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14. ABSTRACT <p>The National Neurovision Research Institute (NNRI), the clinical arm of the Foundation Fighting Blindness (FFB), proposes to establish the National Eye Evaluation Research (NEER) Network to be composed of a collaborative group of five Clinical Treatment and Evaluation Centers (CTECs). The intent of this new Network is to advance the science of therapeutic and preventive interventions for inherited orphan retinal degenerative diseases and dry age-related macular degeneration (AMD) through the conduct of clinical trials and other clinically relevant research. The scope of research to be carried out encompasses: (i) Phase I and Phase II clinical trials to evaluate the safety and efficacy of new therapeutic and preventive approaches, including devices, biopharmaceuticals, small molecules, nutritional supplements, and gene transfer approaches; natural history studies to develop standardized criteria to define disease stage, severity and progression; (iii) observational studies to enhance understanding of the natural history of these diseases for different genotypes and phenotypes; and (iv) evaluations of the reliability and validity of different available treatment outcomes measures to determine those that are most appropriate for various genotypes and phenotypes as well as for specific interventions. The NEER Network will also develop standard protocols for data collection, maintain and expand patient databases, classified by genotype and phenotype, to allow for the timely identification of eligible patients and facilitate patient access for clinical trial participation, and design and conduct, in collaboration with the Department of Defense, training programs for military ophthalmologists in the latest technologies and diagnostic and treatment regimens.</p>					
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Table of Contents

Introduction.....	pg. # 1
Body.....	pg. # 2 - 4
Key Research Accomplishments.....	pg. # 5
Reportable Outcomes.....	pg. # 6 - 7
Conclusion.....	pg. # 8
References.....	pg. # 8
Appendices.....	pg. # 8 - 52

Introduction:

The National Neurovision Research Institute (NNRI), the clinical arm of the Foundation Fighting Blindness (FFB), has established the National Eye Evaluation Research (NEER) Network composed of a collaborative group of five Clinical Treatment and Evaluation Centers (CTECs) and a support Clinical Coordinating Center. The intent of this new Network is to advance the science of therapeutic and preventive interventions for inherited orphan retinal degenerative diseases and dry age-related macular degeneration (AMD) through the conduct of clinical trials and other clinically relevant research. The scope of research to be carried out encompasses: (i) Phase I and Phase II clinical trials to evaluate the safety and efficacy of new therapeutic and preventive approaches, including devices, biopharmaceuticals, small molecules, nutritional supplements, and gene transfer approaches; natural history studies to develop standardized criteria to define disease stage, severity and progression; (iii) observational studies to enhance understanding of the natural history of these diseases for different genotypes and phenotypes; and (iv) evaluations of the reliability and validity of different available treatment outcomes measures to determine those that are most appropriate for various genotypes and phenotypes as well as for specific interventions. The NEER Network will also develop standard protocols for data collection, maintain and expand standardized patient databases, classified by patient genotype and phenotype, to allow for the timely identification of eligible patients and facilitate patient access for clinical trial participation, and design and conduct, in collaboration with the Department of Defense, training programs for military ophthalmologists in the latest technologies and diagnostic and treatment regimens.

The military population mirrors the civilian population, including the incidence of retinal diseases. Soldiers and their families therefore suffer from the same sight-robbing retinal degenerative diseases as the general population. In addition, the military has an expanding retiree population that will suffer from age-related macular degeneration (AMD) and any useful preventative or treatment regimen will greatly enhance these persons lives by preventing them from losing vision.

The NEER network, in cooperation with COL Donald A. Gagliano, MD, MHA, DOD Principal Advisor for Vision, Director, DOD/VA Vision Center of Excellence, and others in DOD as appropriate will actively develop a program to include military hospitals and ophthalmologists in clinical trials for Retinal Degenerative Diseases so that military personnel and their families will directly benefit from the new preventions, treatments and cures for these sight robbing diseases. In addition, the NEER network will work with the appropriate military office to develop a fellowship and senior physician training and continuing education program for military ophthalmologists to obtain specialized training at NEER network academic centers in the latest technologies, including non-invasive imaging such as multifocal electroretinogram (mfERG), optical coherence tomography (OCT), and Adaptive Optic Scanning Laser Ophthalmoscopes (AOSLO).

Body:

The National Neurovision Research Institute (NNRI), the clinical arm of the Foundation Fighting Blindness (FFB), has established the National Eye Evaluation Research (NEER) Network composed of a collaborative group of five (5) Clinical Treatment and Evaluation Centers (CTECs). The intent of the NEER Network is to advance the science of therapeutic and preventive interventions for inherited orphan retinal degenerative diseases and dry age-related macular degeneration (AMD). This will be accomplished within the NEER Network through the conduct of clinical trials and other clinically relevant studies. Pertinent background information on the FFB, the NNRI, the retinal diseases to be studied, and the rationale underlying the need for and feasibility of this new Network are delineated below.

The FFB is the world's largest source of non-governmental support for research on inherited orphan retinal degenerative diseases and dry AMD. Since its inception in 1971, the Foundation has raised more than \$370 million and, in the current fiscal year, is providing over \$14.4 million in funding for 138 research grants to more than 100 of the leading basic and clinical research experts in this area at 76 institutions around the world. To promote collaborations between basic and clinical researchers and accelerate the advancement of promising preventive and therapeutic approaches to the clinic, the Foundation also supports 19 national and international Research Centers. This Research Center Program involves inter-disciplinary groups of investigators conducting multiple research projects with an emphasis on translational research to facilitate clinical applications and the sharing of research tools, knowledge and data.

In 2003, the Foundation established the NNRI, a non-profit entity, to capitalize on the fairly recent emergence of therapeutic and preventive products and devices that require rigorous clinical evaluation for safety and efficacy. The mission of the NNRI is to accelerate the translation of promising research on treatment and prevention approaches into clinical trials.

Inherited orphan retinal degenerative diseases are a family of inherited pathologies with the ultimate consequence of photoreceptor death and severe visual impairment usually ending in blindness. In the United States, the total number of individuals affected by retinitis pigmentosa (RP) and other forms of rare inherited retinal degenerative diseases is estimated at approximately 200,000 individuals. RP, Stargardt disease, and Usher syndrome represent the predominant forms of inherited orphan retinal degenerative diseases and are estimated to affect ~80,000 – 100,000, ~25,000, and ~20,000 individuals in the U.S., respectively. Genetic heterogeneity is a key feature of each of these predominant diseases. To date, over 200 genes with mutations causing one or more forms of inherited orphan retinal degenerative diseases have been cloned, and over 50 more have been identified based on candidate gene studies or linkage mapping.

In the majority of inherited orphan retinal degenerative diseases, visual impairment is detected in the first or second decade of life. Assuming that 30% of individuals will reach legal blindness by their third decade of life, 30% by the fourth decade of life, 30%

by the fifth decade of life, while 10% never reach legal blindness, and considering just the annual cost of blindness to the U.S. government, adjusted annually for inflation at a rate of 2.5%, then the cumulative minimal lifetime costs incurred by the U.S. government for the current civilian and military populations affected by inherited orphan retinal degenerative diseases is more than \$38 billion. This tremendous economic burden will not only continue to be incurred, but will increase unless efforts are made to define the molecular, biochemical and clinical parameters of these diseases and to advance capabilities to a point where rational, safe therapeutic strategies can be designed, tested and adopted as standard care.

While repeat evaluation and study of affected patients are vital to rigorously characterize the unique features of various diseases and the factors that cause disease progression, several obstacles, in addition to the lack of research funding, often prevent the necessary frequency and thoroughness of patient examination. First, patients are often diagnosed by ophthalmologists who have limited training in the diagnosis and management of patients with rare forms of inherited orphan retinal degenerative diseases. Second, once patients are informed of the current lack of treatment options for their disease condition, they have little incentive for engaging in repeat clinical evaluations. Third, and perhaps more rare than the diseases themselves, is the number of clinicians fully trained in both the clinical and genetic aspects of inherited orphan retinal degenerative diseases. Training of additional clinical specialists in diagnostic and genetic evaluation of patients with rare forms of inherited retinal degenerative diseases has been identified as one of the most important resources needed to ensure that therapies for these diseases reach the clinic.

While inherited orphan retinal degenerative diseases account for a small portion of all vision loss, dry age-related macular degeneration accounts for approximately 90 percent of all age-related macular degeneration (AMD), affecting over 7 million individuals in the United States alone. With dry AMD, sometimes called atrophic, nonexudative, or drusenoid macular degeneration, yellow-white deposits composed of waste products from photoreceptor cells, called drusen, accumulate in the retinal pigment epithelium (RPE) tissue beneath the macula. For unknown reasons, RPE tissue can lose its ability to process waste and drusen deposits accumulate in the RPE. These deposits are thought to interfere with the function of photoreceptors and the RPE in the macula, causing progressive degeneration of these cells.

Vision loss from dry AMD occurs very gradually over the course of many years. Central vision may even remain stable between annual eye examinations, and individuals with dry AMD do not usually experience a total loss of central vision. However, vision loss may make it difficult to perform tasks that require finely focused vision (e.g., driving or reading). Although there are extensive research efforts to identify treatments for dry AMD, at this time the only proven treatment for late-stage dry AMD is the Age-Related Eye Disease Study (AREDS) antioxidant supplement regimen and stopping smoking and eating healthfully.

Through the research programs conducted with the support of the FFB and, more recently, through the NNRI, and the National Eye Institute of the National Institutes of Health (NIH), basic scientific discoveries have shown that selected nutritional factors, neuroprotective drugs, and gene therapies are safe and can prevent visual loss or

restore visual function in preclinical animal models of certain genetically defined forms of inherited orphan retinal degenerative disease and dry AMD. While AREDS antioxidant formulation is a widely accepted treatment, clinical trials of other potentially more effective treatments are imminent.

Recent progress in the classification of mutations for various inherited orphan retinal degeneration and dry AMD genotypes and the development of treatment possibilities raise the likelihood that potential treatments will be ready for evaluation in clinical trials in the near future. Unfortunately, there are considerable obstacles to the successful conduct of these clinical trials, including:

- lack of resources for the design and conduct of effective and efficient clinical trials for inherited orphan retinal degenerative diseases and dry AMD;
- the limited number and wide geographic distribution of potentially eligible patients across the U.S., making follow up examinations at one clinical center financially and logistically problematic, if not unfeasible;
- the limited number of retinal specialists with expertise in these diseases;
- the use of diverse, non-uniform approaches to measuring disease severity, stage and progression; and
- unresolved methodologic issues, such as determination of clinically meaningful, reliable and valid outcome measures.

The development of a clinical trials network will be an efficient and valuable approach to overcome these obstacles and to maximize the resources currently available. As new interventions become available for clinical evaluation, the creation of such a network will provide the infrastructure necessary to facilitate the initiation and conduct of properly designed clinical trials of investigational therapeutic and preventive approaches and devices in a timely manner. The development of a clinical trials network in inherited orphan retinal degenerations and dry AMD will require the cooperation of an interdisciplinary team with clinical, genetic, and basic science expertise. A recently established clinical trials network for cystic fibrosis provides a paradigm for a similar network for inherited orphan retinal degenerative diseases and dry AMD.

Key Research Accomplishments:

The NEER Network Steering Committee meeting will take place on December 8, 2009, at which time the committee will be introduced to the EMMES Corporation, the NEER Clinical Coordinating Center. At this meeting CTEC Principal Investigators will be introduced to draft policies and procedures and the online submission system for clinical trials that has been developed.

While finalizing the contracts with the CTECs, the NNRI has continued support of clinical efforts that will impact the NEER network going forward and laid the ground work for the CTECs to participate in ongoing clinical trials for inherited retinal degenerations. One example is the ongoing gene therapy for Leber's Congenital Amaurosis (LCA) being conducted at Children's Hospital of Philadelphia.

1. NNRI has supported the clinical trials of gene therapy for Leber's Congenital Amaurosis at the Children's Hospital of Philadelphia that just reported stunning success in restoring vision to all 12 participants. Of particular note is the 9 year old boy who was legally blind in which the gene therapy has restored functional vision to the point that he can ride his bike and play soccer unassisted. The results from this trial were just published in the Lancet (see references). These results were reported widely in the media, and below are two links to CBS' coverage, including FFB's participation.

