "bracketed" by lines on all four sides.

b. For patients with poor central vision, it may be suggested that the patient fixate eccentrically or turn or move his/her head in any manner if this improves visual acuity. If the patient employs these maneuvers, care must be taken to ensure that the fellow eye remains covered.

c. When the patient cannot read a letter, he/she is told to guess. If the patient states that a letter is one of two letters, then he/she is asked to choose only one letter and, if necessary, to guess.

d. When the patient gives one response but then gives a second response before the first response has been finalized (i.e., before the technician has verified the response as correct or incorrect and before the letter presentation on the EVA screen changes), the patient should be asked if that is his/her final answer; if the patient equivocates, ask the patient to choose one letter. Once the technician has verified the response and the letter presentation has changed on the EVA, no changes can be made in the patient's response.

e. If the patient provides a number or any other response other than one of the 26 letters of the alphabet, the patient should be told again that there are only letters on the chart and to respond with a letter.

C. Poor Vision Testing (Testing Light Perception)

If the patient cannot identify any letters on visual acuity testing of an eye (i.e., letter score = 0), the eye is tested for counting fingers, hand motion, light perception or no light perception. Testing for light perception is performed with the indirect ophthalmoscope as the light source. The testing procedure can be performed according to the investigator's usual routine. The following procedure is suggested:

- Room lighting should remain at the level of normal visual acuity testing. The patient should close the opposite eye and occlude it by making a tight seal with the palm around the orbit and the bridge of the nose. The indirect ophthalmoscope light should be in focus at three feet, and the rheostat set at six volts. From a distance of three feet the beam should be directed in and out of the eye at least four times; the patient should be asked to respond when he/she sees the light. If the examiner is convinced that the patient perceives the light, vision should be recorded as light perception, otherwise as no light perception.

APPENDIX 5: DETERMINING CHILDBEARING POTENTIAL

A female participant who is considered non-childbearing due to a medical condition (i.e., participant has previously undergone a hysterectomy) does not need a pregnancy test, Follicle-stimulating Hormone (FSH) test or contraception.

If a female participant is considered non-childbearing due to menopause, it must be in accordance with the CNS IRB/NIH Ob-Gyn guidance on the definition of menopause. This guidance defines menopause as:

- Women over age 55 who have not had a period for one year will be considered menopausal and do not need a pregnancy test, FSH test or contraception.
- Women between 50 and 55, who have not had a period for one year, should have an FSH test. If their FSH level is ≥ 20 mIU/mL, they will be considered menopausal and do not need pregnancy testing or contraception. If their FSH level is < 20 mIU/mL, they will need pregnancy testing and contraception as required by the protocol.
- Women between 45 and 50 who have not had a period for one year will need both an FSH test and a pregnancy test. If they are not pregnant and their FSH level is ≥ 20 mIU/mL, they will be considered menopausal and will not require contraception or additional pregnancy testing. If their FSH test is < 20 mIU/mL, they will need pregnancy testing and contraception as required by the protocol.