<http://www.cbsnews.com/video/watch/?id=5420150n&tag=contentMain;contentBody>

<http://www.cbsnews.com/video/watch/?id=5422332n&tag=cbsnewsVideoArea.0>

As this trial continues, it will enroll younger individuals (as young as 3 year olds) who are expected to have more significant benefit as they will have lost less vision and their retinas therefore should be able to recover more of their vision. As CHOP is a NEER CTEC, discussions have centered around bringing this next phase of the trial into the NEER network and involving CTEC centers throughout the US.

2. NNRI has negotiated with individual investigators and some biotech companies to have access to new interventional agents to be tested in the NEER network. In addition, the NNRI is funding a gene therapy program with Oxford Biomedica to bring gene therapy for juvenile macular degeneration (Stargardt's disease) and Usher Ib syndrome (deaf-blindness due to a gene defect in a shared gene product) that will use the NEER Network for the phase II clinical trials.
3. NNRI has held multiple clinical investigator meetings to define clinical trial outcomes for orphan inherited retinal degenerative diseases, using juvenile macular degeneration (Stargardt's disease) as a model. These meetings have resulted in a position paper that will guide development of clinical protocol endpoints (i.e. – measures of success) so protocol development in NEER can proceed more quickly.

Reportable Outcomes:

The NNRI issued two Request for Proposals (RFP) to establish the National Eye Evaluation Research (NEER) Network and to solicit proposals from academic institutions and large private practice groups to serve as the NEER Network Clinical Treatment and Evaluation Unit (CTECs) for the study of inherited orphan retinal degenerative diseases and dry AMD. The overall objectives and scope of research for the NNRI RFP and for the NEER Network for which support is requested in this Full Proposal are to design and conduct studies in the following areas:

- Phase I and Phase II clinical trials to evaluate the safety and efficacy of new therapies including, but not limited to, the use of devices, biopharmaceuticals, small molecules, nutritional supplements, and gene transfer approaches;
- Natural history studies to develop standardized criteria to define disease stage, severity and progression;
- Observational studies to enhance understanding of the natural history of inherited orphan retinal degenerative diseases and dry AMD for different genotypes and phenotypes; and
- Evaluations of the reliability and validity of different treatment outcome measures available to determine those that are most appropriate for various genotypes and phenotypes as well as for specific interventions.

The NEER Network will also:

- Develop standard protocols for data collection that can be used in multiple studies of inherited orphan retinal degenerative diseases and dry AMD;
- Establish and maintain patient databases, classified by genotype and phenotype, to allow for the timely identification of eligible patients and to facilitate patient access for participation in clinical trials for specific genotypes and phenotypes; and
- Design and conduct, in collaboration with the Department of Defense, short-term training programs for military ophthalmologists in the latest technologies and diagnostic and treatment regimens for these diseases.

Based on the scores and written critiques resulting from these reviews, NNRI, with TATRC approval, issued contracts to the most highly rated five (5) institutions to establish the NEER Network and its infrastructure of CTECs:

1. University of Pennsylvania and Children's Hospital of Philadelphia
Principal Investigator: Albert Maguire, M.D.
Co-Principal Investigator: Jean Bennett, M.D., Ph.D.
2. Wilmer Eye Institute, Johns Hopkins University
Principal Investigator: Donald Zack, M.D., Ph.D.
Co-Principal Investigator: James Handa, M.D.
3. University of Medicine and Dentistry of New Jersey
Principal Investigator: Marco Zarbin, M.D., Ph.D.

4. John A. Moran Eye Center, University of Utah
Principal Investigator: Paul Bernstein, M.D., Ph.D.
5. University of California, San Diego
Principal Investigator: William Freeman, M.D.

Collectively, the CTECs represent a broad range of scientific and clinical expertise and potential therapeutic/preventive approaches for evaluation in clinical trials, as well as the facilities and other resources required to implement the scope of research of the NEER Network. This group of institutions is also capable of ensuring critical access to the number and range of patients with inherited orphan retinal degenerative diseases and dry AMD that will be necessary to implement the research agenda of the NEER Network, and to provide appropriate training for military ophthalmologist in multiple geographic areas.

The NNRI finalized all contracts with each of the five (5) CTECs necessary to establish the NEER network.

In addition, NNRI executed a contract with the EMMES Corporation, a clinical research support organization (<http://www.emmes.com>) for the NEER network and Western IRB (WIRB) to be the NNRI IRB of record for all clinical trials conducted in the NEER Network.

EMMES will provide the following administrative and statistical support services for the National Neurovision Research Institute (NNRI) National Eye Evaluation Research (NEER) Network:

- Participate in NEER Network Steering Committee meetings and provide statistical and design input on Concept Proposals for clinical trials/studies.
- Develop procedures and a web-based system for submission and review of Concept Proposals.
- Assist NNRI and the NEER Network Steering Committee in the development of a complete set of network policies.
- Conduct qualification visits for the Clinical Treatment and Evaluation Centers (CTECs) which may include GCP and GLP compliance assessments and training and certification in ETDRS Visual Acuity and Refraction.
- Provide clinical study infrastructure tools such as document templates, core data elements, reporting requirements, and study procedures.

NNRI has also contracted with Western Institutional Review Board (Western IRB; WIRB) to be the NNRI/NEER IRB of record for all clinical trials and studies.

Conclusion:

While negotiations with the individual CTEC institutions took much longer than anticipated, they are concluded and all CTECs are on board for NEER participation. In addition, the NNRI has implemented infrastructure support for the network (EMMES as the NEER Network Clinical Coordinating Center [NNCCC] and WIRB as the IRB of record for the NEER Network. Also, NNRI has continued to convene working groups of clinicians to define clinical trial parameters for inclusion/exclusion and endpoints for clinical trials in inherited retinal degenerations expected to be implemented in the NEER Network within the first year. Participants at the latest TATRC PLR review expressed unanimous support for the concept of the NEER network and I, as Principal Investigator, will be engaging the newly created military Vision Center of Excellence in NEER steering committee meetings and deliberations.

References:

1. Cideciyan AV, Aleman TS, Boye SL, et al. Human gene therapy for RPE65 isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. *Proc Natl Acad Sci U S A*. 2008;105:15112-15117.
2. Hauswirth W, Aleman TS, Kaushal S, et al. Phase I Trial of Leber Congenital Amaurosis due to RPE65 Mutations by Ocular Subretinal Injection of Adeno-Associated Virus Gene Vector: Short-Term Results. *Hum Gene Ther*. 2008.
3. Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med*. 2008;358:2240-2248.
4. Bainbridge JW, Smith AJ, Barker SS, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med*. 2008;358:2231-2239.

Appendices:

- Email to CTEC PIs on database survey
- Database Survey
- NEER Concept Proposal Instructions
- NEER Concept Proposal form
- NEER Full Application Instructions
- Lancet article on the LCA2 gene therapy results at the NEER CHOP Center

Subject: FW: NEER- IMPORTANT- REPLY NEEDED by October 26th on attached database survey

Attachments: NEER Database Survey.doc

Importance: High

From: Stephen Rose [mailto:srose@fightblindness.org]
Sent: Monday, October 12, 2009 1:05 PM
To: 'amaguire@mail.med.upenn.edu'; 'donzack@gmail.com'; Jean Bennett; Marco Zarbin; 'paul.bernstein@hsc.utah.edu'; 'rayyagari@ucsd.edu'; 'freeman@eyecenter.ucsd.edu'
Cc: Maria Figueroa; Jennifer Bacik; 'nblustein@niaid.nih.gov'; Steven Bramer; Paul Van Veldhuisen
Subject: NEER- IMPORTANT- REPLY NEEDED by October 26th on attached database survey
Importance: High

Dear CTEC PI,

As I mentioned in my September 29, 2009 email to you, NNRI has tasked the NEER Network Clinical Coordinating Center (NNCCC) with obtaining information about the existing patient databases maintained by each of the participating Clinical Treatment and Evaluation Centers (CTECs). Ascertaining this information from each of the CTECS will help to determine the feasibility of conducting natural history studies within the Network.

The NNCCC is planning on obtaining information about the CTEC's patient databases in 3 stages.

1. The first stage consists of the collection of basic information about each of the CTEC's databases and the identification of an individual or individuals at each CTEC who will serve as a contact for future questions about the databases.
2. In the second stage, the NNCCC will collate the basic information ascertained from each CTEC during stage 1 and present the results at the first Steering Committee meeting to facilitate discussion about the databases and their potential future uses.
3. Following the discussion at the Steering Committee Meeting, the NNCCC will conduct a follow-up telephone survey to attain more extensive information about the identified databases of interest. This step represents the third stage.

In order to complete Stage 1 of this process, we ask that you complete the attached survey and return it to the NEER Network Clinical Coordinating Center via email at neer@emmes.com by October 26, 2009

Thank you for your participation in this endeavor. Please contact Maria Figueroa, the Project Manager (301-251-1161 x:156; mfigueroa@emmes.com) at the NEER Network Coordinating Center if you have any questions about the survey.

Sincerely,

Steve

Stephen M. Rose, Ph.D.
Chief Research Officer
Foundation Fighting Blindness
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Sincerely,

Steve

Stage 1 of the NEER Network
Survey of Existing CTEC Patient Databases

1. Your Name:

2. CTEC: ☐ JHU ☐ UCSD ☐ UMDNJ ☐ UPenn/CHOP ☐ Utah

3. Do you maintain a database of your patients?
☐ Yes ☐ No

If you answered "yes" to question 3, please complete questions a – c.

a. Provide a brief description of the database (e.g., purpose, approximate number of patients, diseases included).

b. What fields do you routinely collect?

c. What date (month/year) was your database established?

4. Does your Center utilize an Electronic Medical Record?
☐ Yes ☐ No

5. Please provide the name, phone number and email address for one or more individuals who will serve as a contact for future questions about the data collected at your Center. This individual may be you, or it may be an individual who is involved in the design, maintenance and/or oversight of the database(s).

Name:

Phone number:

Email address:

Name:

Phone number:

Email address:

6. Any further comments?

Thank you for your time in completing this survey.



National Neurovision Research Institute

National Eye Evaluation Research (NEER) Network

**Instructions for the Preparation and Submission of
Concept Proposals for NEER Network Clinical Trials**

1. INTRODUCTION

1.1 Overview

A critical component of the research activities of the NEER Network involves the design and conduct of Phase I and Phase II clinical trials to evaluate the safety and efficacy of investigational products and approaches for the treatment and prevention of inherited orphan retinal degenerative diseases and dry age-related macular degeneration (AMD). The NEER Network uses a two-staged process for determining the most promising clinical trials to be supported.

- Stage 1 involves the submission and review of **Clinical Trial Concept Proposals** providing NNRI with a brief description of the key study features and rationale as a basis for determining those concepts approved for further development.
- Stage 2 involves the submission and review of **Full Applications** providing the detailed information necessary to evaluate fully scientific soundness, feasibility and costs, and to determine those clinical trials that will be supported. **NOTE:** Full Applications for NEER Network clinical trials will be accepted only for Concept Proposals approved for further development by NNRI.

This document provides instructions for the preparation and submission of Concept Proposals for NEER Network clinical trials. The Clinical Trial Concept Proposal form is located on the NNRI NEER Network website (www.neernetwork.org). Separate detailed instructions and forms for Clinical Trial Full Applications are also located on the NNRI NEER Network website.

1.2 Inquiries: Please address all inquiries regarding Clinical Trial Concept Proposals to the NNRI Project Officer, Stephen M. Rose, 11435 Cronhill Drive, Owings Mills, MD 21117-2220, 800.683.5555 or 410.568.0125, fax #: 410.363.4692, e-mail: srose@fightblindness.org.

1.3 Concept Proposal Submission: Please complete all sections of the Clinical Trial Concept Proposal form and submit, via e-mail and in pdf format, to the NEER Network Clinical Coordinating Center (NNCCC) at neer@emmes.com.

2. INSTRUCTIONS FOR THE PREPARATION OF CLINICAL TRIAL CONCEPT PROPOSALS

To facilitate the preparation of Clinical Trial Concept Proposals, the majority of instructions provided below are also contained on the Concept Proposal form.

Section 1: Clinical Trial Summary Information

- A. *Items 1, 2 and 3:* Provide the title of the proposed clinical trial and identify the phase and disease indication.
- B. *Items 4 and 5:* Identify the Lead Clinical Treatment and Evaluation Center (CTEC), Lead CTEC Principal Investigator (PI), and the Clinical Trial Director (if different from the Lead CTEC PI). The Clinical Trial Director is the individual responsible for the conduct of the clinical trial at the Lead CTEC institution and for the coordination and oversight of the clinical trial at all participating clinical sites.
- C. *Item 6 – Designation of Specific Types of Clinical Trials:* Designate whether the proposed clinical trial involves any of the following:
- gene therapy
 - first in humans
 - investigational products/devices with a high risk profile

The Department of Defense (DOD) requires a second level of review for clinical protocols in any of these 3 categories and, therefore, NNRI needs to be apprised if these types of clinical trials are being proposed.

- D. *Items 7 and 8:* Indicate the total targeted enrollment for the proposed clinical trial and the total number of proposed clinical sites.
- E. *Item 9 –Listing of Proposal Clinical Sites:*
- (a) List each CTEC institution proposed to participate as a clinical site.
 - (b) If applicable, provide a justification for the exclusion of any CTEC institutions as participating clinical sites.
 - (c) If applicable, list the name and location of each non-Network institution/organization proposed to participate as a clinical site.

NOTE: By listing proposed CTEC and non-Network clinical sites, the PI of the Lead CTEC institution affirms that (i) the proposed clinical trial has been discussed with the other CTEC PIs or lead investigators for non-Network institutions/organizations, and (ii) these individuals agree to participate in the proposed clinical trial contingent upon NNRI approval to move to the Full Application stage, NNRI approval of the Full Application, and local Institutional Review Board (IRB) approval.

- F. *Item 10 – Clinical Trial Duration:* Indicate the estimated duration of the proposed clinical trial defined as the time from initiation of recruitment to the last subject visit.

Section 2: Concept Proposal Summary Description

Briefly describe, in no more than 200 words, the rationale, objectives and significance of the proposed clinical trial.

Section 3: Detailed Clinical Trial Description

The detailed clinical trial description consists of the following 5 sections:

3.1 Scientific Rationale:

- (a) Briefly describe the theoretical and/or biological basis for the proposed clinical trial and its clinical significance, expected outcomes and anticipated benefits.
- (b) Include all available pre-clinical and clinical data used to support the scientific rationale. **NOTE:** Up to 5 references for supporting pre-clinical and clinical data may be provided in Section 4.
- (c) Provide a brief description of the investigational product(s)/device(s) proposed and their stage of development.

3.2 Study Objectives and Outcomes: Provide brief descriptions of the following:

- (a) the primary study objective, the primary study outcome, and the methods/measures for assessing the primary outcome; and
- (b) up to 2 secondary objectives and secondary outcomes, and the methods/measures to assess secondary outcomes.

3.3 Study Population: Describe and provide the rationale for the proposed study population, including any exclusions based on age, gender and/or disease stage.

3.4 Overall Study Design: Identify the key design features of the proposed clinical trial, including:

- (a) total sample size and sample size justification, including a brief description of the statistical methods or power considerations used to calculate total sample size;
- (b) randomization, if applicable;
- (c) level of masking, if applicable;
- (d) number and brief description of study arms/groups, if applicable; and
- (e) number and brief description of control group(s), if applicable.

3.5 Assessment of Serious Adverse Events (SAEs):

- (a) Briefly describe all expected, protocol-specific SAEs.
- (b) Identify the clinical evaluations to be used to diagnose each expected SAE and state how often these evaluations will be performed.
- (c) Briefly describe safety findings that would temporarily suspend enrollment and/or study intervention.

Section 4: Additional Concept Proposal Information

This section of the Concept Proposal consists of the following 4 components:

4.1 References: Provide up to 5 references for pre-clinical and clinical data supporting the scientific basis and rationale for the proposed clinical trial. Reprints corresponding to each citation **are required** to be included as attachments to the Concept Proposal.

4.2 Access to Study Subjects:

- (a) List all sources to be used to identify and recruit subjects by the proposed clinical sites.
- (b) Provide an estimate of the approximate number of eligible subjects for all proposed clinical sites combined.

4.3 Investigational Product/Device Information: Provide the following information for each investigational product/device:

- (a) name of manufacturer;
- (b) arrangements/agreements required to ensure provision of the investigational product/device for the proposed clinical trial;
- (c) IND/IDE status;
- (d) IND/IDE sponsor; and
- (e) any intellectual property issues, e.g., pending patents, patent infringements, that may prevent or delay clinical trial implementation.

4.4 Ethical Considerations: Briefly describe the potential risks and benefits for subjects participating in the proposed clinical trial.

National Eye Evaluation Research (NEER) Network

Clinical Trial Concept Proposal Form

Date Submitted:

Section 1: Clinical Trial Summary Information

1. Clinical Trial Title:

2. Phase:

3. Disease Indication:

4. Lead CTEC Institution:

5. a. Name of Lead CTEC Principal Investigator:
b. Clinical Trial Director Name, Title and Institution:

6. Designation of Specific Types of Clinical Trials: *(check all that apply)*

- ☐ gene therapy
☐ first in humans
☐ investigational products/devices with a high risk profile

7. Total Targeted Enrollment:

8. Total Number of Proposed Clinical Sites:

9. Listing of Proposed Clinical Sites:

A. CTEC Clinical Sites:

1. *List the name of each CTEC institution proposed to participate as a clinical site.*

2. *Provide a justification for exclusion of any CTEC institution as a participating clinical site.*

B. Non-Network Clinical Sites: *List the name and location of each proposed non-Network clinical site, if applicable*

10. Clinical Trial Duration: *Indicate the estimated duration of the proposed clinical trial from initiation of recruitment to last subject visit.*

Years

Months

Section 2: Concept Proposal Summary Description: *Briefly describe, in no more than 200 words, the rationale, objectives and significance of the proposed clinical trial.*

Section 3: Detailed Clinical Trial Description

Describe the following key features of the proposed clinical trial.

3.1 Scientific Rationale: *Provide a brief description of the theoretical and/or experimental biological basis for the proposed clinical trial and its clinical significance, expected outcomes and anticipated benefits. Include all available pre-clinical and clinical data used to support the scientific rationale. Also include a brief description of the investigational product(s)/device(s) proposed and their stage of development. NOTE: Up to 5 references for supporting pre-clinical and clinical data may be provided in Section 4. Reprints for all supporting data are **required**.*

3.2. Study Objectives and Outcomes: *Provide brief descriptions of (a) the primary study objective, the primary study outcome, and methods/measures for assessing the primary outcome; and (b) up to 2 secondary objectives and secondary outcomes, and the methods/measures to assess secondary outcomes.*

Primary Study Objective:

Primary Study Outcome:

Methods/Measures for Assessing Primary Outcome:

Secondary Study Objective #1:

Secondary Study Outcome #1:

Methods/Measures for Assessing Secondary Outcome #1:

Secondary Study Objective #2:

Secondary Study Outcome #2:

Methods/Measures for Assessing Secondary Outcome #2:

3.3 Study Population: *Describe and provide the rationale for the proposed study population, including any exclusions based on age, gender and/or disease stage.*

3.4 Overall Study Design: *Identify the key design features of the proposed clinical trial, including: (a) total sample size and sample size justification, including a brief description of the statistical methods or power considerations used to calculate total sample size; (b) randomization, if applicable; (c) level of masking if applicable; (d) number and brief description of study arms/groups, if applicable; and (e) number and brief description of control groups, if applicable.*

3.5 Assessment of Serious Adverse Events (SAEs): *Provide brief descriptions of: (a) all expected, protocol-specific SAEs; (b) the clinical evaluations to be used to diagnose each expected SAE; and (c) how often these evaluations will be performed. In addition, identify the safety findings that would temporarily suspend enrollment and/or study intervention.*

Note: A serious adverse event is defined as any adverse therapy experience occurring at any dose that meets one or more of the following criteria: 1) Death, 2) Life-threatening, 3) In-patient or prolongation of existing hospitalization, 4) Persistent or significant disability or incapacity or 5) Congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Section 4: Additional Concept Proposal Information

4.1 References: *Provide up to 5 references for pre-clinical and clinical data supporting the scientific basis and rationale for the proposed clinical trial. Reprints for all references are required.*

4.2 Access to Study Subjects: *List all sources to be used to identify and recruit subjects by the proposed clinical sites, including CTEC institutional facilities, referrals and patient registries, and provide an estimate of the approximate number of eligible subjects at all proposed clinical sites combined.*

Sources for subject identification and recruitment:

Approximate number of eligible subjects:

4.3 Investigational Product/Device Information: *For each investigational product/device, provide the following information: (a) name of manufacturer; (b) arrangements/agreements required to ensure provision of the investigational product/device for the proposed clinical trial; (c) IND/IDE status, e.g., new or amended IND/IDE required; (d) IND/IDE sponsor; and (e) identification of any intellectual property issues, e.g., pending patents, patent infringements, that may prevent or delay implementation of the proposed clinical trial.*

4.4 Ethical Considerations: *Briefly describe the potential risks and benefits for subjects participating in the proposed clinical trial.*



National Eye Evaluation Research (NEER) Network

**Instructions for the Preparation and Submission of
Full Applications for NEER Network Clinical Trials**

TABLE OF CONTENTS

1. INTRODUCTION	3
1.1 Overview	3
1.2 Assistance Available	3
1.3 Instructions for Full Application Submission	4
1.4 Full Application Review Criteria	4
2. INSTRUCTIONS FOR PREPARATION OF CLINICAL TRIAL FULL APPLICATIONS	6
2.1 Module A: Detailed Clinical Trial Description	7
2.1.1 Clinical Trial Lay Description	7
2.1.2 Background and Scientific Rationale	7
2.1.3 Supporting Pre-Clinical and Clinical Data	7
2.1.4 Study Objectives and Outcomes	8
2.1.5 Study Population	8
2.1.6 Overall Study Design	8
2.1.7 Study Schedule	9
2.1.8 Statistical Considerations	9
2.1.9 Assessment of Adverse Events	9
2.1.10 Product/Device Information	10
2.1.11 Proposed Clinical Sites	10
2.1.12 Clinical Trial Timeline	12
2.2 Module B: Ethical Considerations	12

2.3 Module C: Clinical Facilities and Equipment	13
2.4 Module D: Budget and Justification	14
2.5 Cover Page: Summary Information, Total Budget, and Signatures	15

1. INTRODUCTION

1.1 Overview

A critical component of the research activities of the NEER Network involves the design and conduct of Phase I and Phase II clinical trials to evaluate the safety and efficacy of investigational products and approaches for the treatment and prevention of inherited orphan retinal degenerative diseases and dry age-related macular degeneration (AMD). The NEER Network uses a two-staged process for determining the most promising clinical trials to be supported.

- Stage 1 involves the submission and review of **Clinical Trial Concept Proposals** providing NNRI and the NEER Network Steering Committee with a brief summary of the key study features and rationale as a basis for determining those concepts approved for further development.
- Stage 2 involves the submission and review of **Clinical Trial Full Applications** providing the detailed information necessary to evaluate fully scientific soundness, feasibility and costs, and to determine those clinical trials that will be supported.

This document provides detailed instructions for the preparation of Full Applications for NEER Network clinical trials and pertains only to clinical trials for which initial Concept Proposals have been approved for further development. The Clinical Trial Concept Proposal form and separate instructions for the preparation and submission of Concept Proposals are located on the NEER Network website (www.neernetwork.org).

1.2 Assistance Available

In order to assist the NEER Network Clinical Treatment and Evaluation Centers (CTECs) in preparing Clinical Trial Full Applications, consultation is available in various areas.

- Consultation on statistical design issues is available from The EMMES Corporation, the NEER Network Clinical Coordinating Center (NNCCC). Please contact the following individual:

Jennifer Bacik, MS
Statistician and Co-PI, NNCCC
Phone: 301-251-1161 ext: 2829
Fax: 301-251-1355
Email: jbacik@emmes.com

- For questions regarding investigational products/approaches and budget issues, please contact the NNRI Project Officer:

Stephen M. Rose, Ph.D.
Phone: 800.683.5555 or 410.568.0125
Fax: 410.363.4692
Email: srose@fightblindness.org

- For technical questions on the use of the electronic Full Application submission system (see section 1.3, below), please contact the following individual at the EMMES Corporation:

Jodi DeStefano, Clinical Systems Analyst
 Phone: 301-251-1161 ext: 141
 Fax: 301-251-1355
 Email: jdestefano@emmes.com

- For technical questions regarding the completion of the budget templates (see section 2.5, below), please contact the following individual at the EMMES Corporation:

Jennifer Bacik, MS
 Statistician and Co-PI, NNCCC
 Phone: 301-251-1161 ext: 2829
 Fax: 301-251-1355
 Email: jbacik@emmes.com

1.3 Instructions for Submission of Clinical Trial Full Applications

NNRI and the NEER Network Clinical Coordinating Center have developed an electronic system for the preparation and submission of Clinical Trial Full Applications. Instructions on the use of this electronic system are provided in the User's Guide for the NEER Network Clinical Trial Full Application Submission System located on the NEER Network website (www.neernetwork.org). Clinical Trial Full Applications may be submitted only by the Principal Investigators (PIs) of the CTEC institutions funded to participate in the NEER Network. However, CTEC PIs may designate staff with access to the electronic system for the purposes of entering the required Full Application information.

1.4 Clinical Trial Full Application Review Criteria:

The following criteria will be used in evaluating Clinical Trial Full Applications:

1. Scientific Basis and Rationale:

- To what extent is the proposed clinical trial scientifically sound and based on well-established scientific principles?
- To what extent is there convincing clinical and/or pre-clinical evidence that the clinical trial will have positive results?
- To what extent is the technology/understanding sufficiently advanced to warrant detailed clinical investigation at this time?
- To what extent do previous studies demonstrate promising results regarding safety and potential efficacy? Are there more effective methods of addressing the questions/hypotheses proposed?

2. Clinical Implications:

- How will the proposed clinical trial have a significant impact on disease outcome?

- How will the clinical trial offer insight for subsequent clinical development of the investigational product(s)/approach(es) selected and for subsequent clinical development of related strategies?
- If successful, to what extent will the strategies proposed have potential for extension to other diseases/conditions (i.e.- inherited orphan retinal degenerations and dry AMD) within the purview of the NEER Network?

3. Study Design:

- To what extent is the overall study design scientifically sound and appropriate?
- To what extent have appropriate statistical considerations been included?
- To what extent are the primary and secondary study objectives and corresponding outcome measures appropriate and well-defined?
- To what extent does the clinical trial target appropriate patient populations and provide well-defined and justified subject inclusion and exclusion criteria?
- To what extent is the sample size well justified and the study adequately powered to assess outcome measures?
- To what extent are the criteria to stop the clinical trial appropriate and well justified?

4. Feasibility:

- Will a sufficient number of subjects be available to accomplish the proposed clinical trial and to what extent can enrollment numbers be achieved?
- Are there any serious or potentially serious barriers that could prevent the successful completion of the proposed clinical trial?
- To what extent is the clinical trial timeline realistic and achievable?

5. Investigators and Clinical Sites:

- To what extent do the investigators have a track record of achievement in the design and conduct of clinical trials for the disease(s) and study population(s) proposed in the clinical trial?
- To what extent do the proposed clinical sites have the required facilities, equipment and other resources necessary to conduct the proposed clinical trial? Can/should additional sites and/or expertise be recruited to fill any gaps?

6. Human Subjects:

- To what extent are the procedures for obtaining and documenting informed consent adequate and appropriate?
- To what extent are the potential short- and long-term risks and benefits to human subjects fully described?
- To what extent are the potential short- and long-term risks reasonable in relation to the anticipated benefits to human subjects?
- To what extent are the procedures, and their likely effectiveness, for protecting against or minimizing potential risks well defined and reasonable?

7. Proposed Budget: To what extent is the proposed budget reasonable and appropriate based on the size, level of complexity and duration of the clinical trial?

2. INSTRUCTIONS FOR CLINICAL TRIAL FULL APPLICATION PREPARATION

The NEER Network Clinical Trial Full Application requires the submission of several components as listed below.

- Module A: Detailed Description of Proposed Clinical Trial
- Module B: Ethical Considerations
- Module C: Clinical Facilities and Equipment
- Module D: Budget and Justification
- Cover Page: Summary Information, Total Costs, and Signatures

Information in Modules A-C will be collected via the electronic submission system. Each participating site will submit a detailed budget to the NEER Network Clinical Coordinating Center (NNCCC). The NNCCC will collate the budget information and forward to NNRI for review (see Section 2.4 for further description of the budget process.) The signed cover pages, as described in Section 2.5, will be submitted directly to NNRI.

Upon the submission of the Full Application in the system, the NNCCC will review the submitted materials for missing content. The CTEC PI will be informed of any missing material and will be asked to provide the missing content. When the Full Application is considered complete, the NNCCC will assemble the submitted materials into a report and provide the report to NNRI for review and distribution to the NEER Network Steering Committee.

2.1 MODULE A: DETAILED CLINICAL TRIAL DESCRIPTION

The detailed description of the proposed clinical trial consists of multiple sections as outlined below. Each section represents a data collection form within the electronic submission system. Instructions for the necessary content to be supplied on each form are provided within each section. Please note that any supporting documentation, including figures and charts, for any of the sections of Module A may be provided as attachments within the appropriate form in the system. Documents that are required to be attached are noted as such in the appropriate section.

2.1.1 Clinical Trial Lay Description: Provide a brief description of the proposed clinical trial, not to exceed 200 words, written for a lay audience.

2.1.2 Background and Scientific Rationale: This section provides the background information necessary to understand the scientific rationale behind the proposed clinical trial. Include the following:

1. A description of the nature of the disease(s) of focus and the current state of the art in the treatment/prevention of the designated disease(s).
2. A description of the theoretical and/or experimental biological bases for the proposed clinical trial and how the proposed clinical trial relates specifically to the disease(s) of focus.
3. A discussion of the clinical significance, expected outcomes and anticipated benefits of the proposed clinical trial for improving the treatment or prevention of the disease(s) and the study population(s) selected.

2.1.3 Supporting Pre-Clinical and Clinical Data: The purpose of this section is to describe the existing data that support pursuit of the proposed clinical trial at this time. Include the following:

1. A discussion of all available pre-clinical data supporting the proposed clinical trial and the specific investigational product(s)/approach(es) selected, including any known risks and benefits.
2. A discussion of any and all clinical data available relating to the proposed clinical trial and the specific investigational product(s)/approach(es) selected in the disease(s) of focus, including any known risks and benefits.
3. A description of relevant clinical experience, positive or negative, from other disease areas for the investigational product(s)/approach(es) proposed.
4. An explanation as to why all available data/experience justify the entry of the proposed investigational product(s)/approach(es) into clinical investigation at this time and for the disease(s) and study population(s) of focus.
5. Indicate the number of references to be submitted in support of the proposed clinical trial and provide the citation. Up to 10 references may be submitted. Reprints corresponding to each citation **are required** to be included as attachments to the "Supporting Pre-Clinical and Clinical Data" form within the electronic submission system.

2.1.4 Study Objectives and Outcomes:

1. Primary Objective and Outcome: Describe the primary objective and the primary outcome of the proposed clinical trial, describe all methods or measures to be used to assess the primary outcome, and provide evidence that the methods or measures selected are clinically relevant, valid and reliable.
2. Secondary Objectives and Outcomes:
 - a. Indicate the number of proposed secondary objectives (up to a total of 10).
 - b. Describe each secondary objective and associated outcome and the methods or measures to be used to assess each secondary outcome, and provide evidence that the methods or measures selected are clinically relevant, valid and reliable.

2.1.5 Study Population:

This section of the Full Application provides detailed information on the proposed study population, including:

1. age, gender, and race/ethnicity, and the rationale for any exclusions based on these characteristics;
2. disease indication and disease stage;
3. general health status;
4. overall justification for the study population selected;
5. description of subject inclusion criteria (up to a total of 20); and
6. description of subject exclusion criteria (up to a total of 20).

2.1.6 Overall Study Design:

1. Describe and provide a justification for the study design.
2. Describe each proposed study arm/group (up to a total of 5), including sample size per arm/group.

3. Indicate whether randomization is proposed, and if not, describe methods of assigning subjects to study arms/groups, if applicable.
4. Identify the level of masking.
5. Identify the control group(s) and explain/justify the choice of control group(s).
6. If applicable, indicate whether the proposed clinical trial involves (i) gene therapy, (ii) first-in-humans, and/or (iii) investigational products/approaches with a high risk profile.

2.1.7 Study Schedule:

The Study Schedule section provides information on the (i) time frame/duration, (ii) required evaluations/procedures and their validity, reliability and specificity, and (iii) sequence of events that should occur for the following stages of the proposed clinical trial:

1. eligibility screening
2. enrollment/baseline
3. treatment period
4. post-treatment follow-up period
5. final study visit

In addition, this section includes a description of the primary assessments necessary in the event of early termination.

A Schedule of Events **is required** to be provided as an attachment to the Study Schedule form within the electronic submission system.

2.1.8 Statistical Considerations:

1. Identify and provide a justification for the total sample size. Indicate the outcome upon which the sample size is based, any assumptions made and any statistical methods or corresponding power considerations used to calculate sample size.
2. Provide an overview of the primary features of the planned final statistical analysis for safety and efficacy. At a minimum, the plan should address: (1) the primary safety outcome measure to be studied; (2) the primary efficacy outcome measure to be studied; and (3) the type of analyses to be performed for the primary outcome measures. For example, analyses may take the form of estimation of a parameter with 95% confidence intervals for single-arm studies or comparative analyses for multi-arm studies.
3. Indicate whether any interim statistical analyses are planned and provide an overview of the primary features of the analyses, including the reason for their inclusion (e.g., early stopping for lack of efficacy, safety considerations), the outcome measure(s) upon which they will be based, and their timing.

2.1.9 Assessment of Adverse Events:

1. Describe each expected, protocol-specific serious adverse event.
2. Describe all non-serious adverse events that are eye-related.
3. Describe other relevant non-serious adverse events with an incidence of >5%.
4. Indicate the clinical evaluations that will be used to diagnose each event and how often such evaluations will be performed.
5. Describe how decisions will be made to determine the relationship of each event to treatment.

6. Describe how each event will be managed until resolved or considered stable.
7. Describe safety findings that would temporarily suspend enrollment and/or study intervention.

2.1.10 Product/Device Information

1. Provide the following information for each product and/or device proposed for use in the clinical trial:
 - a. whether the product/device is legally marketed, and if so, the approved indication(s); and
 - b. generic name, brief description, and manufacturer.
2. Briefly describe the arrangements/agreements required to ensure the provision of each product/device to be used in the proposed clinical trial.
3. For clinical trials to be conducted under INDs and/or IDEs, indicate whether a new or an amended IND/IDE Application will be required, and if so, the current IND/IDE status, the IND/IDE sponsor, if known, and the indications for which new or amended INDs/IDEs will be necessary.

2.1.11 Proposed Network and Non-Network Clinical Sites

This section of the Full Application (i) identifies all proposed clinical sites, (ii) names those investigators with responsibility for study conduct and management/oversight, and (iii) provides information on access to eligible subjects and subject recruitment and retention strategies. **For multi-site clinical trials, please note the following with respect to (i) the Lead CTEC institution, (ii) other CTEC institutions proposed as clinical sites, and (iii) the conditions under which Non-Network institutions may be proposed as clinical sites:**

- The Lead CTEC institution must provide a **Clinical Trial Director** with responsibility for the conduct of the clinical trial at the Lead CTEC institution and for the coordination and oversight of the clinical trial at all participating clinical sites.
- Each proposed clinical site must provide a **Lead Clinical Investigator** responsible for the conduct and management of the clinical trial at the site.
- **Non-Network Clinical Sites:** Qualified institutions outside of those funded as NEER Network CTECs may be proposed for participation in multi-site clinical trials in order to ensure access to adequate numbers of subjects. CTEC PIs proposing to include non-Network institutions are required to discuss their proposals with and receive concurrence from the NNRI Project Officer prior to Full Application submission. NNRI concurrence constitutes agreement to allow for non-Network clinical sites to be proposed in Full Applications. Such concurrence does not in any way constitute approval for the participation of non-Network clinical sites in any NEER Network clinical trial. In addition, this section of the Full Application requires specific additional information on the qualifications of all proposed non-Network clinical sites.
- By including clinical sites from CTEC institutions and/or non-Network institutions, the Lead CTEC PI affirms that (i) the design and requirements of the proposed clinical trial have been discussed with the key investigators at all such sites, and (ii) all such sites agree to participate in the clinical trial if approved by NNRI, contingent upon local Institutional Review Board (IRB) approval.

1. Identify the role of each of the 5 NEER Network CTECs in the proposed clinical trial, i.e., Lead CTEC, Other Participating CTEC or Not Participating.
2. For the Lead CTEC, indicate whether the CTEC PI will serve as the Clinical Trial Director, and if not, identify the individual who will serve as the Clinical Trial Director. For each of the Other Participating CTECs, identify the Lead Clinical Investigator. For CTECs identified as Not Participating, indicate the reason for non-participation.
3. For the Lead CTEC and for each Other Participating CTEC, provide the following information:
 - a. approximate number of eligible subjects;
 - b. target enrollment;
 - c. sources for the identification and recruitment of subjects;
 - d. geographic location of potential subjects;
 - e. strategies for subject recruitment and retention;
 - f. anticipated drop-out rate; and
 - g. a description of any inducements, financial or otherwise, the clinical trial offers to potential subjects and, if applicable, the terms and conditions of any such arrangements.
4. If non-Network clinical sites are proposed, provide the following information:
 - a. name of non-Network clinical site and Lead Clinical Investigator;
 - b. all information on eligible subjects and recruitment/retention specified in 3.a. through g. above;
 - c. descriptions of (i) the overall size and organization of the site and the staff, facilities and other resources dedicated to the diagnosis, treatment and management of inherited orphan retinal degenerative diseases and dry AMD, (ii) organizational experience with the study population of focus for the clinical trial, (iii) the geographic area served and the incidence of the disease of focus within that area, and (iv) the expertise and experience of the clinical site in the design and conduct of similar clinical trials, including a list of relevant ongoing clinical trials and clinical trial completed over the past 3 years indicating: (i) title and phase, (ii) status (ongoing or completed), (iii) study population, (iv) investigational produce/device, (v) total sample size, target enrollment for the clinical site, (vi) sponsor, and (vii) study results (if publicly available).

Curricula Vitae, limited to 3 pages and focused on qualifications, expertise and experience relevant to the proposed clinical trial, **are required** to be provided as attachments to the "Proposed Network Clinical Sites" and "Proposed Non-Network Clinical Sites" forms within the electronic submission system for the proposed Clinical Trial Director and, for multi-site clinical trials, for the proposed Lead Clinical Investigator for each clinical site. For Full Applications where the Clinical Trial Director and/or the Lead Clinical Investigators are CTEC PIs or Co-PIs, CVs do not need to be provided.

2.1.12 Clinical Trial Timeline: Provide:

1. the estimated duration of the clinical trial from initiation of recruitment to the last subject visit.
2. the total time period required to conduct the clinical trial for each individual subject.
3. the duration of time required for each stage of the clinical trial, i.e., completion of final draft protocol for submission to NNRI, subject screening and completion of enrollment, treatment phase, and follow-up phase.

2.2 MODULE B: ETHICAL CONSIDERATIONS

The Ethical Considerations section of the Full Application and the corresponding form in the electronic submission system focus on the ethical and human research subject protection issues relating to the proposed clinical trial. In this section, address the following issues:

1. *Informed Consent*: Briefly describe the consent procedures to be followed and comment on the following items, where applicable:
 - a. Who will seek consent, the nature of the information to be provided to prospective subjects, and the method of documenting consent.
 - b. If the proposed clinical trial involves pediatric subjects, describe the process for obtaining both parental/guardian consent and pediatric assent.
 - c. If proposing the retrospective use of specimens and diagnostic and other tests/evaluations, describe if and how informed consent will be obtained.
 - d. If proposing genetic testing, describe how informed consent will be obtained.

NOTE: The NEER Network will use the definition of children provided for in the Code of Federal Regulations, 45 CFR 46, Subpart D – Additional Protections for Children Involved in Research (<http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm>), i.e., “persons who have not attained the legal age for consent to treatments or procedures involved in research, under the applicable law of the jurisdiction in which the research will be conducted.” For multi-site clinical trials conducted in states with different definitions of pediatric subjects, assent may be required in some cases and not others. In such instances, local Institutional Review Boards (IRBs) will determine the consent/assent documents required.

2. *Risks and Benefits*: Provide a description of the potential risks and benefits for subjects participating in the proposed clinical trial and describe procedures, and their likely effectiveness, for protecting against or minimizing potential risks.

2.3 MODULE C: CLINICAL FACILITIES AND EQUIPMENT

In order to prepare Module C for proposed multi-site clinical trials, it will be necessary for the Lead CTEC PI to obtain information from each clinical site regarding the specific facilities and equipment to be made available on site. To facilitate the collection of this information, the NEER Network Clinical Coordinating Center has prepared templates to be used by CTEC PIs. These templates are provided for on the NEER Network website: www.neernetwork.org.

2.3.1 Clinical Facilities:

1. Identify and describe each clinical facility required for the conduct of the proposed clinical trial and where each facility is located. The focus should be on facilities beyond what is typically found in ophthalmic suites. For example, an exam room to assess ETDRS best-corrected visual acuity does not need to be included. Include facilities where specialized testing for protocol-specific procedures will be performed. This may include facilities for: (i) subject screening and determination of eligibility for enrollment (ii) subject enrollment, (iii) receipt, distribution, and/or destruction of investigational product (i.e., research pharmacy), (iv) administration of investigational product/device, (v) performance of protocol-specific clinical evaluations and safety monitoring, (vi)

- subject follow up, and (vii) final subject evaluations. Centralized facilities to be used by all participating sites, such as a Centralized Reading Center, should also be included.
2. For each clinical facility, indicate whether this is a centralized facility to be used by all clinical sites.
 3. For each non-centralized clinical facility, indicate whether the facility is available on site at each of the proposed clinical sites, and if not, describe the arrangements necessary to provide patient access to that facility.

2.3.2 Equipment:

1. Identify and describe each piece of equipment required for the conduct of the proposed clinical trial. Include equipment for: (i) subject screening and determination of eligibility for enrollment, (ii) subject enrollment, (iii) administration of investigational product/device, (iv) performance of protocol-specific clinical evaluations and safety monitoring, (v) subject follow up, and (vi) final subject evaluations. Equipment used for both standard and specialized testing should be included.
2. For each piece of equipment, indicate whether the equipment is available on site at each proposed clinical site.
3. For equipment not available at any clinical site, describe the arrangements necessary to provide patient access to the equipment.
4. Describe the specifications (e.g., make, model) for each piece of equipment identified.

2.3.3 Note on Other Resources:

NNRI, through the NEER Network, will provide the data management, statistical and DSMB support for all Network studies.

2.4 MODULE D: BUDGET AND JUSTIFICATION

All Clinical Trial Full Applications require detailed budgets. Each site participating in the clinical trial is required to submit a budget. The budget template will be provided to the Lead CTEC PI/Clinical Trial Director by the NEER Network Clinical Coordinating Center (NNCCC). The process is as follows.

1. NNRI will inform the NNCCC when the Concept Proposal has been approved.
2. The NNCCC will contact the Lead CTEC PI/Clinical Trial Director to gather details regarding the clinical trial and request a Schedule of Events table.
3. Based on the Schedule of Events table, the NNCCC will tailor the budget template to accommodate the specifics of the proposed clinical trial.
4. The NNCCC will provide the budget template to the Lead CTEC PI/Clinical Trial Director for further customization, who will return it to the NNCCC when customization is complete.
5. The NNCCC will distribute the customized budget template to the Lead Clinical Investigators of each of the participating sites. This process will allow each of the participating sites to submit budgets which are similar in content and format.
6. Upon completion of the budgets, each participating site including the Lead CTEC will submit its budget to the NNCCC.
7. The NNCCC will review the individual budgets, identify any obvious issues or discrepancies, and work with the individual sites to resolve any issues.

8. The NNCCC will provide the individual site budgets to NNRI for distribution to the NEER Network Steering Committee. This process protects the confidentiality of sensitive salary information.

Note: (1) Budgets for NEER Network clinical trials should include **only** those costs associated with the conduct of the clinical trial. Costs associated with the delivery of standard care that are reimbursable by third party insurers should **not** be included in the budget. (2) Requests for the purchase of proposed new equipment are permitted; however, all such requests **require** a full justification to be provided in the budget as described above.

2.5 COVER PAGE: SUMMARY INFORMATION, TOTAL COSTS AND SIGNATURES

The Cover Page template for Clinical Trial Full Applications is located on the NEER Network website (www.neernetwork.org) and is not part of the electronic submission system.

- For each proposed clinical site, including the Lead CTEC, the **NEER Network Clinical Trial Cover Page for a Participating Site** must be completed and signed by the CTEC PI or Lead Clinical Investigator for a non-Network clinical site (if proposed) and the institution's authorizing official.
- The signed Full Application Cover Pages are to be sent via e-mail, in pdf format, to the NNRI Project Officer, Stephen M. Rose, Ph.D., at srose@fightblindness.org.

[Note on Conflict of Interest Policy: All NEER Network-supported investigators participating in the design, conduct and analysis of clinical trials are required to comply with the NEER Network Conflict of Interest (COI) policy, and must submit COI disclosure forms when the clinical trial is approved for funding, as well as annually during the execution of the clinical trial, or more frequently if there is a significant change in the investigator's outside interests.]

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Age-dependent effects of RPE65 gene therapy for Leber congenital amaurosis: a phase 1 dose-escalation trial



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Summary

Background Gene therapy has the potential to reverse disease or prevent further deterioration of vision in patients with incurable inherited retinal degeneration. We therefore did a phase 1 trial to assess the effect of gene therapy on retinal and visual function in children and adults with Leber congenital amaurosis (LCA).

Methods We assessed the retinal and visual function in 12 patients (aged 8–44 years) with LCA-RPE65 given one subretinal injection of adeno-associated virus (AAV) containing a gene encoding a protein needed for the isomerohydrolase activity of the retinal pigment epithelium (AAV2-hRPE65v2) in the worst eye at low ($1 \cdot 5 \times 10^{10}$ vector genomes), medium ($4 \cdot 8 \times 10^{10}$ vector genomes), or high dose ($1 \cdot 5 \times 10^{11}$ vector genomes) for up to 2 years.

Findings AAV2-hRPE65v2 was well tolerated and all the patients showed sustained improvement in subjective and objective measurements of vision (ie, dark adaptometry, pupillometry, electroretinography, nystagmus, and ambulatory behaviour). Patients had at least a 2 log unit increase in pupillary light responses, and an 8-year-old child had nearly the same level of light sensitivity as that in normal-sighted individuals. The greatest improvement was noted in children, all of whom gained ambulatory vision. The study is registered with ClinicalTrials.gov, number NCT00516477.

Interpretation The safety, extent, and stability of improvement of vision in all patients support the use of AAV-mediated gene therapy for treatment of inherited retinal diseases, with early intervention resulting in the best potential gain.

Funding Center for Cellular and Molecular Therapeutics at the Children's Hospital of Philadelphia, National Eye Institute-National Institutes of Health, Foundation Fighting Blindness, Telethon, Research to Prevent Blindness, FM Kirby Foundation, Mackall Foundation Trust, Regione Campania Convenzione, European Union, Associazione Italiana Amaurosi Congenita di Leber, Fund for Scientific Research, Fund for Research in Ophthalmology, and National Center for Research Resources.

Introduction

One of the most severe forms of inherited retinal degeneration is Leber congenital amaurosis (LCA), which is a group of diseases that are caused by mutations in any of 13 genes. Patients with LCA have severe loss of vision and abnormal eye movements (nystagmus) in early infancy and during early childhood. Diminished pupillary light reflexes and flat or nearly undetectable responses during electroretinography confirm the clinical diagnosis.^{1–4} LCA2, caused by mutations in a gene that encodes a protein needed for the isomerohydrolase activity of the retinal pigment epithelium (RPE65), accounts for about 6% of cases.⁵ There is no treatment for LCA and severe visual impairment during childhood usually progresses to total blindness by the third or fourth decade of life.⁴ Clues for how to treat LCA2 (LCA-RPE65) came from studies in which mutations in RPE65 resulted in substantially diminished amounts of 11-cis retinal [A: okay (we only use "significant" to mean statistically significant)?].^{6–9}

Replication-deficient adeno-associated virus (AAV)-mediated delivery of the wildtype RPE65 cDNA to the

RPE in animal models of LCA resulted in rapid development of retinal and visual function through the enzyme-mediated generation of 11-cis retinal.¹⁰ Furthermore, the success rate for recovery and magnitude of improvement was related to the age at treatment, with best results obtained in young animals before widespread cellular degeneration.^{11,12} This result and additional findings for safety and efficacy¹³ provided the basis for a phase 1 trial of gene augmentation therapy in individuals with LCA-RPE65, and for the inclusion of children who might get the most benefit from the intervention.¹⁴ AAV-mediated RPE65 therapy in young adults^{15–20} resulted in most individuals reporting a perception of increased brightness in the injected eye after treatment, as judged with various methods, including dark adaptometry, perimetry, and pupillary light reflexes.^{15–18} Two individuals in two studies^{15,16} showed improvements in ambulation. Significant improvements in visual acuity in all three individuals were reported in one study.¹⁵

Here we present the results from the complete phase 1 dose-escalation study done at the Children's Hospital of Philadelphia (CHOP, PA, USA) with the aim to assess

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For more about mutations see <http://www.sph.uth.tmc.edu/retnet/sum-dis.htm>

For additional details about the study see http://www.webconferences.com/nihoba/13_dec_2005.html

See Online for webappendix

the safety and efficacy of AAV2-hRPE65v2.¹⁵ We also assessed the role of an individual's age (or stage of disease progression) on the extent of reversal of blindness.

Methods

Patients

Inclusion and exclusion criteria for patients are reported by Maguire and colleagues.¹⁵ 12 patients (8–44 years) with LCA-RPE65 were enrolled and consecutively treated, with an interval of at least 6 weeks between individuals (table). All surgery was done at CHOP and follow-up tests were done at CHOP or Seconda Università degli Studi di Napoli (Naples, Italy) for the Italian patients.

This study was approved by a national ethics committee in Italy. Patients from Italy provided written informed consent (if ≥18 years) or written assent and parental permission (if <18 years) at two study sites—the Referral Centre of Hereditary Retinopathies, Department of Ophthalmology, Seconda Università degli Studi di Napoli [A: correct?], and Foundation Fighting Blindness CHOP-University of Pennsylvania (CHOP-PENN) Pediatric Center for Retinal Degenerations (Philadelphia, PA, USA). The remainder of the patients provided written informed consent (or assent) only at the Foundation Fighting Blindness CHOP-PENN Pediatric Center for Retinal Degenerations. All patients appearing in webvideos provided written media consents or assents.

Vector and surgical delivery

The transgene cassette in the AAV2-hRPE65v2 vector had a chicken β-actin promoter for expression of the human RPE65 cDNA with an optimised Kozak sequence.²¹ The Center for Cellular and Molecular Therapeutics at CHOP manufactured the vector using good manufacturing practices.^{13,15}

For each patient, we selected the eye with the worst function for treatment with AAV2-hRPE65v2.^{13,15} We did a standard three-port pars plana vitrectomy, with removal of the posterior cortical vitreous, as described by Maguire and colleagues.¹⁵ Patients in the low-dose cohort were injected with $1 \cdot 5 \times 10^{10}$ vector genomes ($1 \cdot 0 \times 10^8$ per μL) and those in the medium-dose with $4 \cdot 8 \times 10^{10}$ vector genomes ($3 \cdot 2 \times 10^8$ per μL) of AAV2-hRPE65v2 in a volume of 150 μL into the subretinal space (table). Patients in the high-dose cohort were injected with $1 \cdot 5 \times 10^{11}$ vector genomes ($5 \cdot 0 \times 10^8$ per μL) in 300 μL (table) after the focal area was buttressed from hydrodynamic stress during injection with perfluorooctane liquid (Perfluoron, Alcon, Fort Worth, TX, USA), which is heavier than water. The liquid was aspirated after the AAV2-hRPE65v2 had been delivered.

Assessment of safety and efficacy

Patients were assessed before and at designated timepoints after surgery as described elsewhere.^{13,15} Efficacy for each individual was monitored with objective and subjective measurements of the changes in vision.¹⁵

The response duration was measured from 3 months to 2 years. Additional details are provided in the webappendix (pp 1–23).

The study is registered with ClinicalTrials.gov, number NCT00516477 [A: style is not to have margin link for this]

Role of the funding source

The main sponsor of the study and personnel working for the sponsor were involved in study design, data gathering, analysis, and interpretations, and writing of the report. None of the other funding sources had any direct role with respect to the design or execution of the study, data gathering, analysis, interpretation, or writing of the report. The corresponding author had full access to all data throughout the study and had final responsibility for submission for publication.

Results

Maguire and colleagues¹⁵ have described the short-term results from the first three patients (NP01, NP02, and NP03 in the low-dose cohort). The vector was injected into the macula in nine patients, but not in three patients (NP01,¹⁵ CH12, CH13) with substantial atrophy in this region. About half the macula of patient NP15 was exposed (figure 1). An epiretinal membrane that was noted during baseline studies in the injected eye of patient CH10 was removed before injection. A foveal dehiscence was noted at the time of injection in this individual as some of the vector escaped from the foveal defect, reducing the total volume in the subretinal space by about 70% and resulting in the exposure of a third of the macula (figure 1).

All of the retinal detachments had resolved by the next assessment (within 14 h after surgery); and foveal abnormalities were noted in only one patient (NP02), as noted previously,¹⁵ with optical coherence tomography. The foveal dehiscence in patient CH10 had completely resolved with no evidence of a macular hole after surgery at the first assessment with optical coherence tomography on day 7 (webappendix p 21). With the exception of pigment atrophy at the lower border of the original detachment site in patient NP15, all the other postoperative retinal assessments were unremarkable.

None of the patients had serious adverse events, and the vector was found in samples of tears and blood only transiently after surgery (webappendix pp 8–9). Exposure to subretinal AAV at the doses used was not immunogenic (webappendix p X) [A: sentence correct? Please provide page number(s) where indicated by p X or p X and p Y].

All 12 individuals reported improved vision in dimly lit environments in the injected eyes starting 2 weeks after surgery. Improvements in visual acuity were substantial and stable in the three patients given the vector at a low dose,^{15,20} three given the middle dose (NP04, CH10, and CH11), and one administered the high dose (NP15). Visual acuity worsened in one patient (CH06; figure 2; webappendix p X and p Y). For the other

	Low-dose cohort			Medium dose cohort				High-dose cohort				
	NP01	NP02	NP03	NP04	CH06*	CH08	CH09	CH10	CH11	CH12	CH13	NP15
Date at administration	Oct 11, 2007	Dec 13, 2007	Jan 24, 2008	April 10, 2008	May 22, 2008	July 10, 2008	Sept 25, 2008	Nov 18, 2008	Dec 30, 2008	March 5, 2009	April 16, 2009	June 2, 2009
Age (years)	26	26	19	17	20	9	8	10	24	44	35	11
Sex	Female	Male	Female	Male	Female	Male	Male	Male	Female	Female	Male	Male
RPE65 or [A:ok?] additional retinal-specific mutations	E102K/ E102K	E102K/ E102K	R234X/ R234X	R91W/ T149N	IVS1+5g>a/ L341S	F530fs/ F530fs[A:ok?]	R124X/ Lys297del1aggA	IVS1+5g>a/ Phe530del1ttc	V473D/ V473D	K303X/ W431C	IVS1+5g>a/ IVS1+5g>a	D167W/ H313R
Injected eye†	Right	Right	Right	Left	Right	Right	Left	Right	Right	Right	Right	Right
Nystagmus frequency before and 90 days after gene therapy (Hz)	3.8/2.0	2.3/2.1	4.2/3.0	1.0/0	1.1/1.3	3.7/2.0	2.5/2.0	4.0/2.0	0.5/0.5	1.0/0.25	0.3/0.4	0.4/0.4

*Also heterozygous for RDH12 S203R. †Eye with worst site selected for surgery.

Table: Summary of patient demographics and nystagmus

individuals, no substantial gains or losses in visual acuity were noted in the injected or non-injected eyes (webappendix p 22). The improvement was not associated with age; however the baseline visual acuity was higher in children than in adults ($p=0.04$; webappendix p 16).

There was no clearcut dose effect with respect to improvements in visual acuity in the injected or non-injected eyes. Figure 2B shows that, with the exception of CH06, visual acuities improved or remained stable. Although, the visual acuity of the injected eye in patient CH08 might have worsened at the most recent visits, further results will be needed from tests done on the designated days (webappendix p 5) to find out whether this change is significant.

We noted an improvement in the visual field of all 12 patients (figure 1). Although visual-field tests in patients with severe impairment show substantial variability,^{22,23} the enlargements exceeded the variation in the contralateral non-injected eye (figure 1).

The extent of improvement in visual fields in the injected eyes correlated with the amount of salvageable retina that was targeted, effects of immediate postoperative head-positioning on the borders of the detachment, and map of the visual field at baseline (figure 1). For example, the visual fields improved substantially in patients CH08, CH09, and CH10, given injections to regions that had initially had restricted function but had viable retina as noted with ophthalmoscopy and optical coherence tomography. Further, if the injection covered regions of healthy retina that had previously had scotomas, the visual field increased as the scotomas were obliterated (eg, CH11; figure 1). Nevertheless, the post-injection visual fields often expanded in regions larger than the region targeted during surgery (eg, CH10, NP15; figure 1). Although the volume injected was larger (300 μ L vs 150 μ L), covering a large part of the retina, the fields did not improve as much in older individuals (>19 years—eg, patients CH12 and CH13) as they did in younger individuals (≤ 19 years—eg, patients CH08, CH09, and NP15). This difference is

probably caused by the loss of viable photoreceptors with advanced disease in older individuals.

Most individuals given middle and high doses were tested for full-field sensitivity to white light before and after injection; NP04 and NP15 were not tested because the equipment was not available. All individuals had bilaterally diminished full-field sensitivity at baseline. After injection, a large interocular difference (ie, difference in sensitivity between injected and non-injected eyes) in full-field sensitivity was noted in five (CH08, CH09, CH10, CH11, and CH13) of seven individuals when we used stringent criteria to assess the response (3 SDs from the average of the interocular difference in normal-sighted individuals; figure 2C). Only the injected eyes showed improved sensitivity (figure 2C). Improvements in full-field sensitivity were especially noteworthy in the youngest patients, who gained several log units of sensitivity.

Pupillary responses improved in the injected eyes of all 11 individuals tested. Figure 3 shows the representative responses from patients given the middle and high doses of AAV2-hRPE65v2 (including children and an adult). The pupil diameter (for the largest of the two pupils) immediately before the first exposure to light for patient CH08 was 8.6 mm at baseline, 7.8 mm at day 14, 8.55 mm at day 365; CH10, 8.0 mm at baseline, 9.1 mm at day 270; CH13, 7.8 mm at baseline, 5.6 mm at day 60, 5.6 mm at day 90; and NP15, 8.0 mm at baseline, and 8.3 mm at day 7. Improved responses were detectable as early as day 7 after injection (in patient NP15) and were present even in the eye that was not injected with the entire subretinal dose because the patient (CH10) had a foveal dehiscence. When the injected eye was illuminated with light, both pupils constricted; when the control, non-injected eye was illuminated with light, minimum constriction of the pupil was seen [A: should we add fig 3 citation?]. Analyses of the variables of the pupillary light response showed substantial differences between the injected and control eyes in the amplitude and velocity of constriction (webappendix p 17).

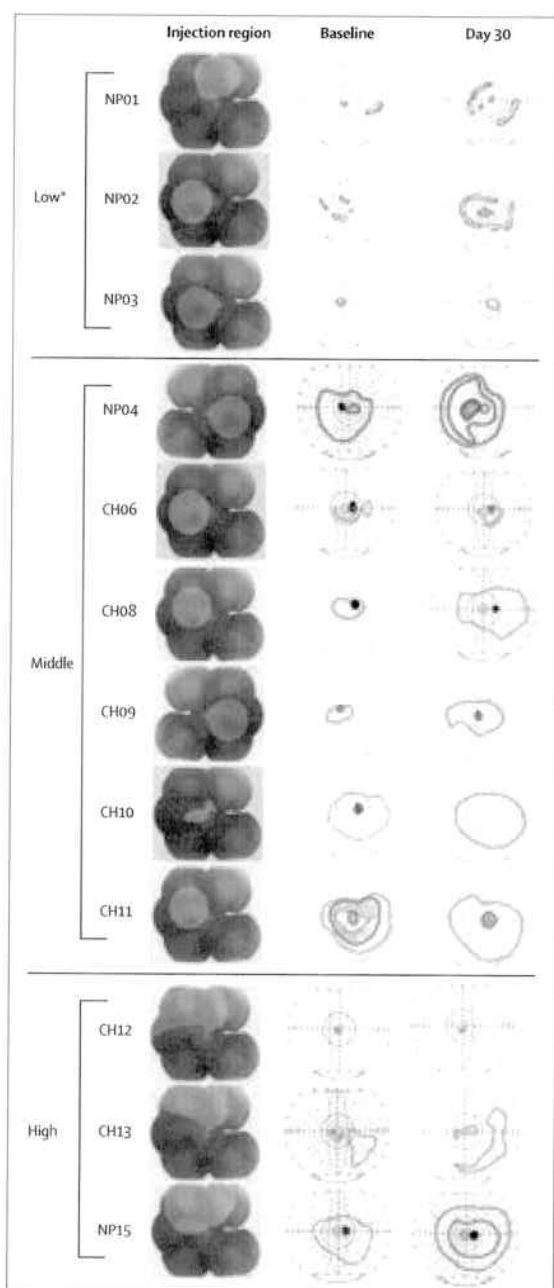


Figure 1: Area of retina exposed to adeno-associated virus-mediated delivery of wild-type retinal pigment epithelium (AAV2-hRPE65v2). Column 1 was drawn over composite photos of a normal retina, and columns 2 and 3 over the baseline and follow-up Goldmann visual fields, respectively, in the injected eyes. All follow-up visual fields are shown at day 30, except for patients NP01 (4.75 months) and NP02 (2.75 months). Stimuli used to measure Goldmann visual fields were V4e (red) and II4e (blue). Scotomas and the natural blind spot are shown in black. *Visual field data from these patients were reported previously¹ but are presented here for completeness.

Baseline tests showed that the pupillary light responses in individuals with LCA-RPE65 were much less sensitive than those reported in unaffected individuals (controls;

figure 3)[A: possible to provide some info about these controls—eg number, age, male/female?]. Baseline responses to a dim stimulus (<0.04 lux) after a 40-min dark adaptation in patients given low, middle, and high doses of the vector were negligible (figure 3).

The responsiveness to light of the injected eye was consistently greater than that of the contralateral non-injected eye in patients after injection of AAV2-hRPE65v2. For CH08, for example (figure 3A), when a dim stimulus (0.04 lux for 200 msec) was initially delivered to the injected eye at baseline, minimum response was noted in either eye. After injection, the eye responded vigorously. Repetition of the pattern of the relative afferent pupillary defect was noted with successive alternating flashes up to the latest timepoint (eg, from day 14 and to day 365 for CH08, day 270 for CH10, and day 90 for CH13 (figure 3A), showing that the pupillary light responses were happening in the injected eye, while the non-injected eye remained defective.

Significant differences were noted in amplitudes and velocities between the injected eyes versus non-treated eyes in most individuals; the exception was patient CH11 (webappendix p X). The differences persisted throughout the follow-up with different intensities of light. Although, little difference was noted in response between patient NP04's injected and non-injected eyes (webappendix p X), stimulation with light at 0.04 lux resulted in a significant difference in velocity between the injected and control eyes ($p=0.003$). Every individual had at least a 2 log unit increase in pupillary-light-response sensitivity in the injected eye. An 8-year-old patient (CH09) had nearly the same (high) level of light sensitivity as did normal-sighted individuals.

The final level of sensitivity in all patients after injection correlated with age (Spearman correlation coefficient (r) -0.80 , $p=0.002$) and baseline sensitivity (0.50 , $p=0.09$; figure 3B). In the analysis of correlation between age and the successive reductions from baseline in light intensity, r was -0.61 ($p=0.03$), suggesting that young individuals are more likely to have step changes in light intensity in the eye injected with AAV2-hRPE65v2. Such changes were not noted in the contralateral non-injected eye. The average change in light sensitivity in the injected eyes was about 2.2 log units in individuals aged 8–11 years (highest change was noted in patient CH09 [3.8 log units]), and about 1.2 log units in those aged 19–44 years ($p=0.04$ for difference in light sensitivity).

Full-field scotopic and photopic electroretinographic responses were flat in all individuals before and after injection even with the use of fast Fourier analysis.²⁴ However, multifocal electroretinography could be done in two patients after injection as a result of a reduction in nystagmus. Because of nystagmus, multifocal electroretinography could only be done at baseline for patient NP15. By contrast, results suggested photopic responses in one part of the injected retina at day 30 and then in several other parts at day 60 (webappendix p X)

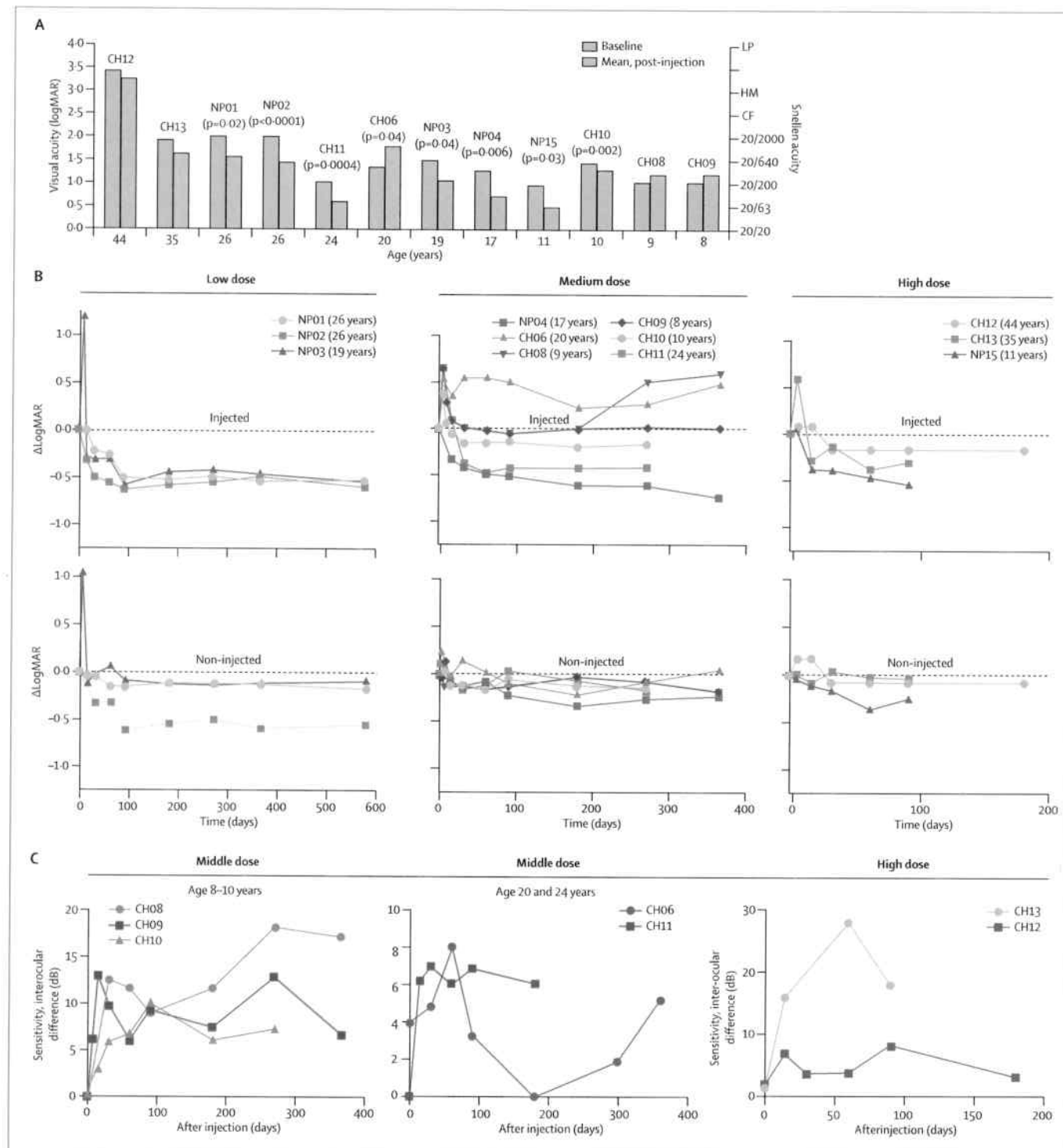


Figure 2: Visual acuity and full-field sensitivity and dark adaptometry changes after injection with adeno-associated virus-mediated delivery of wild-type retinal pigment epithelium (AAV2-hRPE65v2)

(A) Correlation of age with visual acuity in the injected eye. Visual acuity at baseline was compared with the mean visual acuity after injection (all timepoints included); a worsened visual acuity was noted in CH06. p values for significant differences are reported. (B) Change in logarithm of the minimum angle of resolution (LogMAR) scores in the injected and contralateral non-injected eyes is indicated as a function of time for patients given low, medium, and high doses of vector. LogMAR score was normalised to 0 at baseline for each individual. (C) Most patients in the middle and high dose groups were tested for full-field sensitivity to white light before and after injection. LP=light perception. HM=hand motion. CF=counting fingers.

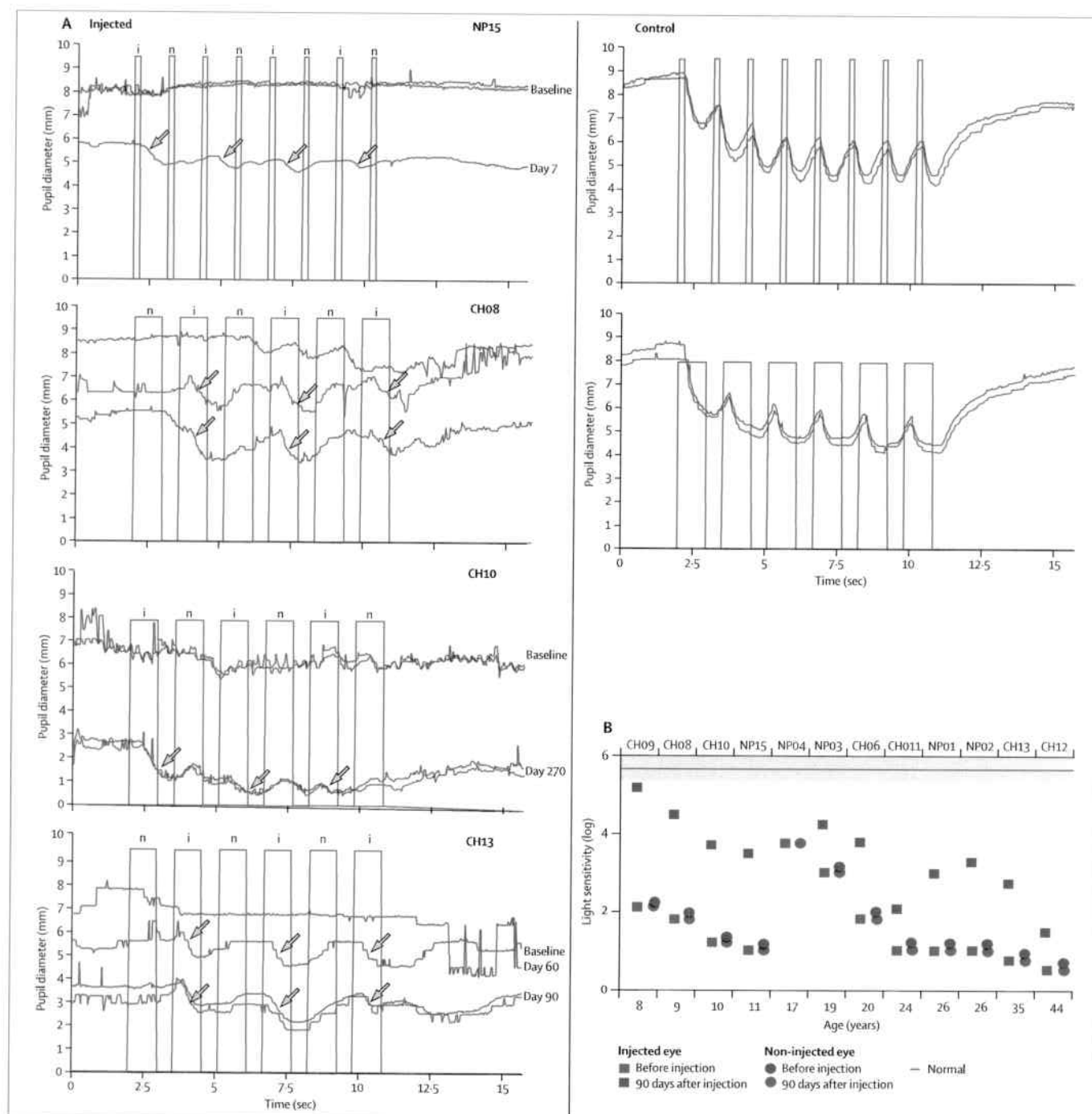


Figure 3: Objective evidence of improvement in pupillary light reflexes

(A) Improved pupillary light reflexes—as a function of time after injection and after alternating stimulation of the injected (i, red columns) and non-injected (n, blue columns) eyes—are shown in representative recordings from patients after injection of middle and high doses of the vector. Red and blue curves represent diameters of the right and left pupils, respectively; however, only one pupil is shown for patient NP15 (day 7 after surgery) because the other was atropinised. Recorded light intensity was 0.04 lux for patients NP15 and CH08, 0.4 lux for CH10, and 10.0 lux for CH13. Days after injection are indicated. Alternating stimuli were presented 2 sec after recording was initiated. In the panel for patient NP15, each stimulus was presented in 200 msec with 1 sec spaces between the flashes. In the panels for patients CH08, CH10, and CH13, stimuli were presented in 1 sec with 600 msec spaces between the flashes. Traces in each panel are shifted vertically to compare responses obtained at different timepoints. Control pupillary light responses (actual pupil diameters) measured in normal-sighted individuals at 4 lux are shown for comparison. (B) Correlation of improvements in full-field sensitivity with age (and baseline retinal sensitivity). The light sensitivities are not shown for patient NP15 because his data were analysed at day 60. The intensity at which the pupillary light response was eliminated from the test eye before injection and at which the relative afferent pupillary defect developed after injection was identified as the lower limit of sensitivity. The mean and SD of sensitivity of normal-sighted individuals in the age range of the patients is indicated by a blue line and shading, respectively.

and day 90 (data not shown). Similarly, tests done after subretinal injection in patient NP04 suggested waveforms in the left part of the retina (figure 1) but not in the contralateral (non-injected) retina (figure 3). Similar results were noted in the injected retina of patient CH09 at day 365 (data not shown) although the results of the contralateral non-injected eye were not recorded.

Nystagmus results for patients given low-dose gene therapy are presented elsewhere.²⁰ When patients were tested for their ability to navigate a standardised obstacle course before administration of AAV2-hRPE65v2, 11 of 12 had great difficulty, especially in dim light, as assessed by the number of errors and time taken. Patient NP04 was not tested at low-light levels. After injection, four children (CH08, CH09, CH10, and NP15) given AAV2-hRPE65v2 had substantial improvement in their ambulation when tested with only the injected eye covered (webappendix p X; webvideos 1–6). They were unable to navigate the course accurately when only their non-injected eye was not covered. These patients could also navigate the course with fewer errors and often more quickly than at baseline with their injected eyes not covered (webappendix p X).

Discussion

All 12 patients given AAV2-hRPE65v2 in one eye showed improvement in retinal function. The effect was stable during follow-up. The results support our hypothesis that the response to subretinal gene therapy depends on the extent of retinal degeneration and, therefore, the age of the patient.¹⁴

Assessment of global retinal function showed clinically meaningful vision in patients. The most noteworthy result was the ability of children to navigate an obstacle course independently and accurately, even in dim light. Objective tests provided quantitative evidence for the improved retinal function and sensitivity in these and other individuals. Pupillometry, a sensitive and robust test that provides quantitative information about the response of the entire retina to light, showed a strong miotic response after illumination of the injected eye (but not the control eye). The improvements in the pupillary responses were easily assessed through measurement of the amplitude and velocity of constriction.^{25,26} There was a stronger pupillary light reflex after illumination of the injected eye when compared with the non-injected eye (ie, an acquired relative afferent pupillary defect or Marcus Gunn pupil) as early as 7 days after injection (patient NP15). The gain in light sensitivity in the injected eye was up to 4 log units. Objective measurement of eye movements showed a reduction in nystagmus in most patients after injection of the gene vector. Suppression of nystagmus indicates improvement in fixation—ie, the ability of the eye to maintain alignment with an object.²⁷ Most subretinal injections targeted the macula, and by contrast with a patient in another study,²⁸ there was no change in fixation (or increase in amplitude of nystagmus). Because of the improvement in nystagmus

in our patients, we were able to do multifocal electroretinography in three individuals after injection; a signal was seen in the electroretinographs of all of these patients. Improvement was not seen with full-field flash electroretinography because the total area of the treatment zones in all patients was too small to generate a gross electrical response.

Results of subjective tests corroborated the improvements noted with those of objective tests. Visual behaviour in the children—as assessed by the ability to walk—showed substantial improvements after treatment (webvideos 1–6). Six individuals had substantial improvements in standard tests of visual acuity or visual fields that could alter their designation as legally blind. We could not find a correlation with dose, baseline vision, or other variables with improvement in visual acuity after treatment. Ultimately, patients may not be able to attain normal acuity (eg 20/20) because of the amblyopic effect of congenital nystagmus that prevents high-resolution central vision as a result of image blur from unsteady fixation. Although central vision is important for normal activities of daily living, visual acuity represents only a small proportion of total retinal and visual function, so the other features of vision might benefit when patients are treated with retinal gene therapy.

Increases in the size of the visual fields in the injected eyes roughly correlated with the area of the retina covered by the injected genome vector. A greater than predicted increase in the size of the visual field, however, probably resulted from immediate postoperative positioning of the patient (webappendix p X). Small shifts in the original retinal detachment in the immediate postoperative period might have contributed to the enlargement of the visual fields in individuals with viable retinal cells. Such shifts in the patients with extensive degeneration were unlikely to expand the visual fields[A: okay?]. Diffusion of the vector into other parts of the retina did not seem to contribute to the enlargement of the visual fields[A: correct?] as these other parts might have previously undergone complete degeneration. This hypothesis is substantiated by the finding that the retinas of older patients had widespread degeneration and improved less.

The injected eyes were more sensitive to light than were the non-injected eyes, which showed no change, during full-field sensitivity testing. The light stimulus in this test is projected externally rather than directed at selected areas of the retina by focal laser, as in microperimetry.^{17,19,28} Although full-field tests and pupillometry show improvements in only the vector-injected retinas, a mild bilateral improvement of visual function (eg, visual acuity) was noted in many patients. Although the underlying mechanisms remain to be elucidated, three potential explanations are that, like microperimetry, full-field tests are subjective, and the results might improve because of patient learning effect; an improvement in nystagmus after injection of one eye

See Online for webvideos 1–6

could result in improved resolution of the other eye;^{15,20} and changes in how the visual signal is processed (central nervous system plasticity) might affect the visual outcome in the non-injected eye after administration of gene therapy to just one eye. Thus bilateral simultaneous or immediately consecutive treatment of both eyes might have a synergistic effect.

Overall, the results of objective and subjective tests support our hypothesis that the greatest improvement in visual function with subretinal gene therapy will occur in young individuals. Although young patients had better visual function at baseline than did older individuals, they also had the greatest overall improvement in vision.

Subretinal gene therapy seemed safe at all administered doses. Treatment with the vector did not elicit local or systemic adverse events. The foveal dehiscence that was apparent during subretinal injection in a patient resolved immediately after surgery and did not seem to be related to the investigational product. We subsequently modified the procedure so that hydrodynamic stress, and therefore the likelihood of, foveal dehiscence or development of a macular hole was kept to a minimum. We did not note any signs of inflammation or acute retinal toxicity after injection. However, the presence of PCR-detectable (but non-quantifiable) vector in blood after injection in two patients with widespread retinal degeneration suggests that transient systemic exposure can occur after administration of a high dose or in individuals with widespread outer retinal atrophy. In future studies, we do not plan to use doses higher than 1.5×10^{11} vector genomes per injection in case we have reached the dose ceiling in terms of potential toxicity.

The clinical benefits of subretinal gene therapy were sustained at the 2-year follow-up. The visual recovery noted in the children confirms the hypothesis that efficacy will be improved if treatment is applied before retinal degeneration has progressed. Assessment of whether the treatment alters the natural progression of the retinal degeneration will be possible in follow-up studies.

The success of this gene therapy study in children provides the foundation for gene therapy approaches to the treatment of other forms of LCA and of additional early onset retinal diseases.

Contributors

[Note to me: must check all signed statements once they've been gathered by NH] AMM, JWM, FS, and JB participated in the design of the clinical trial. AMM, EAP, FT, SR, KAM, XZ, and FS did the surgical and clinical procedures. AMM, AA, JFW, FT, FM, JLB, SR, KAM, BH, OZ, EMS, CA, TMR, JS, FS, and JB obtained and maintained the equipment and supplies for the study. AMM, EAP, FM, JLB, KSS, NJV, FS, and JB participated in the design of specific assays [A: agree with you, not good idea to list all assays—but changed "a specific assay" to "specific assays" if ok with you?]. AMM, FT, SR, AF, KAM, SB, LR, FC, EDB, ES, BPL, and FS participated in patient selection. AMM, KAH, AA, KAM, EMS, CA, JWM, and FS were responsible for the regulatory issues. KAH, JFW, JLB, BH, OZ, and JB generated and validated the clinical vector. EAP, FT, SR, KAM, DCC, XZ, LR, CA, FS, and JB tested

the use of the vector in patients. AMM, EAP, FT, FM, JLB, GY, KAM, SB, JIWM, BH, OZ, FC, EDB, AL, JWM, BPL, FS, and JB did the data analysis. AMM and JB wrote the report. AMM was the principal investigator for the clinical trial. All authors participated in reviewing the report [A: all okay?].

Conflicts of interest

AMM and JB are co-inventors of a pending patent for a method to treat or slow the development of blindness, but both waived any financial interest in this technology in 2002. JB served on a scientific advisory board for Ceregene in 2006–08, and presented a seminar at Novartis in 2009. KAH has served as a consultant for Tacere and as a scientific advisory board member for Amsterdam Molecular Therapeutics, but there was no retinal research involved; been a speaker at the invitation of Genzyme, a company with a research programme in AAV; and is the director Center for Cellular and Molecular Therapeutics at CHOP, which sponsors the clinical trial. FM has served as a consultant for Arthrogen, but there was no retinal research involved; is an inventor of a patent of composition and methods for the detection and modulation of T-cell responses to the AAV capsid. FS, FT, SR, CA, AL, NJV, GY, and AF declare that they have no conflicts of interest.

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